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Synthesis and evaluation of novel bis-and trisphosphonates

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SYNTHESIS AND EVALUATION OF NOVEL BIS- AND TRISPHOSPHONATES

by

Jacqueline Patricia Smits

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the
Doctor of Philosophy degree in Chemistry
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Thesis Supervisor: Professor David F. Wiemer

ABSTRACT

Phosphorus is an essential element for life, observed in all biological systems usually as inorganic phosphate and various organic phosphate esters. Phosphonates are metabolically stable analogues of natural phosphorus-containing compounds and have been used in a variety of industrial and medicinal applications.

Bisphosphonates have been found to be especially good inhibitors of enzymes in the isoprenoid biosynthetic pathway. A screen of bisphosphonates against the enzyme squalene synthase (SQS) resulted in the identification of a lead target. The lead compound was resynthesized via a new method along with two other analogues. It was determined that, although the lead bisphosphonate had potent and selective activity against SQS and resulted in the reduction of cholesterol levels, use of the lead drug in combination with either a statin (lovastatin) or a nitrogenous bisphosphonate (zolendronate) had an even greater impact. Furthermore co-treatment of cells with the lead compound and either lovastatin or zoledronate significantly prevented a reduction of cell viability caused by lovastatin or zoledronate alone. The combination of an SQS inhibitor with either an HMGCR or FDPS inhibitor may be beneficial for reducing cholesterol synthesis while preventing non-sterol isoprenoid depletion.

A series of stilbenoid bisphosphonates has been developed to afford potential inhibitors of enzymes in the isoprenoid biosynthetic pathway, a potential target for cancer therapies. Although the new compounds had limited activity against major enzymes in the isoprenoid biosynthetic pathway, it was determined that one of the targets had modest activity in a screen of inhibitors for decaprenyl diphosphate synthase, an enzyme vital to synthesis and maintenance of cell walls in mycobacteria. A second generation synthesis

of stilbenoid bisphosphonates that contain a *para*-substituted electron withdrawing group was initiated, resulting in the identification of a more potent inhibitor of decaprenyl diphosphate synthase. The use of novel bisphosphonate inhibitors could have an impact on treatment of bacterial diseases such as tuberculosis.

The development of novel phosphorus-containing compounds could provide new inspiration for industrial and medicinal products. The α–trisphosphonic acid esters provide a unique spatial arrangement of three phosphonate groups, and may represent an attractive motif for inhibitors of enzymes that utilize di- or triphosphate substrates. A general route to alkyl derivatives of the parent system has been developed through phosphinylation and subsequent oxidation of tetraethyl alkylbisphosphonates, and the reactivity of these new compounds has been studied in representative reactions that afford additional examples of this functionality. During the course of synthesis of the parent trisphosphonate system, an unusual oxidized bisphosphonate phosphate was discovered, and the methods to synthesize this species have been investigated.

Abstract Approved: _____
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Title and Department

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Date

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CERTIFICATE OF APPROVAL

PH.D. THESIS

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

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has been approved by the Examining Committee
for the thesis requirement for the Doctor of Philosophy
degree in Chemistry at the December 2011 graduation.

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For the walruses

A journey of a thousand miles begins with a single step.

Laozi

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9-BBN	9-Borabicyclo[3.3.1]nonane
Ac	Acetyl
ADP	Adenosine diphosphate
AIBN	Azobisisobutyronitrile
Anal.	Analysis
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
br	Broad
Bu	Butyl
C	Celsius
C_{50}PP	ω-*E*,poly-*Z*-decaprenyl diphosphate
Calcd	Calculated
CNS	Central Nervous System
d	Doublet
DHDDS	Dehydrodolichol diphosphate synthase
DMAPP	Dimethylallyl pyrophosphate
DNA	Deoxyribonucleic Acid
DMF	Dimethylformamide
dt	Doublet of Triplets
ESI	Electrospray ionization
Et	Ethyl
Et_2O	Diethyl ether

EtOAc	Ethyl acetate
EtOH	Ethyl alcohol
Eq	Equivalents
FDPS	Farnesylpyrophosphate synthase
FPP	Farnesylpyrophosphate
FTase	Farnesyltransferase
g	Gram
GGDPS	Geranygeranyldiphosphate synthase
GGPP	Geranylgeranylpyrophosphate
GGTase	Gernaylgeranyltransferase
GI_{50}	Growth Inhibition at 50%
GPP	Geranylpyrophosphate
Grubbs II	Grubbs second generation catalyst
GTP	Guanosine triphosphate
h	Hour
HepG2	Human hepatoma cell line
HIV	Human immunodeficiency virus
HMG-CoA	3-Hydroxyl-3-methylglutaryl coenzyme A
HMGCR	3-Hydroxyl-3-methylglutaryl coenzyme A reductase
HRMS	High resolution mass spectroscopy
HWE	Horner–Wadsworth–Emmons
Hz	Hertz
IC_{50}	Inhibitory concentration of 50% activity

IPP	Isopentylpyrophosphate
iPr	Isopropyl
J	Coupling constant
K562	Human leukemia cell line
KHMDS	Potassium hexamethyldisilyl azide
Kg	Kilograms
LAD	Lithium aluminum deuteride
LDA	Lithium diisopropyl amide
LDL	Low density lipoprotein
LDLR	Low density lipoprotein receptor
LHMDS	Lithium hexamethyldisilyl azide
m	Multiplet
min	Minutes
m-CPBA	*meta*–Chloroperbenzoic acid
M	Molar
Me	Methyl
MEP	Non–mevalonate pathway
MeOH	Methyl alcohol
mg	Milligram
mL	Milliliter
mmol	Millimole
MDR	Multi-drug resistant
n–BuLi	*n*–Butyl Lithium

NaHMDS	Sodium hexamethyldisilyl azide
NBS	N–bromosuccinimide
NMR	Nuclear magnetic resonance
Pi	Orthophosphate
PPi	Pyrophosphate
ppm	Parts per million
q	Quartet
rt	Room temperature
s	Singlet
SAR	Structure-activity relationship
sat.	Saturated
SF–295	CNS cancer cell line
SQS	Squalene synthase
t	Triplet
tt	Triplet of triplets
TB	Tuberculosis
TEA	Triethylamine
TEMBP	Tetraethyl methylenebisphosphonate
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TLC	Thin-layer chromatography
TMS	Trimethylsilyl
TMSBr	Trimethylsilyl bromide

XDR	extensively drug resistant

CHAPTER I

NATURAL AND SYNTHETIC ORGANO-PHOSPHORUS COMPOUNDS

Phosphorus is one of the six essential elements required for all known life on earth. Carbon, hydrogen, nitrogen, oxygen, sulfur and phosphorus are the major components of life and necessary for the composition of nucleic acids, proteins, and lipids. These six elements along with some trace metals are found in every living thing and play a vital role in biological structures and processes. Phosphorus is frequently observed in biology as a phosphate or a pyrophosphate and is a required component of deoxyribonucleic acid (DNA) and ribonucleic acids (RNA) as well as phospholipids and adenosine triphosphate (ATP) (Figure 1). Although there have been recent reports that arsenic can be substituted in the place of phosphorus in some living organisms,[1] there has not been full scientific consensus on this matter and the topic is currently widely debated.[2, 3,4]

Phosphorus, in the form of pyrophosphates, plays a vital role in cellular biochemistry. One example of this is the reversible conversion of ATP (**1**) to adenosine diphosphate (ADP, **2**) or adenosine monophosphate (AMP, **3**). In the forward reaction ATP is hydrolyzed resulting in either the formation of ADP and orthophosphate (Pi) or the formation of AMP and pyrophosphate (PPi). If ADP is formed it can undergo further hydrolysis to produce AMP and a second equivalent of Pi. The cleavage of the phosphoanhydride bonds releases a large amount energy, which can be harnessed to drive enzymatic processes that require energy. Because cells have a limited supply of ATP, the

reverse reaction can occur in which ATP is synthesized from ADP by the enzyme ATP synthase[5] through a process known as chemiosmosis.[6]

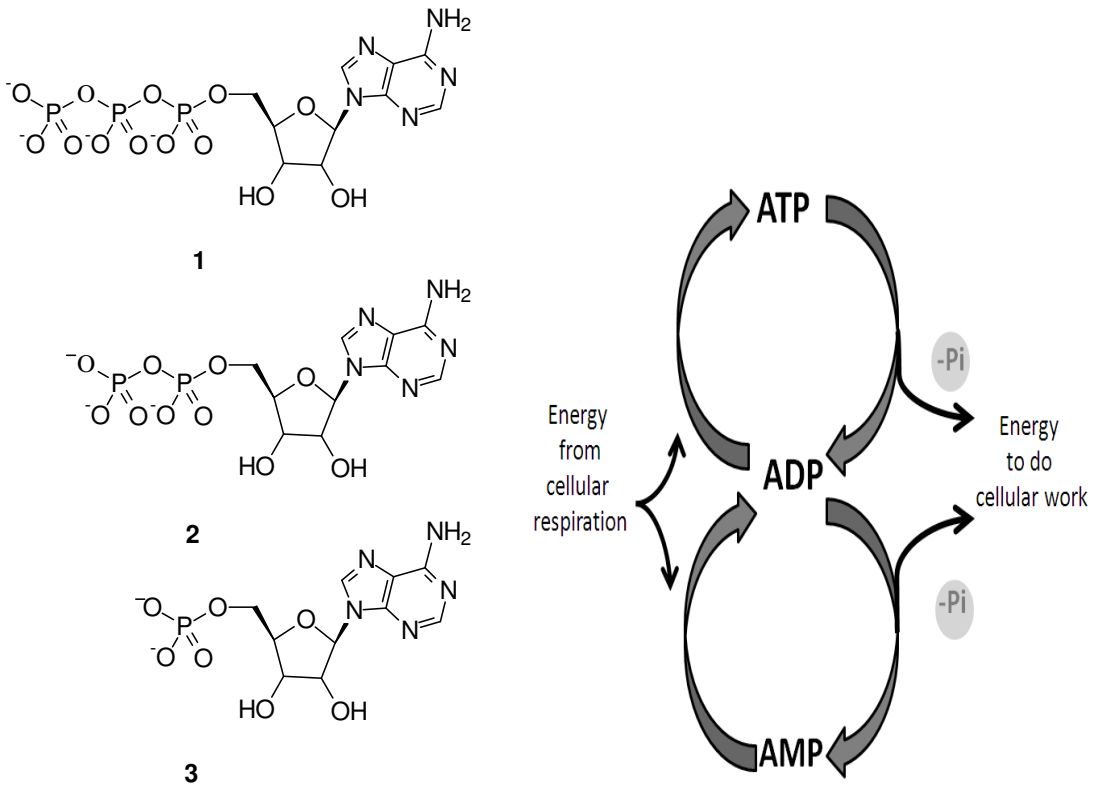


Figure 1. ATP is converted into energy

Pyrophosphates also play an important role in enzyme-catalyzed transformations that result in the formation of larger biomolecules. For example, these processes are necessary for the synthesis of higher order terpenes via progression through the isoprenoid biosynthetic pathway (mevalonate pathway). The first dedicated step in the mevalonate pathway is the reduction of 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) by the enzyme HMG-CoA reductase. This is also the rate-limiting step in this pathway. The reduction of HMG-CoA results in the formation of mevalonate.

Mevalonate undergoes transformation by a series of kinases and a decarboxylase to provide isopentenyl pyrophosphate (IPP), which can be interconverted with its isomer dimethylallyl pyrophosphate (DMAPP). Next an equivalent of IPP and one of DMAPP are joined in a head-to-tail fashion to produce the ten-carbon geranyl pyrophosphate (GPP) in a reaction catalyzed by the prenyltransferase farnesylpyrophosphate synthase (FDPS).[7] Another equivalent of IPP can be added to the GPP chain by FDPS to produce the fifteen-carbon farnesyldiphosphate (FPP).[8]

Fanesylpyrophosphate is a major branch point of the isoprenoid biosynthetic pathway. If FPP undergoes condensation with IPP through the action of dehydrodolichol diphosphate synthase (DHDDS), it can proceed to the formation of the polyprenols known as dolichols which play a role in *N*-glycosylation. Alternatively, one molecule of FPP can undergo head-to-head condensation with an additional molecule of FPP, a process catalyzed by the enzyme squalene synthase (SQS). The resulting molecule is known as pre-squalene which is eventually converted to squalene. Squalene is processed through multiple steps to form sterols. The molecule FPP also can undergo direct transfer to a protein in a process mediated by farnesyltransferase (FTase). If FPP becomes a substrate for the enzyme geranylgeranyldiphosphate synthase (GGDPS), then an additional equivalent of IPP can be added to the scaffold resulting in the formation of the twenty-carbon geranylgeranylpyrophosphate (GGPP). Like FPP, GGPP also can undergo direct transfer to proteins through a process catalyzed by a geranylgeranyltransferase (GGTase). The attachment of isoprenoid “tails” to proteins is an important post-translational modification and is essential for the function of

farnesylated (e.g. Ras, RhoB etc.) and geranylated proteins (GTPases, e.g., RhoA, RohB, Rap1a etc.).[9,10]

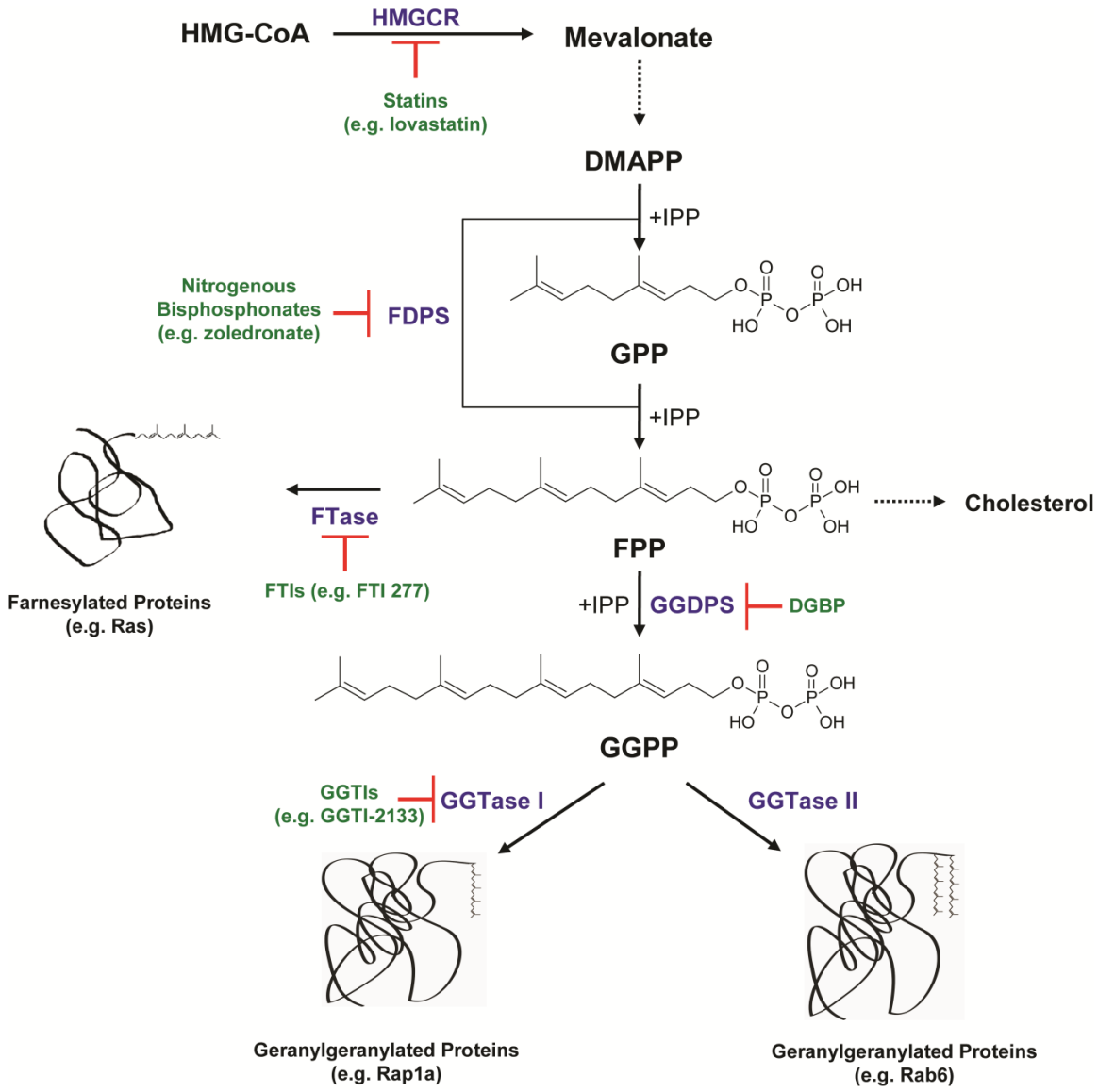


Figure 2. The Isoprenoid biosynthetic pathway.

Because of the importance of phosphorus in biological systems, it has frequently been an inspiration for the development of synthetic phosphorus agents. Pyrophosphates

are demonstrably unstable as seen in the inter-conversion of ATP and AMP. Phosphonates are structurally similar to phosphate esters and can often act as inhibitors of enzymes in which phosphates and pyrophosphates are substrates. The high stability of the carbon-phosphorus bond gives these inhibitors a significant lifetime in vivo.[11,12]

Natural phosphonates are less common than their pyrophosphate counterparts,[13,14] though numerous synthetic phosphonates have been prepared for use in a variety of industrial applications. Phosphonates are able to chelate divalent cations such as $Ca^{2+}$and Ba^{2+} ions presumably due to their charge distribution and tetrahedral orientation.[15,12] The ability of phosphonates to chelate metal ions and inhibit the deposit of scale has been exploited in water softeners, detergents, and in the textile industry where they are used to complex metals in chlorine-free peroxide-based bleaching solutions.[15] Phosphonates also have been used as pesticides (MCP, **4**),[16] fungicides (o,o-biphenyl methyl phosphonate, **5**), and herbicides (glyphosphate, **6**).

Some phosphonates have medicinal applications. One example is the drug tenofovir which is an acyclic nucleoside phosphonate.[17] Tenofovir (**7**) is an antiretroviral drug that inhibits the reverse transcriptase enzyme and is used in the treatment of viral diseases such as human immunodeficiency virus (HIV) and hepatitis B.[17] The α-fluoro-α-alkylphosphonates (**8**) (Figure 3) are a class of phosphonates that have been developed to mimic biological phosphate[18] and are being studied for their ability to inhibit a variety of phosphate-utilizing enzymes.[19,20]

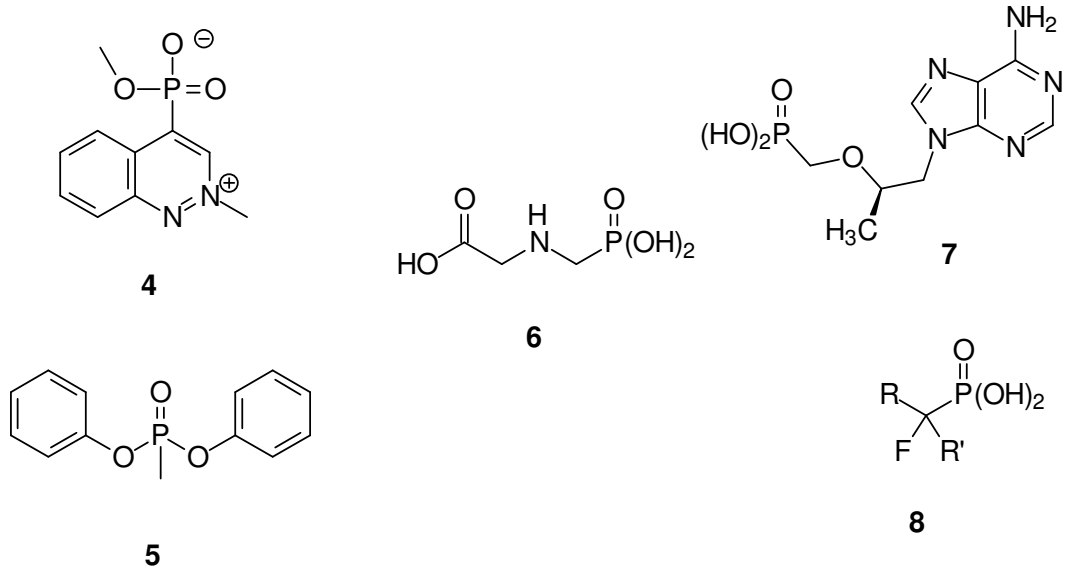


Figure 3. Examples of synthetic phosphonates.

Another relevant class of clinical phosphonates is the bisphosphonates. Bisphosphonates can be viewed as metabolically stable analogues of pyrophosphates (Figure 4). Formal replacement of the central bridging oxygen of a pyrophosphate with a carbon atom not only increases the stability, but it also makes the structure amenable to modification with a variety of substituents.

Figure 4. Pyrophosphoric acid and bisphosphonic acid.

The first bisphosphonates were synthesized in 1897.[21] Etidronate, the first bisphosphonate to be used clinically in humans, was first synthesized over 100 years ago.[22] Originally, bisphosphonates were used as corrosion inhibitors and complexion

agents in textile, fertilizer, and oil industries.[22] Bisphosphonates also were found to act as sequestering agents for calcium and were used as "water softeners" by limiting the ability of calcium carbonate to precipitate, reducing the build-up of calcium carbonate scaling.[15] In the 1960's, the ability of bisphosphonates to sequester calcium was applied to the inhibition of boneresorption[23] which eventually lead to the use of bisphosphonates in bone-related diseases.

The clinical bisphosphonates (Figure 5) fall into two general categories. One includes non-hydrolysable analogues of ATP such as etidronate (**11**) and clodronate (**12**) which interfere with ATP-dependent intercellular pathways. The second includes nitrogenous bisphosphonates (e.g., alendronate (**13**), pamidronate (**14**), residronate (**15**) and zolendronate (**16**)) which act as substrate mimics and inhibit the key enzyme FDPS in the mevalonate biosynthetic pathways [24,22] by chelating to one of the magnesium ions in the active site of the enzyme.

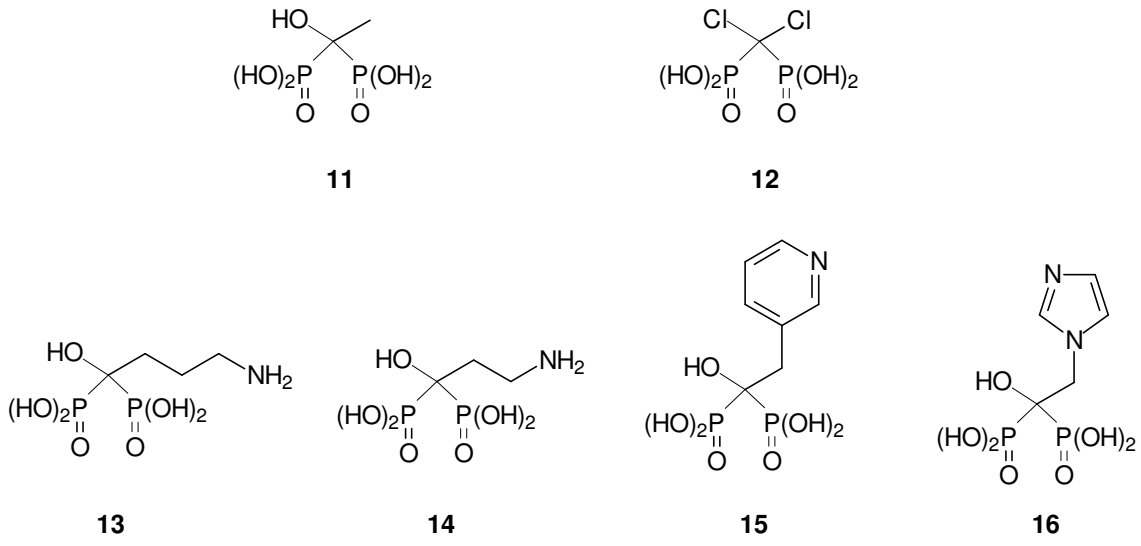


Figure 5. Clinically used bisphosphonates.

Several other enzymes within the isoprenoid biosynthetic pathway have specific bisphosphonate inhibitors as well. Bisphosphonate inhibitors of SQS have been discovered that may prove useful in the treatment of hypercholesterolemia.[25] The first selective inhibitor for GGDPS, known as digeranyl bisphosphonate, was reported in 2006.[26,27] Phosphonate inhibitors of transferase enzymes within the pathway have also been discovered[24] and investigations of new analogues are underway.

Because phosphonates have proven to be incredibly useful, there have been a substantial number of investigations regarding their syntheses. Several processes to synthesize mono-phosphonates through C-P bond formation have been established (Figure 6). The Abramov reaction utilizes an aldehyde and a trialkylphosphite to produce an α-hydroxy phosphonate.[28] A similar transformation is the Pudovik reaction, where a dialkylphosphite under basic conditions adds to a carbonyl group to provide an α-hydroxy phosphonate.[28] The Michaelis-Arbuzov reaction is the most commonly used process for the synthesis of phosphonates from alkyl halides through reaction with triethylphosphite.[29] More recently, the direct conversion of alcohols to phosphonates through a Lewis acid-mediated variation of the Arbuzov reaction has been reported.[30,31]

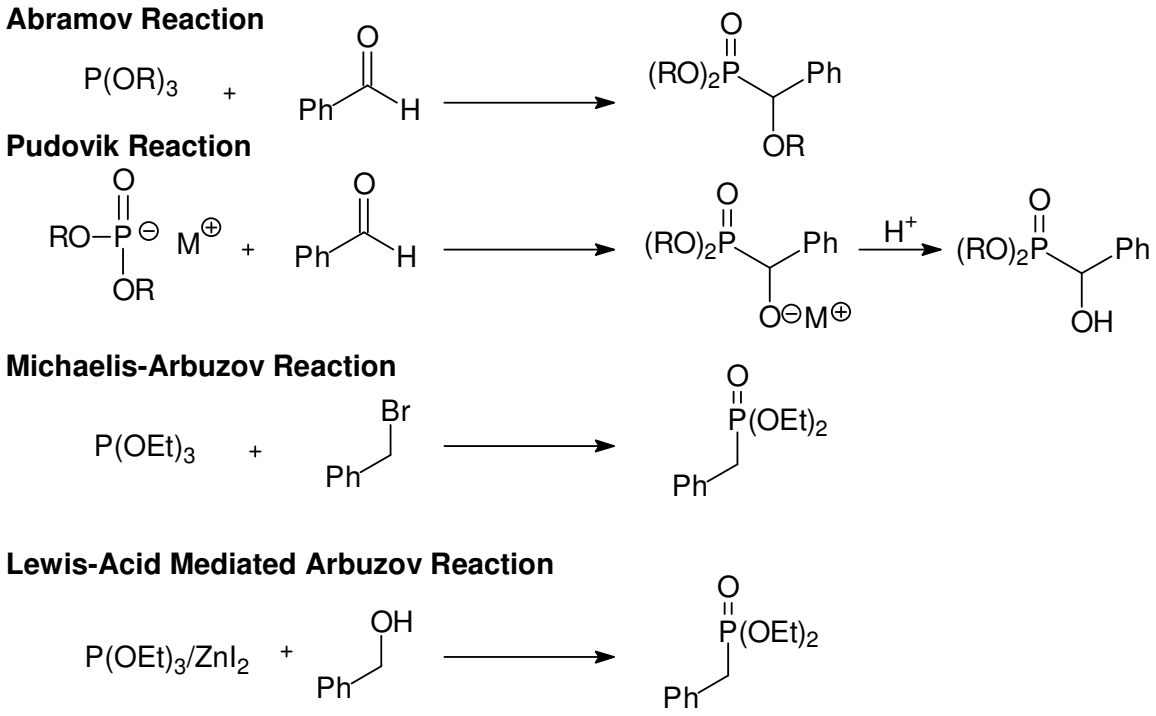


Figure 6. Selected ethods to synthesize monophosphonates based on nucleopholic phosphorus.

Like monophosphonates, the bisphosphonates have an established history of syntheses (Figure 7). One of the most common methods to synthesize bisphosphonates is through the alkylation of tetraethyl methylenebisphosphonate (TEMBP). Treatment of TEMBP with base followed by addition of an allylic halide results in the formation of both the mono-and dialkyl products.[26, 32] A second method for the synthesis of alkyl bisphosphonates is through Michael addition to the vinyl bisphosphonate.[33] Bisphosphonates also can be synthesized through C-P bond-forming reactions. For example, treatment of a monophosphate with base followed by addition of a dialky chlorophosphate results in formation of a bisphosphonate. More recently, it has been demonstrated that bisphosphonates also can be formed through a Lewis acid- mediated

process from benzaldehydes.[31] The α-hydroxy bisphosphonates often are synthesized through reaction of the acid chloride with diethylphosphite.[34] This process also can result in the formation of an O-P rearranged product.[35]

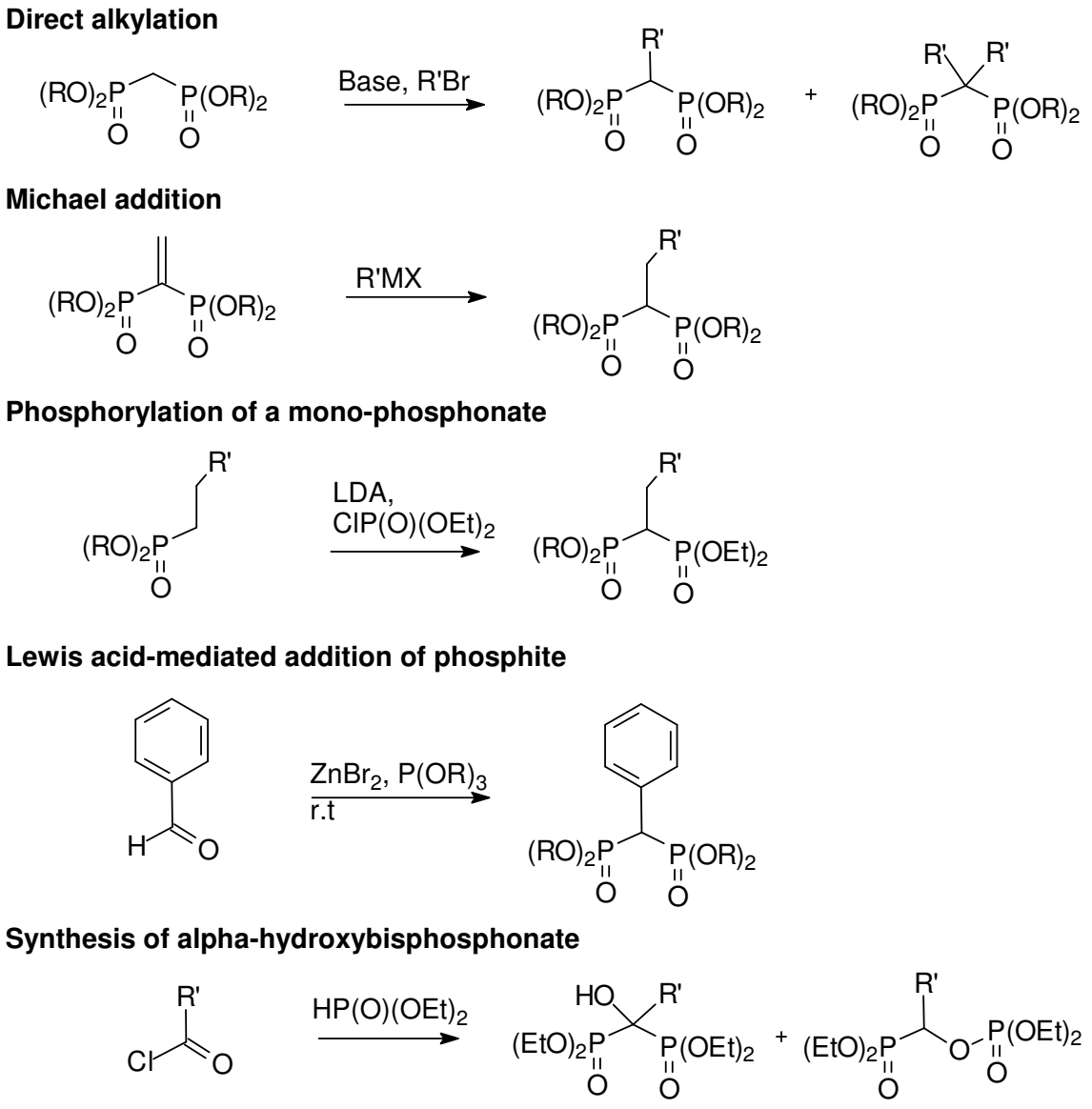


Figure 7. Examples of methods to synthesize alkylbisphosphonates.

Our group has had a long standing interest in development of new C-P bond formation reactions, C-C bond formation using phosphorus reagents,[36] and synthesis of new phosphorus compounds. Our investigations which utilize phosphorus have led to new C-P bond formation methodologies,[30,37,38,39,40] total synthesis of natural products and natural product analogues,[41,36,42] synthesis of phosphorus-containing biological probes and enzyme inhibitors,[43, 44,32] and the development of new phosphonate classes.[45,46] Because synthetic phosphonates have proven to be abundantly useful in a variety of applications, phosphorus-containing compounds remain a rich source of new chemistry. The use of phosphorus for chemical, industrial, and medicinal applications will likely provide inspiration and tools for many other scientific endeavors. In the following chapters, a series of studies on the synthesis and biological evaluation of new phosphonates will be described.

CHAPTER II

NOVEL BISPHOSPHONATE INHIBITORS OF CHOLESTEROL BIOSYNTHESIS

In humans, the mevalonate pathway is responsible for the synthesis of cholesterol, (**17**) a polycyclic compound produced from the biosynthetic precursor squalene. Squalene is formed through a series of enzymatic reactions beginning with the reduction of 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) by HMG-CoA reductase to produce mevolonate. Mevolonate undergoes transformation to isopentenyl pyrophosphate (IPP) which can interconvert with its isomer dimethylallyl pyrophosphate (DMAPP). Farnesyl diphosphate synthase (FDPS) an enzyme in the pathway utilizes both IPP and DMAPP to produce farnesyl pyrophosphate (FPP) which can then undergo reaction with a second equivalent of FPP to produce pre-squalene diphosphate. Pre-squalene diphosphate undergoes subsequent reaction to form squalene which is eventually converted into cholesterol (Figure 8).[10]

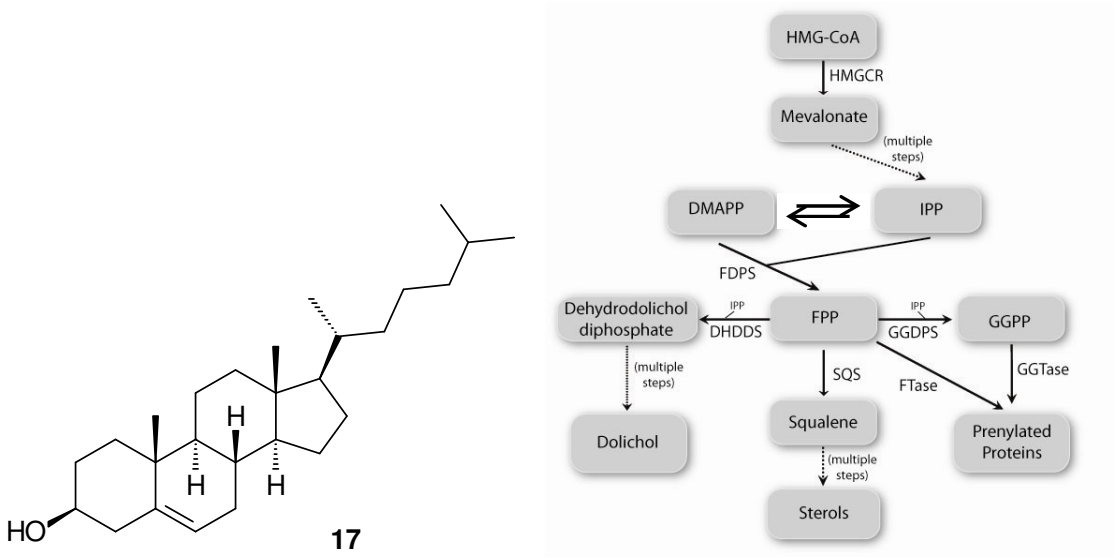


Figure 8. Structure and biosynthesis of cholesterol

Due to the impact of cholesterol production on human physiology, the mevalonate pathway has been a target of numerous pharmaceutical endeavors. One of the most common pharmaceutical drug classes used for the reduction of cholesterol levels is the statins (e.g., lovastatin (**18**) and atorvastatin (**19**) Figure 9).

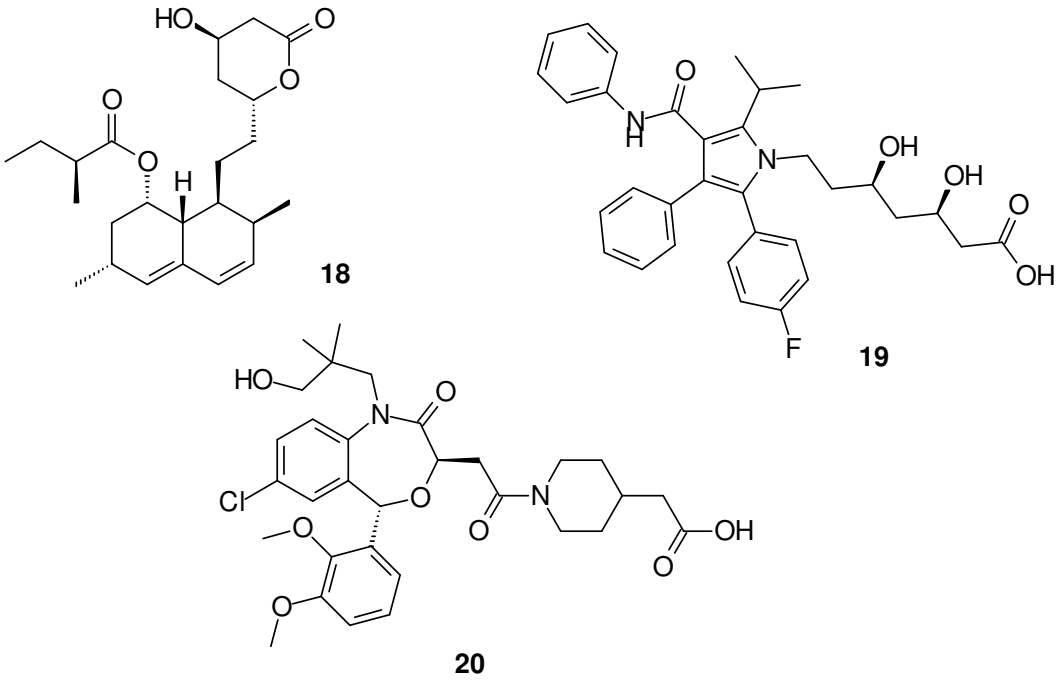


Figure 9. Structures of lovastatin (**18**), atorvastatin (**19**), and lapaquastat (**20**)

The statins act through inhibition of HMGCR [47] which is the rate limiting step in the biosynthesis of cholesterol.[48] Inhibition of HMGCR causes an up-regulation of the low density lipoprotein (LDL) receptor (LDLR) in the liver causing a reduction in the uptake of LDL from the bloodstream.[49] Although statins have been prescribed abundantly and used effectively, there can be side effects from their use. Myopathy and hepatoxicity have been associated with the depletion of non-sterol components in the mevalonate pathway.[50,18] A more specific inhibitor could alleviate some of the side effects that are associated with statin usage.

The inhibition of squalene synthase provides a potentially desirable target for the reduction of cholesterol levels.[51, 52] Specific inhibition of SQS should not only reduce the production of squalene and subsequently reduce cholesterol, but also allow other enzymes in the pathway to produce substrates required for normal biological function.

Other inhibitors of SQS have had limited success. Lapaquastat (TAK-475, Takeda, **20** Figure 9) had progressed to Phase III clinical trials, but research has since been discontinued due to high dose (100 mg/kg) hepatoxicity.[52] It is still unknown if these side effects are attributed to the drug class or if they are due to the specific inhibition of SQS. Inhibition of SQS can cause an accumulation of FPP as well as FPP metabolites, some of which could be responsible for the hepatoxicity observed in the lapaquistat studies. For example the accumulation of farnesol can be pro-apototic at high concentrations (IC_{50} 25 to 250 μM).[53]

Another class of compounds that has shown promise as inhibitors of enzymes in the mevalonate pathway is the bisphosphonates. The α-hydroxy bisphosphonates are used clinically in the treatment of osteoporosis,[54] because thay accumulate in osteoclasts and inhibit FDPS. The nitrogenous bisphosphonates residronate (**15**) and zoledronate (**16**) have been shown to inhibit FDPS, a major branch point of the mevalonate pathway. They also have been used clinically in the treatment of osteoporosis and metastatic bone disease.[55] Monogeranyl (**21**) and digeranyl bisphosphonate (**44**) were the first compounds synthesized that had specificity for inhibition of GGDPS, an enzyme that is also responsible for prenylation events that appear down-stream of FDPS. [26]

A screen for inhibitors of SQS from the library of bisphosphonates that had been synthesized by our group resulted in discovery of a lead compound (**25**, Figure 10). The compounds **21**,[56] **22**,[44, 57] and **25** [44, 57] already had been synthesized. The lead inhibitor **25** is characterized by a biphenyl moiety attached at the C9 position of a geranyl chain, and showed differential activity for the enzyme SQS. Due to a limited supply of the lead compound, it needed to be resynthesized for further evaluation as an SQS inhibitor. In

addition to the lead compound, two analogous biphenyl compounds, where the substitution pattern of the second phenyl ring is either *ortho* (**23**) or *meta* (**24**), also were prepared for investigation as potential inhibitors of SQS.

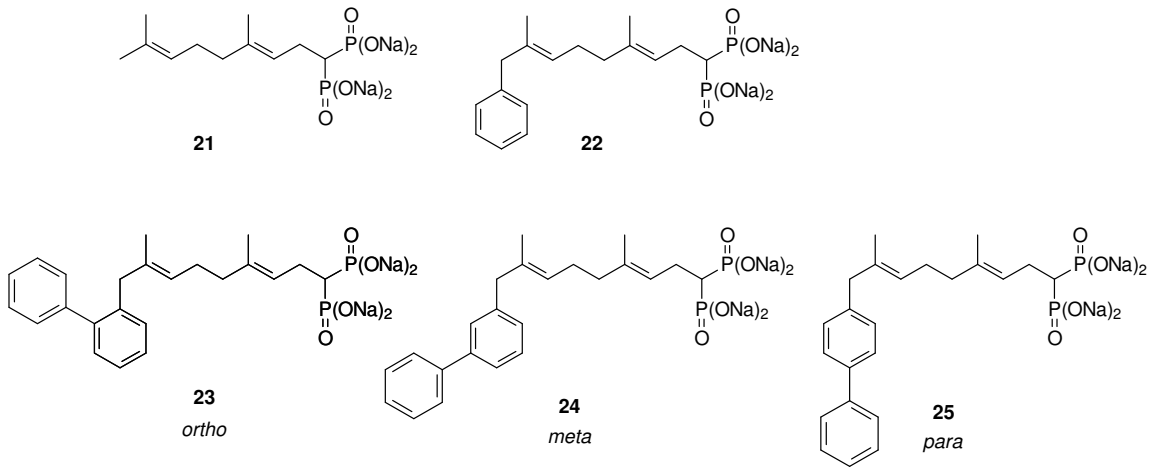


Figure 10. Structures of compounds tested for inhibitory activity against SQS

The original route selected to synthesize the compounds involved the reported synthesis of **25**, in which a THP-protected alcohol located at the C9 position of monogeranyl bisphosphonate undergoes a copper-mediated displacement with a Grignard reagent resulting in the coupled product (**25**).[57] It was expected that this method would provide an efficient route to the *para* substituted lead (**25**) as well as the desired *ortho* (**23**) and *meta* (**24**) targets.

Treatment of tetraethyl methylenebisphosphonate with geranyl bromide resulted in formation of a mixture of mono- (**26**) and dialkylbisphosphonates which were separable via flash chromatography. The ^{31}P and ^{1}H NMR data for compound **26** were consistent with reported literature values.[56] Oxidation of the mono-geranylated bisphosphonate with SeO_2 resulted in formation of a separable mixture of an aldehyde

and an alcohol (compounds **27** and **28**, respectively).[57] The aldehyde **27** was subjected to reduction with sodium borohydride to provide more of the alcohol **28**. Treatment of the free alcohol with 3,4-dihydro-2H-pyran resulted in formation of the tetrahydropyranyl ether (THP) **29**. The ^{31}P and ^{1}H NMR data for compound **29** were consistent with reported literature values.[57] With the THP protected alcohol **29** in hand it was subjected to treatment with the requisite Grignard reagent (Figure 11). However, despite numerous trials, no appreciable amount of the Grignard displacement product could be obtained.

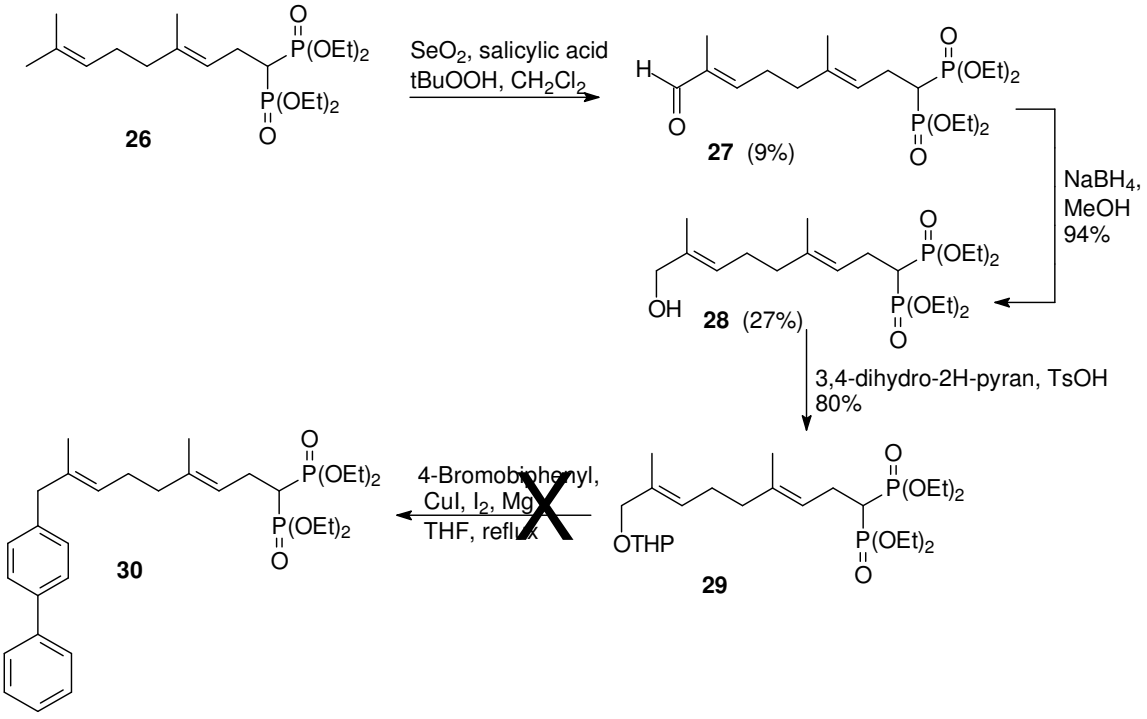


Figure 11. Attempts to synthesize **30** via reported literature procedures

To explain the failure of this reaction, it was hypothesized that a competitive homo-coupling might have been occurring. A very nonpolar compound was isolated from the reaction mixture. Analysis of this sample yielded numerous peaks in the

aromatic regions of the ^{1}H and ^{13}C NMR spectra as well as a molecular ion consistent with the formation of quarterphenyl. This data is consistent with the formation of a product arising from either an Ullman reaction or a homo-coupled Wurtz-Fittig product.[58] It is has been demonstrated that Grignard reagents and aryl cuprates[59]can undergo reaction with a second equivalent of the same species resulting in the homo-coupled product (Figure 12). [60]

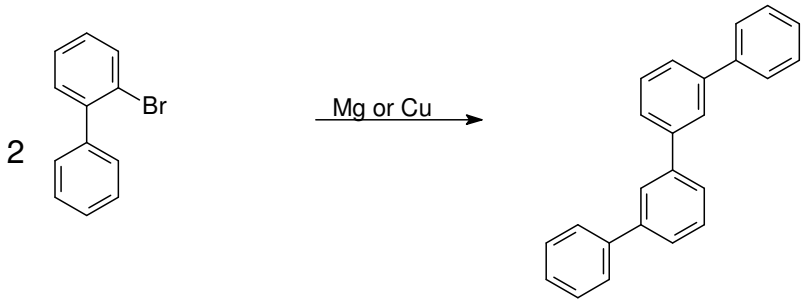


Figure 12. Representative formation of a homo-coupled product.

To circumvent this problem, an alternative route was employed. Prior work had shown that a THP- protected allylic alcohol could be displaced to provide the coupled product.[61] This route had been employed in synthesis of FTase inhibitors, but none of the biphenyl geranyl bisphosphonate derivatives had been synthesized through this method. Utilization of this methodology would provide the desired alkyl group which could then be subjected to standard nucleophilic bromination with PBr_3. With the requisite bromides in hand, alkylation of the anion of tetraethyl methylenebisphosphonate would provide the desired bisphosphonate esters which could then be hydrolyzed to the salts.

Geranyl acetate (**31**) was oxidized with SeO_2 under standard conditions to provide a mixture of both the aldehyde and alcohol **32** which were separable by flash chromatography.[62] The newly formed hydroxyl moiety was protected by reaction with

3,4-dihydro-2H-pyran and the acetate protecting group was removed using K_2CO_3 to provide the THP-protected ether **35**.[63,64] The THP group of alcohol **35** was subjected to copper-mediated Grignard displacement with the *p*-biphenyl Grignard reagent.[44, 57, 61] The Grignard reagent was prepared by dropwise addition of a solution of the 4-bromobiphenyl to Mg metal and a crystal of iodine in diethyl ether followed by gentle heating. The mixture was then treated with copper iodide and the THP-protected alcohol **35**. The desired product **38** was isolated in 64% yield which is consistent with other copper-mediated THP ether displacements with a Grignard reagent.[61,57] Standard conversion of the free hydroxyl group to the allylic bromide (**41**) was performed using PBr_3. After standard workup, the bromide was carried forward in the synthesis without further purification and used for the alkylation of tetraethyl methylenebisphosphonate. Treatment of tetraethyl methylenebisphosphonate with KHMDS followed by addition of the allylic bromide provided the desired phosphonate ester **30** in modest yield.[65] The phosphonate esters were hydrolyzed under standard McKenna procedures to provide the corresponding tetrasodium salt (**25,** Figure 13).[66]

This process was repeated using the *ortho* biphenyl analogue without complication to provide the *ortho* substituted bisphosphonate ester **42**, which was hydrolyzed under the same conditions to yield the tetrasodium salt **23**. Attempts to perform the copper-mediated THP displacement with the *meta* substituted biphenyl bromide proved more arduous. Despite the formation of the desired product **37,** the yields for this transformation on this substrate were considerably lower (55%) than those observed for the *ortho* (**36**) and *para* (**38**) analogues (87% and 64%, respectively).[61,57]

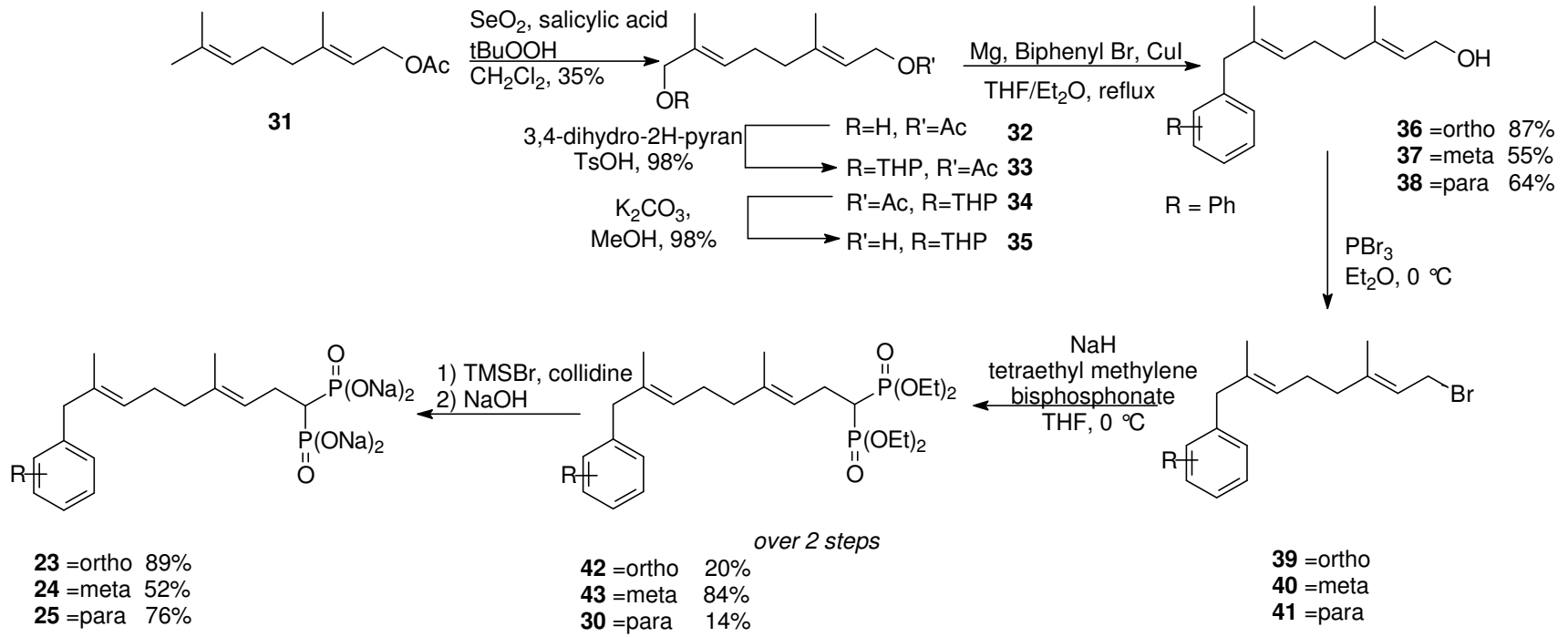


Figure 13. Synthesis of *ortho* (**23**), *meta* (**24**), and *para* (**25**) biphenyl methylenebisphosphonate salts.

To determine the activity of these new bisphosphonates the purified enzyme SQS was treated with inhibitor and radiolabeled [1-3H]-FPP and incubated for 10 min at 37 °C. The products of the reaction were extracted into petroleum ether and transferred to a vial containing scintillation fluid. The radioactivity was quantified with a scintillation counter and the data was graphically analyzed. A plot of the dose response curves was used to determine the concentration of compound that is required to inhibit 50% of SQS activity (IC_{50}). Monogeranyl bisphosphonate (**21**) had an IC_{50} of 1361 nM in this assay. Addition of a phenyl ring at the C-9 (**22**) position dramatically enhances potency to an IC_{50} of 26.5 nM. Introduction of an additional phenyl group at either the *ortho* (**23**), *meta* (**24**), or *para* (**25**) position of the first ring further increases the inhibitory activity resulting in IC_{50} values of 5.7 nM, 13.4 nM and 7.1 nM, respectively (Figure 14). Tukey post hoc analysis indicated that compounds **23**-**25** were statistically different from compound **21,** but were not statistically different from each other.

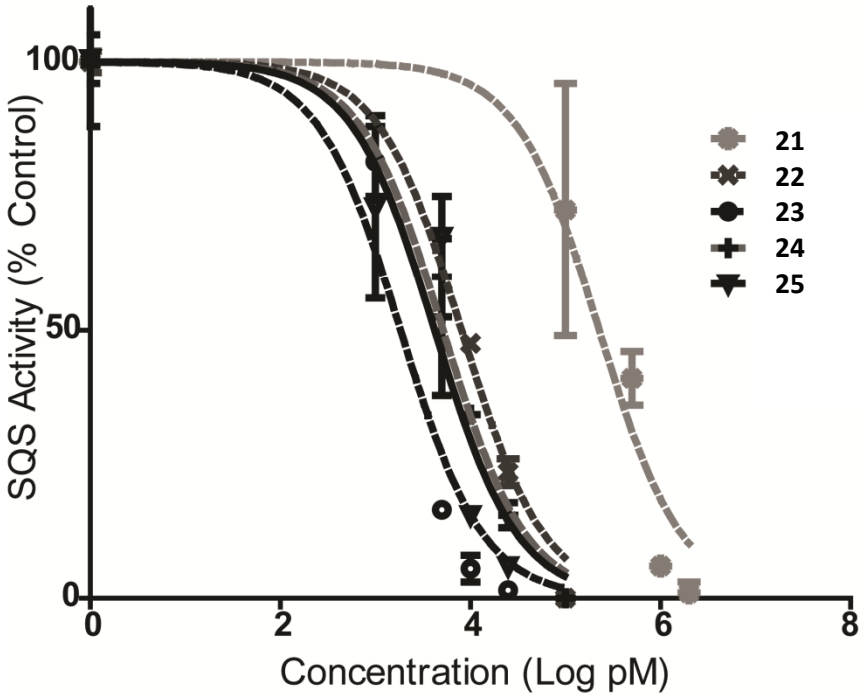


Figure 14. Dose response curves for compounds **21**-**25**. Studies were performed in 20 μL reactions with 50 mM phosphate buffer (pH 7.4, 5 mM $MgCl_2$, 4 mM CHAPS, 10 mM DTT), 400 ng recombinant enzyme, 0.25 μM [1-3H]-FPP, and NADPH.

Because bisphosphonate inhibitors could have off-target effects due to inhibiting other FPP utilizing enzymes, the selectivity of compounds **21**-**25** was tested in HepG2 cells for the inhibition of protein prenylation. Geranyl bisphosphonate has been shown to be a potent and selective inhibitor of GGDPS.[26] It was uncertain whether the addition of the biphenyl moiety would increase specificity for SQS or if the biphenyl compounds would also have inhibitory activity elsewhere in the mevalonate biosynthetic pathway.

After treatment of the cells with a potential inhibitor, western blot analysis was performed and the prenylation status of various proteins was determined. Under normal cellular conditions the RAS protein undergoes complete farnesylation which is reflected by the presence of a slower migrating (lower) band on the gel. The appearance of an additional faster migrating (upper) band on the gel means that normal farnesylation processes in the cell have been disrupted and is indicative of inhibition of farnesylation. Protein geranylgeranylation is detected through analysis of the Rap1a proteins. For this protein only unmodified protein is detected by the commercial antibody, and thus the appearance of a band indicates that protein geranylgeranylation has been diminished. The protein α-tubulin is added as a loading control for the gel. The first lane of the western blot is the control lane, protein isolated from cells that have not been treated with any drug. These cells are undergoing normal prenylation events, only geranylgeranylated Rap1a should be present, and no spots should be apparent after treatment with the antibody. The second lane is from cells that have been treated with lovastatin (**18**). Lovastatin inhibits HMG-CoA reductase at the beginning stage of the mevalonate pathway. This activity depletes mevalonate and results in a reduction of downstream processes including protein farnesylation as well as geranylgeranylation.

The ability of compounds **21-25** to inhibit prenylation was investigated in HepG2 cells, a cell line derived from a human liver carcinoma. Compound **21** shows selective inhibition of geranylgeranylation but no inhibition of farnesylation (Figure 15 A). Likewise, treatment of HepG2 cells with compound **22** shows some inhibition of geranylgeranylation, but there is no observable impact on protein farnesylation. Treatment of cells with compounds **23**-**25** show no significant impact on either

farnesylation or geranylgeranylation, signifying that these compound are not disrupting prenylation processes even though they inhibit SQS. The effects of lovastatin (25 μM) on farnesylation and geranylgeranylation can be reversed by addition of the substrates FPP and GGPP respectively. A combination of lovastatin (25 μM) and compound **25** results in restoration of farnesylation, but a reduction in geranylgeranylation is still observed. This may be the result of changes in flux of substrates within the pathway. Treatment of HepG2 cells with lovastatin alone shows a significant impact on both protein farnesylation and protein geranylgeranylation (Figure 15 B).

Co-treatment of HepG2 cells with lovastatin and FPP restores normal Ras farnesylation events. Co-treatment of HepG2 cells with compound **25** (25 μM) and lovastatin (25 μM) prevents lovastatin induced impairment of Ras farnesylation, but does not entirely restore Rap1a geranylgeranylation. Likewise, co-treatment with lovastatin and GGPP restores cellular Rap1a geranylgeranylation. Treatment of HepG2 cells with 10 μM zoledronate (**16**), a nitrogenous bisphosphonate that inhibits FPPS, causes disruption of both Ras farnesylation and Rap1a geranylgeranylation. Co-treatment with zoledronate and FPP or GGPP restores farnesylation or geranylgeranylation, respectively. Co-treatment of HepG2 cells with zoledronate (10 μM) and the biphenyl bisphosphonate **25** (25 μM) prevented both zoledronate–induced impairment of farnesylation and geranylgeranylation (Figure 15 C).

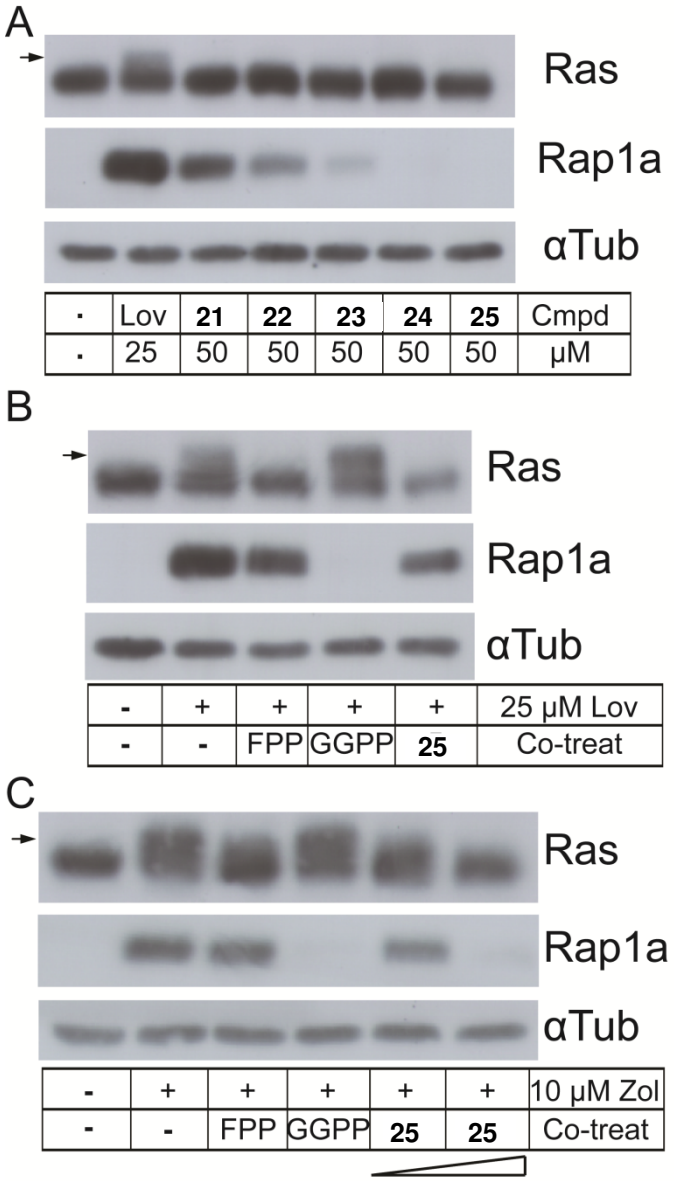


Figure 15. Protein prenylation: All data were collected from HepG2 cells A: Cells were treated with 25 μM lovastatin of 50 μM of the compounds **21**-**25** for 24 h. B: Co-treatment with 25 μM lovastatin and 25 μM FPP, 25 μM GGPP, or compound **25** for 24 h. C: Co-treatment of 10 μM zoledronate and 25 μM FPP, 25 μM GGPP, or compound **25** for 24 h.

The levels of FPP and GGPP from HepG2 cells were quantified after treatment with either compound **25** alone or treatment with compound **25** plus lovastatin. Cells that had been treated with compound **25** alone (25 μM) had an approximately 16-fold increase of FPP when compared to control cells. The GGPP levels for these cells were increased approximately 1.6-fold. Treatment with lovastatin (25 μM) reduced both FPP and GGPP levels. Treatment of cells with a combination of lovastatin (25 μM) and compound **25** (25 μM) resulted in increased FPP levels when compared with lovastatin treated cells, but GGPP levels remained diminished. These data are consistent with the western blot data which show that co-treatment with compound **25** can prevent lovastatin induced impairment of farnesylation, but does not affect lovastatin induced reduction of geranylgeranylation (Figure 16).

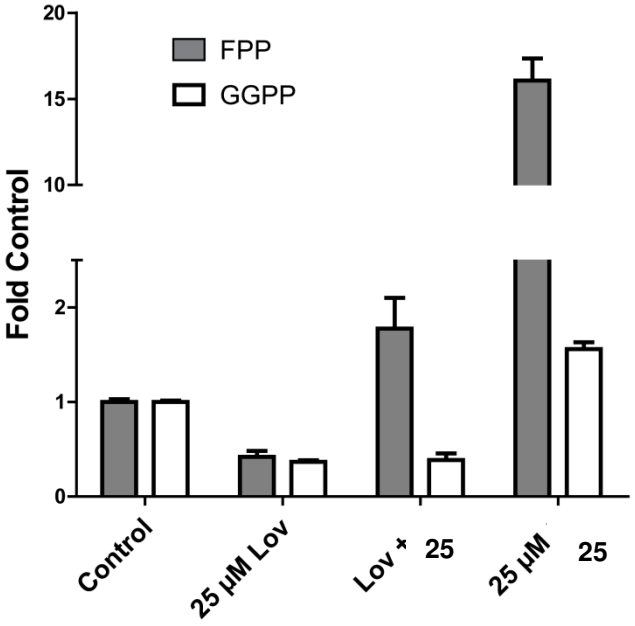


Figure 16. FPP and GGPP levels measured from HepG2 cells after 24 h incubation with the indicated compounds.

Next, the effects of compound **25** on cholesterol biosynthesis were tested. HepG2 cells treated with either lovastatin or compound **25** at 50 μM show significantly inhibited *de novo* cholesterol biosynthesis when compared to untreated cells. When these two drugs are combined there is an observable trend toward enhanced inhibition of cholesterol biosynthesis compared to the independent treatments. Treatment of HepG2 cells with zoledronate also significantly reduced cholesterol biosynthesis. The combination of compound **25** (25 μM) and zoledronate did not significantly decrease cholesterol biosynthesis when compared to independent treatments (Figure 17).

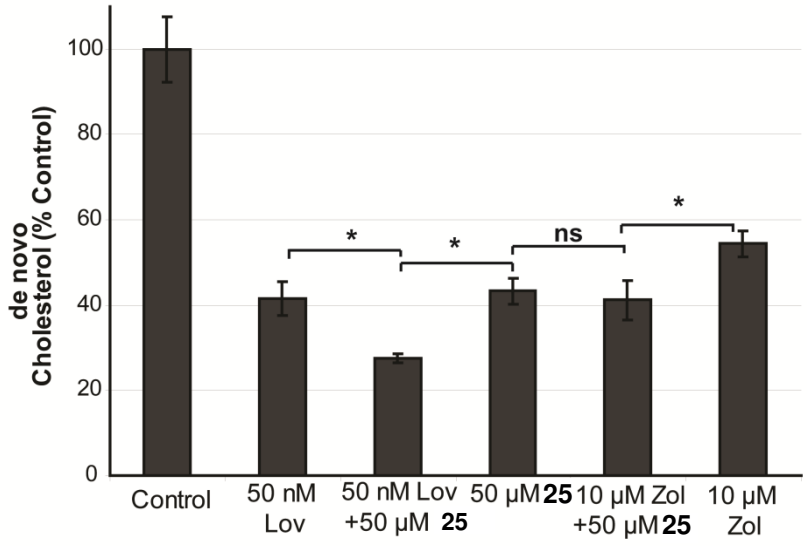


Figure 17. *De novo* cholesterol biosynthesis. HepG2 cells were treated with the indicated compounds for 1 h followed by addition of ^{14}C –acetate for 4 h, and then radiolabeled cholesterol was measured.

A test of cell viability also was initiated. The MTT analysis is a colorimetric assay that measures cellular viability through the determination of the ability of mitochondrial enzymes to reduce a tetrazolium salt to a formazan dye resulting in a color change. In the control portion, cell growth is standardized to 100%. Treatment of cells with lovastatin results in a reduction of cell viability. Addition of the substrates FPP or GGPP to cells that had been treated with lovastatin restores cell viability. The addition of compound **25** to cells that had been treated with lovastatin also leads to better cellular viability. Cells treated with compound **25** do not demonstrate a significant reduction in cellular viability.

Similarly, treatment of cells with zoledronate cause a significant (~50%) reduction in cellular viability. Addition of FPP to cells treated with zoledronate does not significantly increase cellular viability but addition of GGPP restores some cellular viability. Co-treatment of cells with zoledronate and compound **25** increased cellular viability (Figure 18).

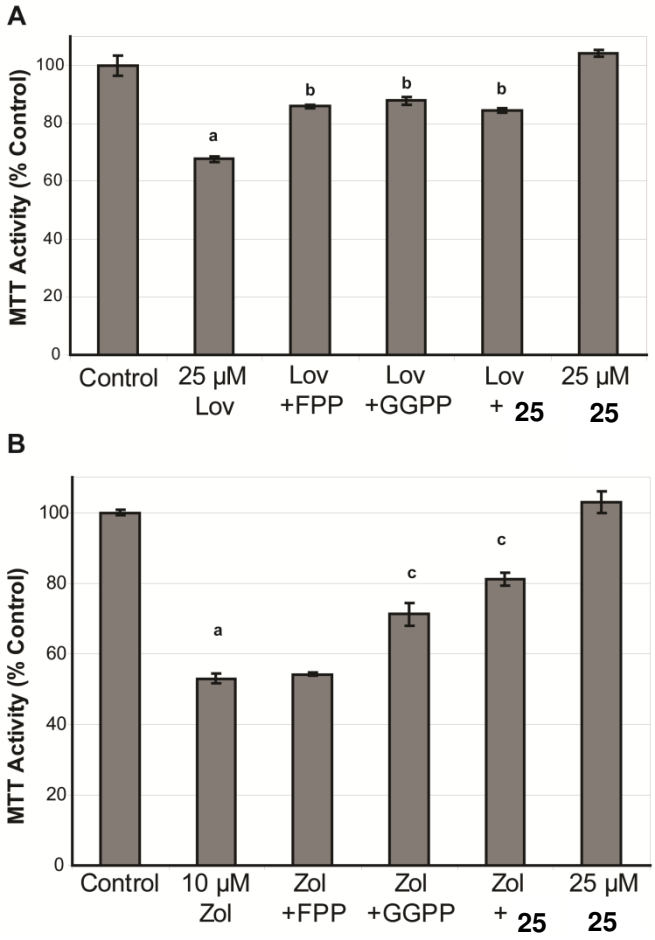


Figure 18. MTT assay of HepG2 cells treated for 48 h.

Although there has been a lack of success in clinical trial of SQS inhibitors as mono-drug treatments for lowering cholesterol, the use of a bisphosphonate SQS inhibitor along with a statin drug may prove to be medicinally useful. Although statin drugs are widely prescribed for the treatment of high cholesterol levels, when used alone they sometimes cause a number of downstream effects in the mevalonate pathway and some patients may suffer unwanted side effects. The ability of this combination of drugs

to reduce cholesterol levels while maintaining normal protein prenylation status may be clinically useful in avoiding statin-related side effects.

CHAPTER III

SYNTHESIS AND BIOLOIGICAL ACTIVITY OF STILBENOID BISPHOSPHONATES

The isoprenoid biosynthetic pathway is responsible for production of a variety of isoprenoids including squalene, dolichols, and ubiquinones.[67] These substrates are required for cell membrane maintenance, steroid and hormone production, synthesis of glycoproteins, and cell respiration.[68] It also is responsible for synthesis of smaller chain isoprenoids that are implicated in post translational modification of regulatory proteins including Ras and Rho GTPases. The GTP-binding proteins have direct roles in cell signaling which has impact on cellular proliferation, malignant transformation, tumor progression (metastasis), and cell death.[68] Consequently the development of inhibitors of enzymes within the mevalonate pathway is a rational approach to the synthesis of medicinal compounds for therapeutic use against cancer.

Two examples of enzymatic targets that may provide a rational approach to drug design are FDPS and GGDPS which are necessary for formation of isoprenoids required for the farnesylation and geranylgeranylation of proteins. Both of these processes can be traced back to the production of FPP, a fifteen carbon isoprenoid, from DMAPP and IPP by FDPS. At this point the newly formed FPP can be utilized in at least two pathways. It can either be attached to a protein by FTase, or alternatively it can have an additional equivalent of DMAPP added to the carbon scaffold by GGDPS in a head-to-tail fashion, resulting in the formation of the twenty carbon GGPP, or it can dimerize in a head-to-head fashion to afford squalene. Once formed GGPP can proceed in a manner similar to

FPP where it can be attached to proteins by either GGTase or RGGTase (Figure 2, page 4).

Digeranyl bisphosphonate (**44**) has been found to be a selective and specific inhibitor of GGDPS (0.2 μM).[69] An MTT assay of SF-295 cells, treated with digeranyl bisphosphonate shows growth inhibition of this cell line derived from human glioma, a cancer of the central nervous system (CNS) (Figure 19). Although the ability of bisphosphonates to traverse the blood brain barrier may be limited because they are highly charged species, there is potential to modify the alkyl chains of the bisphosphonate to increase lipophilicity and the potential to mask the negative charges of the bisphosphonate using a pro-drug approach.[70,71]

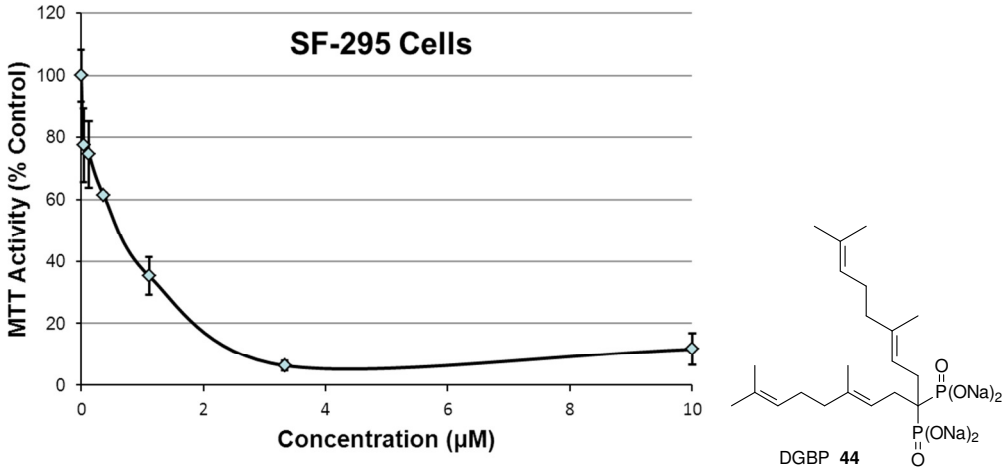


Figure 19. An MTT assay of monogeranyl bisphosphonate (**44**) in SF-295 cells

It has been demonstrated that a number of stilbenes are capable of traversing the blood brain barrier.[72] It was imagined that installation of a stilbenoid moiety into a geranylated bisphosphonate may result in a compound that retains the potent and

selective activity for GGDPS while increasing the lipophilicity of the bisphosphonate. This strategy might allow for design of stilbenoid bisphosphonate inhibitors that target cancers of the brain and central nervous system.

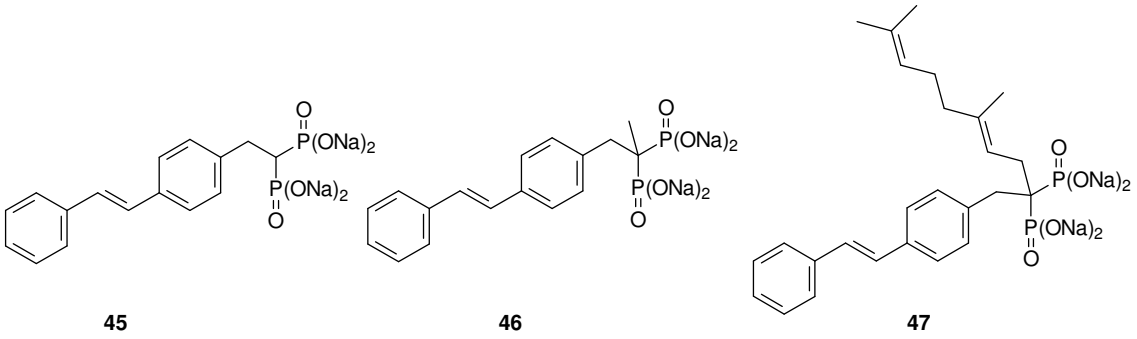


Figure 20. First-generation stilbenebisphosphonates.

Preparation of the stilbenoid bisphosphonates commenced with synthesis of the stilbene moiety. Horner-Wadsworth-Emmons (HWE) condensation of diethyl benzylphosphonate (**48**) and *p*-tolualdehyde (**49**) proceeded to provide the desired stilbene **50** in good yield. Bromination of the methyl group using *N*-bromosuccinimide (NBS) resulted in the formation of bromomethyl stilbene **51**. Stilbene **51** was recrystallized and then was carried forward in the reaction sequence. It should be noted that attempts to synthesize the benzylic bromide **51** via bromination of a benzylic alcohol using phosphorus tribromide resulted in a final product that could not be fully purified despite multiple attempts. This may be the consequence of addition of HBr, a byproduct of the bromination technique, across the stilbene olefin.

Alkylation of tetraethyl methylenebisphosphonate with bromide **51** resulted in formation of a mixture of the mono- (**52**) and dialkylated bisphosphonate products which were chromatographically separable. The mono-stilbene had a ^{31}P NMR shift downfield

(22.9 ppm) from the bisphosphonate starting material (~19.5 ppm). The aromatic region of the ^{1}H and ^{13}C NMR spectra had multiple new resonances. There also was a triplet of triplets (2.67 ppm; J_{PH} = 24.0 Hz, J = 6.3 Hz) observed in the ^{1}H NMR spectrum, a feature that results from phosphorous and methylene couplings to the α-hydrogen of the bisphosphonate, and is indicative of the formation of a mono-alkylbisphosphonate.

The resulting mono-stilbenebisphosphonate **52** was treated with base to form an anion followed by geranyl bromide, which provided stilbene bisphosphonate **53**. There is an observable upfield shift to 25.5 ppm in the ^{31}P NMR spectrum when compared to the mono-alkylbisphosphonate starting material **52** (22.9 ppm). This is consistent with the formation of a dialkylbisphosphonate.[26,44] In the ^{1}H NMR spectrum, there are new resonances that are characteristic of a geranyl chain. Most notable are the two new resonances in the vinylic region as well as the three new methyl signals. Parallel features are found in the ^{13}C NMR spectrum as well. There are four new carbon resonances observed in the vinylic region and three new methyl resonances observed in the methyl region.

The mono-stilbenebisphosphonate **52** was elaborated further by alkylation with methyl iodide. Treatment of compound **52** with base provided the anion which was then alkylated by addition of methyl iodide, resulting in bisphosphonate **54**. The triplet of triplets from the starting material is no longer evident in the ^{1}H NMR spectrum and there is a new triplet (1.45 ppm, J_{PH} = 16.5 Hz) which corresponds to the methyl hydrogens of the newly added methyl group. The stilbenenoid bisphosphonate esters **52**, **53**, and **54**, were subsequently hydrolyzed via the McKenna procedure to their corresponding tetra sodium salts **45**, **46**, and **47** (Figure 21). [66]

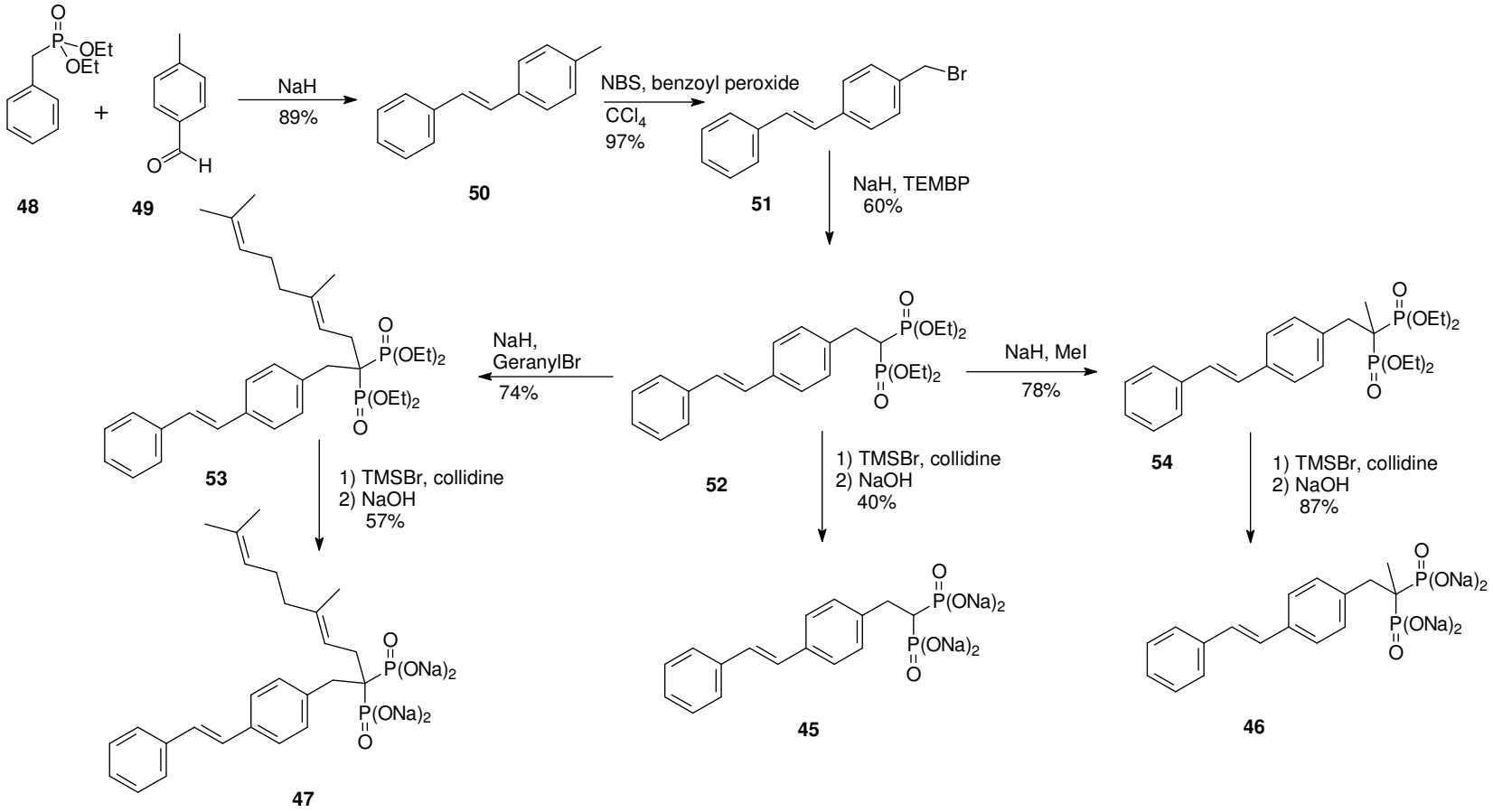


Figure 21. Synthesis of stilbenebisphosphonate targets **45**, **46**, and **47**.

The stilbenebisphosphonates **45**, **46** and **47** were tested for inhibitory activity on GGDPS and FDPS in K562 cells. Using western blot analysis, the prenylation status of each protein was determined. In a normal cell the RAS proteins undergo farnesylation and sometimes geranylgeranylation. Farnesylation is determined by the appearance of a slower migrating (lower) band on the gel. The appearance of an additional faster migrating (upper) band on the gel means that normal farnesylation processes in the cell have been disrupted and is indicative of inhibition of farnesylation. Geranylgeranylation of proteins can be detected through analysis of Rap1a's prenylation status. In this case only unmodified protein is detected by western blot analysis with a commercial antibody. Thus the appearance of a band on the western blot indicates that protein geranylgeranylation has been diminished. The first lane of the western blot (Figure 22) is the control lane, which means that the cells used have not been treated with any drug and that the cells are undergoing normal protein prenylation events. The second lane is a positive control. Cells used in this lane have been treated with lovastatin, an inhibitor of HMG-CoA reductase which acts at the beginning of the mevalonate pathway. Inhibition of HMG-CoA reductase subsequently depletes mevalonate causing a reduction in downstream processes including both farnesylation and geranylgeranylation.

Unfortunately preliminary investigations of this class of compounds show very limited inhibition of either GGDPS or FDPS. Neither bisphosphonate **45** nor bisphosphonate **46** show any significant inhibitory activity against farnesylation or geranylgeranylation in the Ras or the Rap1a tests. This indicated that compounds **45** and **46** are not disrupting prenylation events in the cells. Likewise the bisphosphonate **47** does not appear to affect the Ras blot, an indicator of farnesylation, and only has a very

minor effect on geranylation as indicated by analysis of Rap1a, even at high concentration (~100 μM Figure 22).

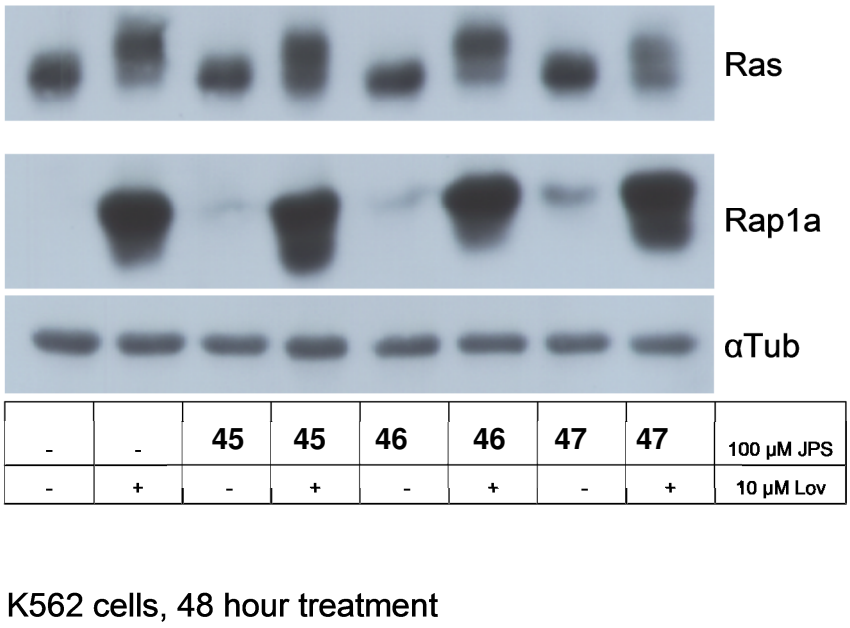


Figure 22. Western blot analysis of stilbenebisphosphonates.

Despite the determination of limited activity against GGDPS and FDPS, these compounds were tested for inhibitory activity against a number of other enzymes. A fortuitous screen of the bisphosphonates **45**, and **47** against decaprenyl diphosphate synthase (Rv2361c), an enzyme found in mycobacteria, showed positive results with bisphosphonate **45.** These compounds were screened against *M. tuberculosis* decaprenyl diphosphate synthase in vitro at 10 μM. Compounds with an IC_{50} within this range were tested further using dose-response curves to determine an IC_{50} value. Compound **55** was the most active compound in this investigation and had an IC_{50} of ~1 μM. The dialkylated compound **47** had very limited activity against decaprenyl diphosphate

synthase while the monoalkyl stilbene **45** had moderate activity (IC_{50} ~10 μM) suggesting that other monoalkyl stilbenebisphosphonates may be inhibitors of decaprenyl diphosphate synthase, and may have some therapeutic value as antibiotics (Figure 23).

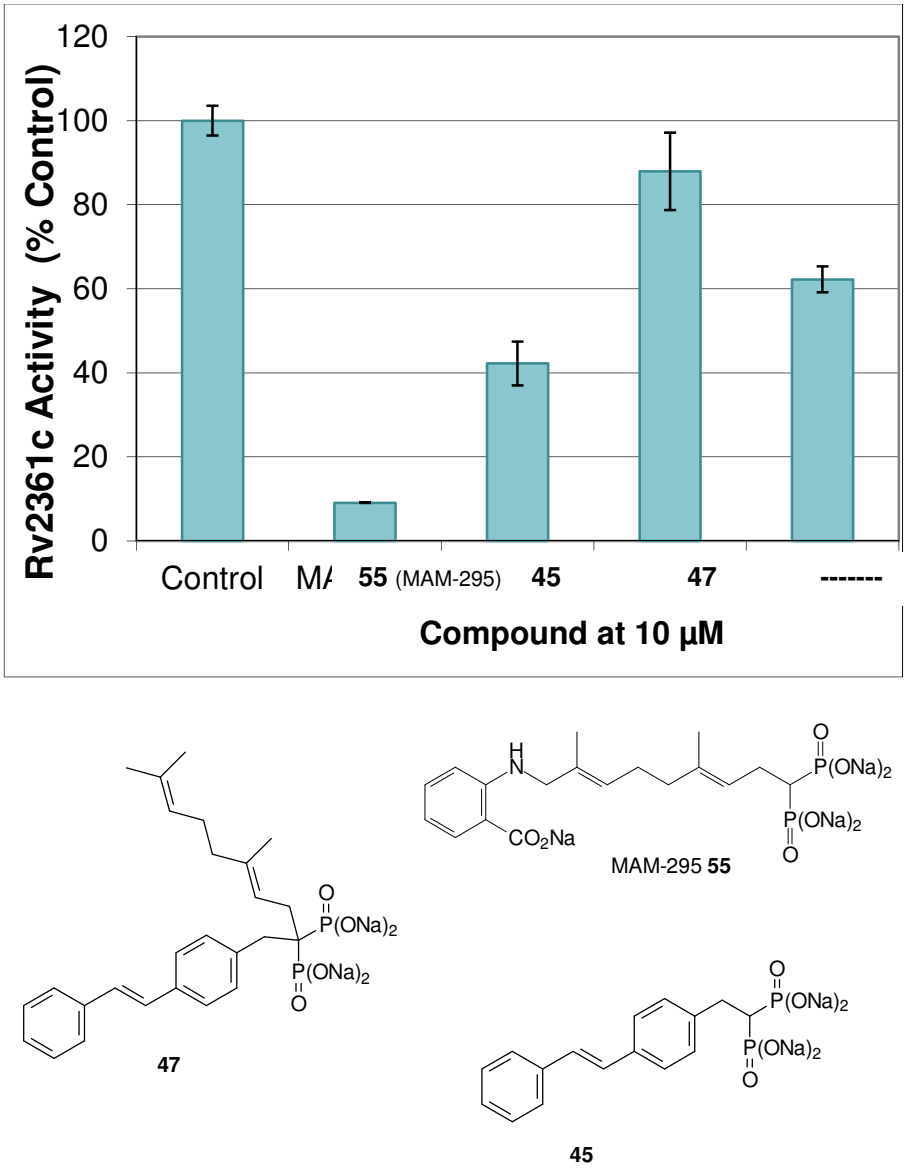


Figure 23. Inhibitory activity of selected bisphosphonates against Rv2361c.

Mycobacteria utilize the non–mevalonate pathway (MEP) to synthesize isopentenyl pyrophosphate (IPP). The substrate IPP undergoes reaction with its isomer dimethylallyl pyrophosphate (DMAPP) to produce geranyl pyrophosphate. An additional unit of IPP is added to GPP to produce ω-*E,Z*-farnesyl pyrophosphate (ω-*E,Z*-FPP). The enzyme decaprenyl diphosphate synthase (Rv2361c) then adds additional isoprene units derived from IPP onto ω-*E,Z*-FPP in a stereospecific manner to form a molecule of ω-*E*,poly-*Z*-decaprenyl diphosphate (C_{50}PP). The fifty carbon isoprenoid C_{50}PP is utilized to synthesize numerous components of the mycobacterium cell wall known as the MAP-c complex (Figure 24). The ability to inhibit decaprenyl diphosphate synthase could disrupt the mycobacterium cell wall biosynthesis and might represent a new antibiotic target for therapeutic compounds.

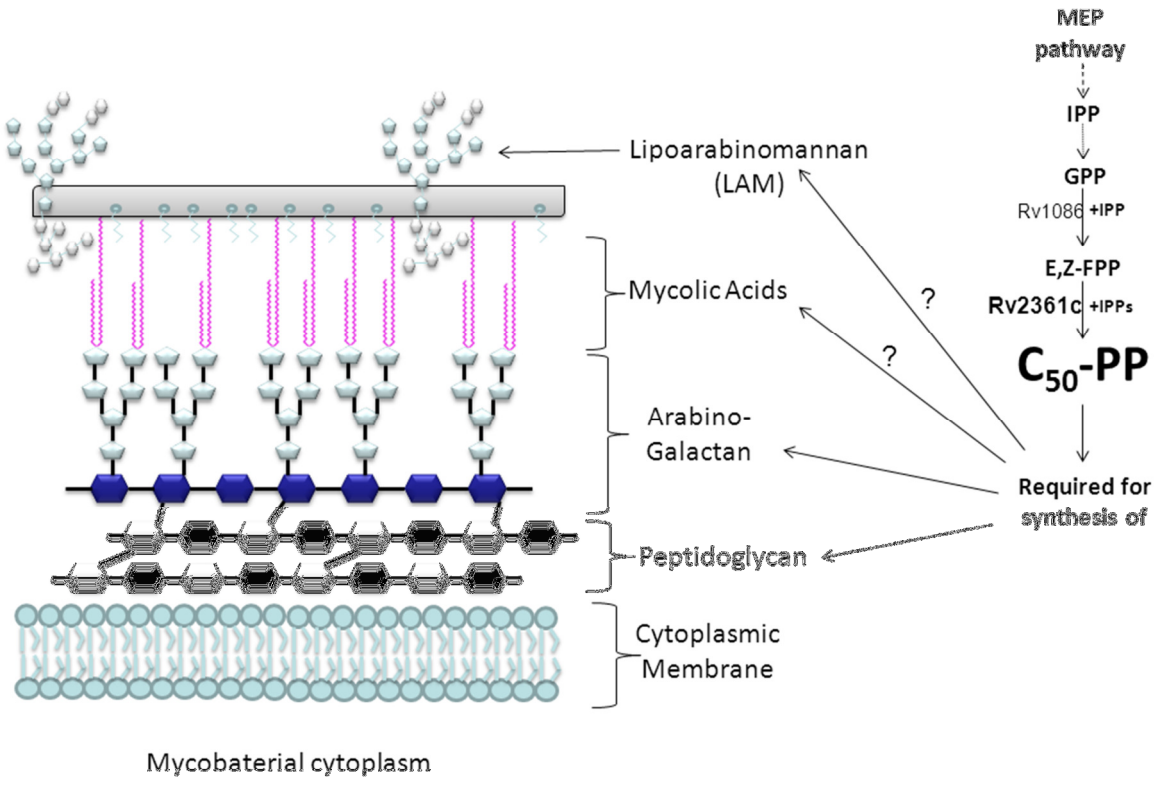
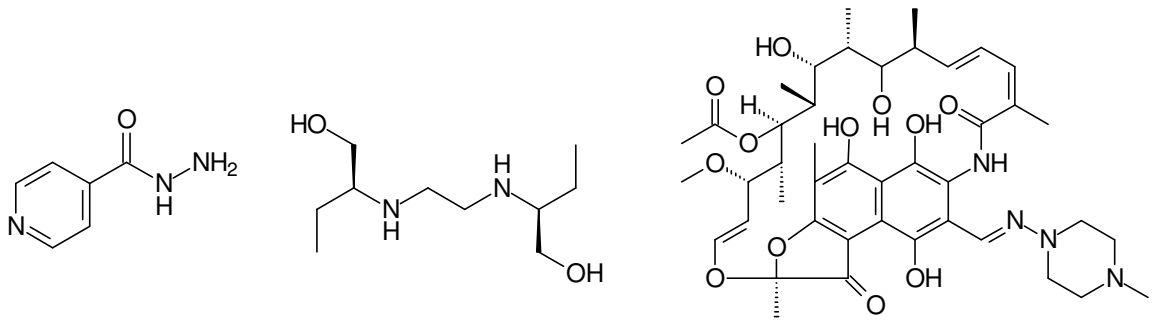


Figure 24. The MAP-c complex of mycobacterium.[73]

One specific mycobacterium of particular concern is *M. tuberculosis*. *M. tuberculosis* is the bacterium which causes the disease tuberculosis (TB), and it is estimated that over 1/3 of the world population is infected with this organisim. In most cases of TB, the infection is latent and the individual may remain asymptomatic for their lifetime. In approximately 5-10% of these cases, however, the infection will become active and then if left untreated there is a high mortality rate. Because of the extensive treatment regime required for patients with TB, there are often difficulties with medicinal compliance which is in turn a major contributor to antibiotic the resistance of the bacterium. There have been newly emergent strains of TB discovered including a multi-

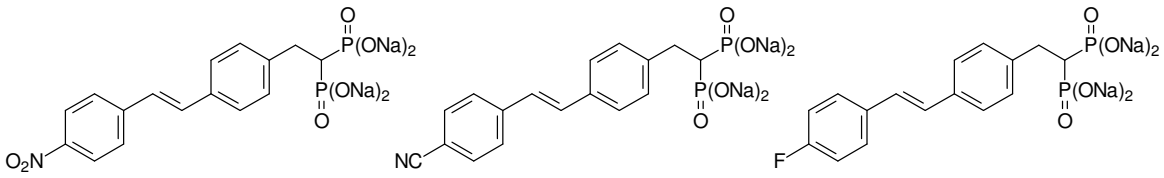
drug resistant (MDR) strains which is resistant to both of the frontline drugs isoniazide (**56**) and rifampicin (**57,** Figure 25) as well as an extensively drug resistant (XDR) strain which is resistant to both the frontline and some of the secondary drugs now available.



isonazide **56** ethambutol **57** Rifampicin **58**

Figure 25. Drugs used to treat tuberculosis infection.

A closer investigation of the lead target **55** resulted in the hypothesis that inclusion of an very polar group might be an important factor for inhibitory activity. This hypothesis was tested by the preparation of stilbenoid bisphosphonates that included an electron withdrawing moiety at the para position of the stilbene moiety (Figure 26).



59 **60** **61**

Figure 26. Second generation stilbenebisphosphonate targets.

The requisite phosphonate for compound **59** was prepared via Arbuzov reaction of 4-nitrobenzyl bromide (**62**) with triethyl phosphite to provide the 4-nitrobenzylphosphonate (**63**). Compound **63** was then deprotonated with base and treated with *p*-methylbenzaldehyde (**49**) using Horner-Wadsworth-Emmons olefination conditions to provide the condensation product, stilbene **64**. The *p*-nitrostilbene (**64**) was isolated and converted to the bromide via bromination with *N*-bromosuccinimide (NBS), then carried on in the reaction sequence without further purification. Tetraethyl methylenebisphosphonate was treated with base followed by addition of the stilbene bromide resulting in the formation of the mono stilbenebisphosphonate **65**. The product was purified and characterized using standard techniques. The ^{31}P NMR resonance of the product was observed at 22.7 ppm, a frequency that is in the region of mono-substituted bisphosphonate esters.[26, 33] The ^{1}H and ^{13}C NMR spectra were both indicative of the formation of the mono-stilbenebisphosphonate ester **65** and the multiplicities that were observed in the ^{1}H and ^{13}C NMR spectra were consistent with what was expected for the desired product. For example, in the ^{1}H NMR spectrum there was a notable triplet of triplets (~2.66 ppm) that corresponds to the α-hydrogen of the mono-substituted bisphosphonate, and a triplet of doublets (~3.28 ppm) that correspond to the methylene hydrogens. The carbon spectrum had three distinct sets of triplets. The most obvious was from the α-carbon (38.9 ppm) which had a phosphorus coupling of ~132 Hz. Once characterization of compound **65** was complete, the bisphosphonate ester was subsequently converted to the tetra sodium salt under standard McKenna hydrolysis procedures to provide compound **59** (Figure 27).[66]

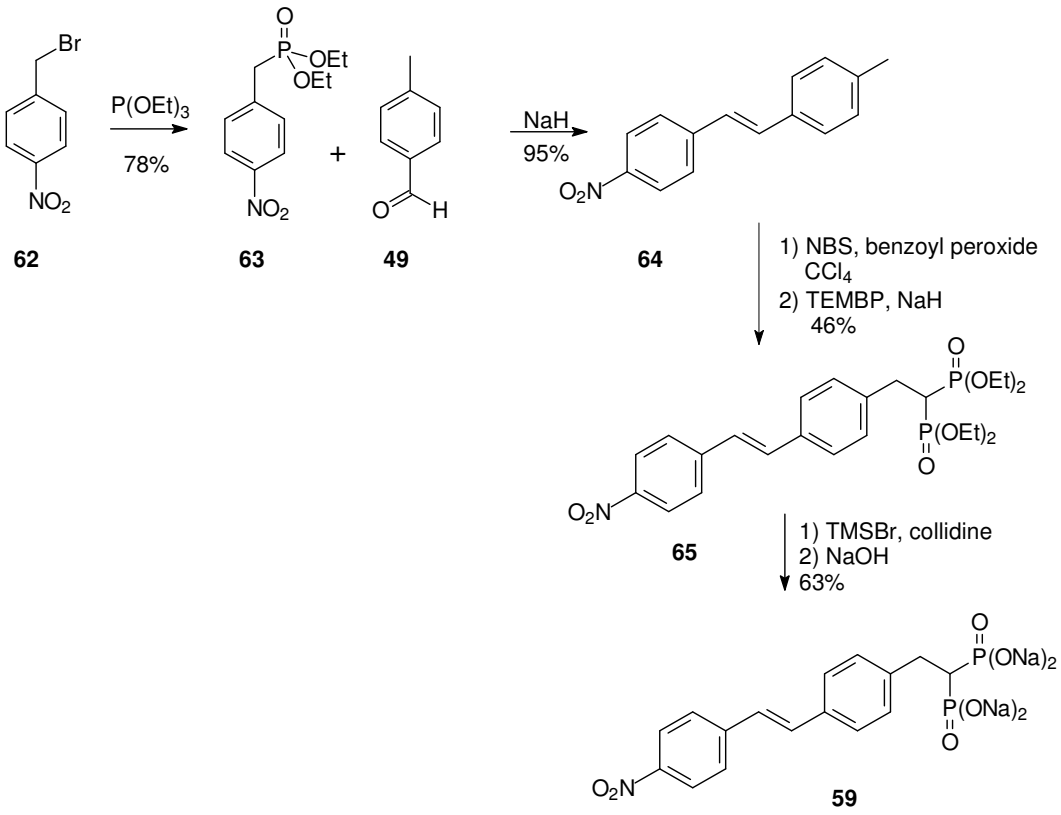


Figure 27. Synthesis of *p*-nitrostilbenebisphosphonate **59**

The tetra sodium bisphosphonate salt **59** was tested for inhibitory activity against decaprenyl diphosphate synthase and a dose response curve was generated (Figure 28). It was determined that the compound **59** had an IC_{50} of 890 nM, a value that is somewhat more active than the lead compound **55** (IC_{50} ~1 μM).

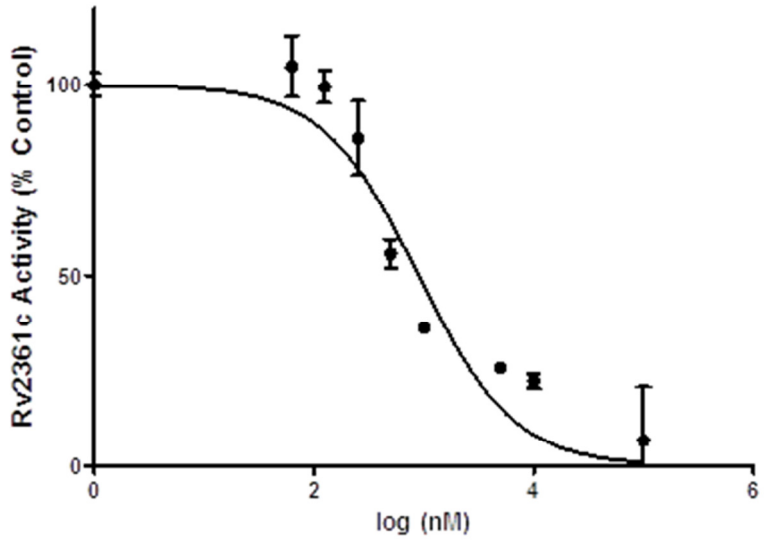


Figure 28. Dose-response curve of compound **59** against decaprenyl diphosphate synthase.

The activity of the bisphosphonate **59** led to the desire to synthesize other substituted stilbenebisphosphonates with an electron withdrawing group at the para position. Nitro groups can be metabolized *in vivo* by bacteria leading to a variety of secondary metabolites which may have adverse side effects.[74] It was hypothesized that a nitrile may have similar activity against decaprenyl diphosphate synthase while maintaining better metabolic stability. To obtain the desired target, bromide **67** was allowed to react with triethyl phosphite to form the benzylphosphonate **68**. Compound **68** was condensed with *p*-methylbenzaldehyde (**49**) under standard Horner-Wadsworth-Emmons olefination conditions. The resulting stilbene **69** was converted to the corresponding bromide using NBS. The bromide was used without further purification in the alkylation of TEMBP (**79**). Several isolation attempts resulted in a mixture of TEMBP and the desired bisphosphonate **70**. The mixture had a ^{31}P NMR resonance (~23

ppm) consistent with the formation of the desired product as well as a signal at 19.5 ppm from the bisphosphonate starting material. Analysis of the mixture using HRMS revealed a molecular ion also indicative of the desired product. Despite multiple attempts to purify the mixture of TEMBP and mono-stilbenebisphosphonate **70,** complete purification of the desired bisphosphonate **70** was unsuccessful (Figure 29).

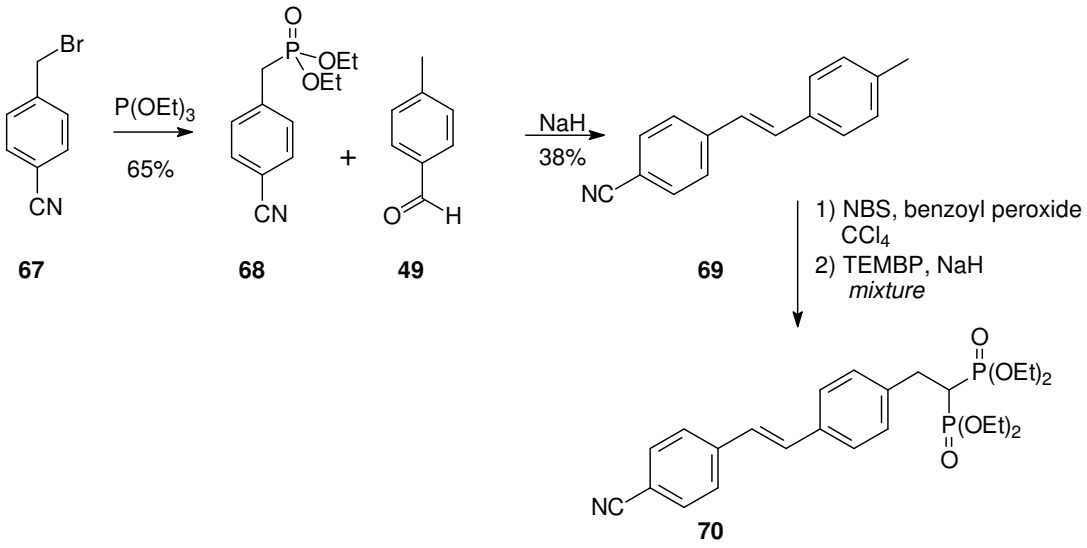


Figure 29. Attempted synthesis of stilbenebisphosphonate **70**

Instead of pursuing compound **70** focus was shifted to a *p*-fluorostilbene analogue **73**. The commercially available phosphonate **71** was treated with base and *p*-methylbenzaldehyde (**49**) under Horner-Wadsworth-Emmons olefination conditions. The resulting stilbene **72** was converted to the bromide under electrophilic bromination conditions and after standard workup it was used in the alkylation of TEMBP (Figure 30). Formation of the desired bisphosphonate **73** was evident from TLC analysis as well as ^{31}P NMR analysis of the unpurified reaction mixture. Upon isolation there were two

noticeable peaks in the ^{31}P NMR spectrum, one at ~23 ppm that could be associated with formation of the desired bisphosphonate **73** and one that occurred at ~13 ppm from an unidentified by-product. The ^{1}H NMR spectrum also exhibited features consistent with the formation of the desired product, such as a triplet of triplets (~2.6 ppm) and triplet of doublets (~3.3 ppm) as well as multiple peaks in the aromatic region of the spectrum. The peaks associated with the ethyl groups of the phosphonate esters had overlapping resonances presumably from the unidentified byproduct. Analysis of the reaction mixture using HRMS revealed a molecular ion consistent with the desired bisphosphonate **73**. Despite attempts to resynthesize and isolate the desired product **73,** the byproduct persisted and complete purification of the desired bisphosphonate (**73**) was not successful. Ultimately, isolation of stilbene bisphosphonates **73** and **66** may require that they are prepared on a larger scale followed by extensive purification, or a new synthetic strategy may need to be enlisted.

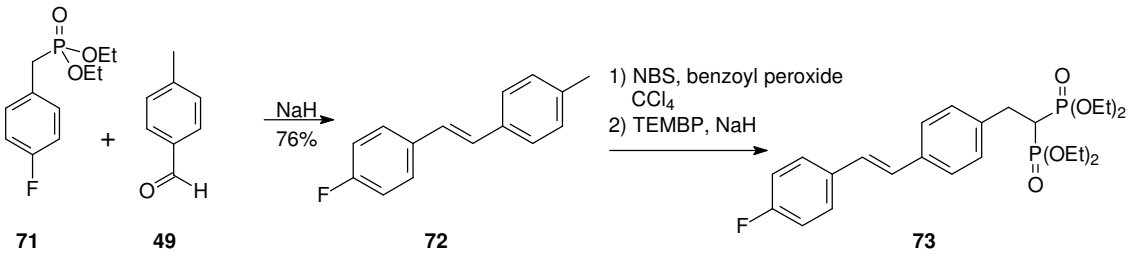


Figure 30. Attempted synthesis of the *p*-fluoro stilbenoidbisphosphonate ester **73**

CHAPTER IV

DESIGN AND SYNTHESIS OF ALKYL 1,1,1,-TRISPHOSPHONATES

Numerous biological processes utilize phosphorus-containing molecules as substrates in enzyme catalyzed reactions. Of these biomolecules, di-and triphosphates have gained significant attention and are frequently observed as intermediates in various biological pathways. Due to the extensive incorporation of phosphorus in biology, synthetic chemists have actively pursued the development of small molecule analouges that contain a variety of different phosphorus moieties. Some synthetic classes that have proven to be synthetically adaptable and medicinally useful in enzyme inhibition include phosphates, phosphonates and bisphosphonic acids.

Despite the ability to create diverse collections of phosphorus-containing small molecule inhibitors little attention had been devoted to synthesis of α-tris phosphorylated compounds (Figure 31). The trisphosphonic acids provide a unique arrangement of phosphonate groups in space as well as the potential to invoke varied charge states. The development of a method that would allow for the synthesis of a variety of α-alkyltrisphosphonic acids would be useful in the expansion of biologically interesting phosphonate-containing compounds with tunable properties, compounds that could be exploited for a variety of uses.

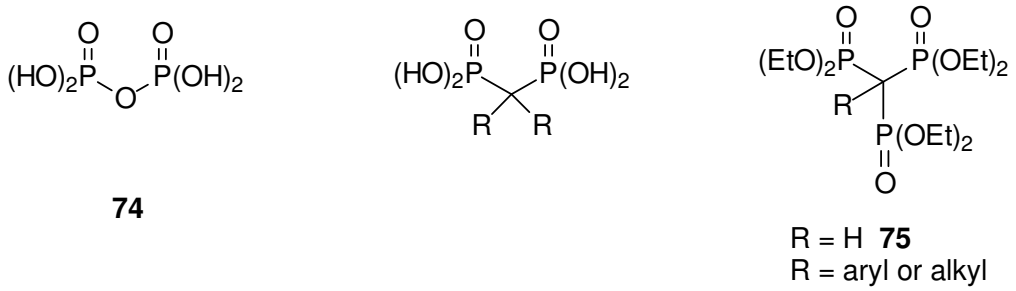
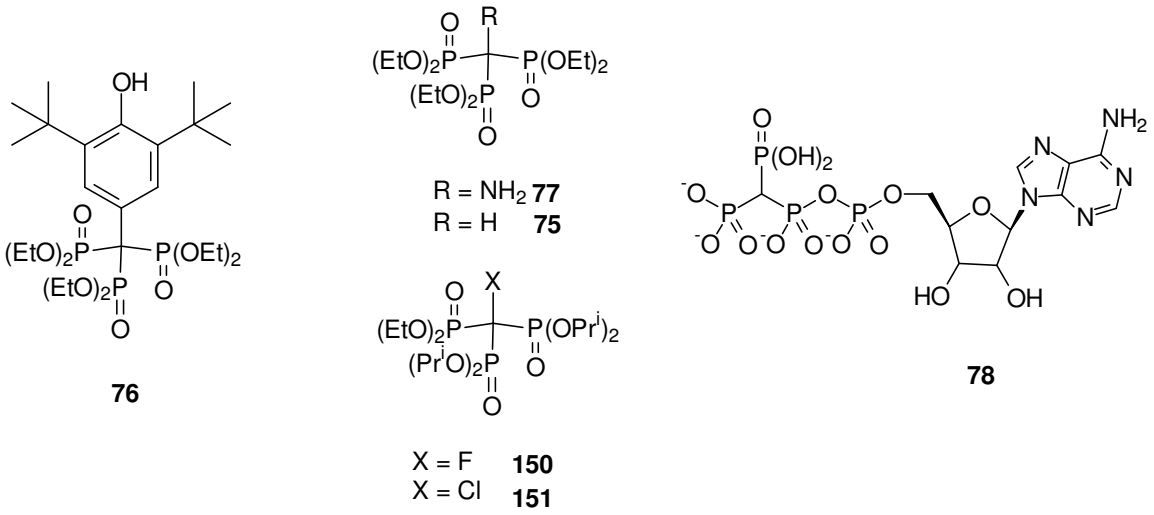


Figure 31: Pyrophosphoric acid (**74**), bisphosphonic acid, and trisphosphonate (**75**).

The first α-trisphosphonate was reported by Gross in 1972.[75] Professor Gross later established that by phosphinylation of tetraethyl methylenebisphosphonate and subsequent air oxidation an α-tris phosphorylated species (**75**) could be produced in modest yields (~30%).[76] The synthesis of alkyl trisphosphonates has been extremely limited (Figure 32). It has been demonstrated that trichloromethylamine can undergo reaction with triethyl phosphite to afford amino trisphosphonate **77** through a reaction sequence that is assumed to be based on an elimination–addition reaction.[75,77,75] Gross was also able to synthesize the aryl trisphosphonate **76** through a similar strategy that might involve a quinone methide, but further elaboration of the alkyl substituent in this case is impractical.[78] Most of the functionality that has been imparted to α-trisphosphonates has been required for their synthesis and leaves few options to modify the compound further.

Despite the limited access to alkyltrisphosphonates, the trisphosphonate family had been expanded to include some α-halo trisphosphonates that have been investigated as phosphoric acid analogs. Formation of an analogue of the parent trisphosphonate **75** followed by deprotonation and treatment with an electrophilic halogenating agent provided α-fluoro (**150**) and α-chloro (**151**) species.[79] Modification of the phosphonate

esters also has been investigated resulting in the synthesis of trisphosphonates that are adenosine triphosphate analogues.[80, 81]



76 78

Figure 32. Examples of trisphosphonates that have been previously synthesized.

The extensive interest in the therapeutic properties of phosphonates and bisphosphonates has lead to the development of phosphonates and bisphosphonates with appreciable medicinal value. Alkyl trisphosphonates would be a unique addition to this group and may exhibit interesting and useful biological properties. The ability to prepare a variety of novel trisphosphorylated species would require developing a methodology that is broadly applicable.

One might argue that the most direct route from tetraethyl methylenebisphosphonate (**79**) to a family of alkyl trisphosphonates would involve preparation of the parent trisphosphonate **75** followed by deprotonation to form an anion and treatment with an alkyl bromide (Figure 33).[46] To explore the viability of this reaction route, compound **75** was synthesized in modest yields according to literature

procedure.[76] The ^{1}H and ^{13}C NMR spectra were consistent with the literature values and the ^{31}P NMR resonance matched the reported value of 14 ppm.

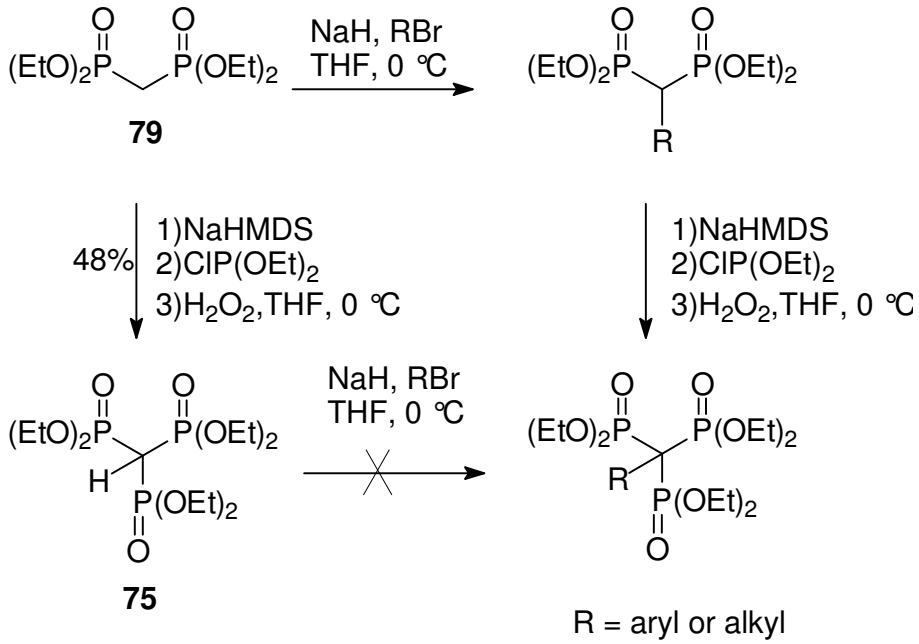


Figure 33. Possible synthetic routes to an alkyl trisphosphonate

Alkylation attempts were initiated using the general reaction conditions for the alkylation of tetraethyl methylenebisphosphonate. The trisphosphonate starting material was dissolved in anhydrous THF and the solution was placed into an ice bath. The addition of sodium hydride to the reaction vessel resulted in notable effervescence. After stirring for thirty minutes at 0 °C, benzyl bromide was added to the reaction vessel. The mixture was allowed to stir an additional 30 minutes after which the reaction progress was monitored periodically by ^{31}P NMR. A single peak was observed in the ^{31}P NMR spectrum at 32 ppm, a significant shift from the starting material **75,** which has a ^{31}P NMR resonance at 14 ppm.[76] The downfield shift in the ^{31}P NMR spectrum may be

indicative of a trisphosphonate anion (**81**). This hypothesis is supported by correlating the shift of the anion of tetraethyl methylenebisphosphonate (**80**) to the neutral species (**79**). In the bisphosphonate series, the neutral species (**79**) has a ^{31}P NMR resonance at 19 ppm whereas the anion (**80**) can be observed at ~42 ppm (Figure 34). The magnitude of the downfield shift of the anion of the trisphosphonate and the region in which it occurs is similar to the magnitude and region of resonance observed with the anion of tetraethyl methylenebisphosphonate.

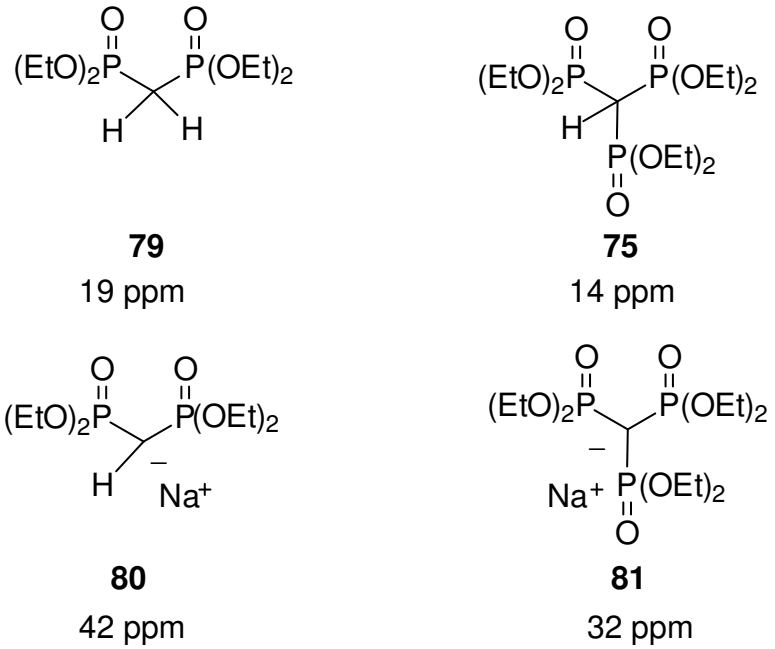


Figure 34. ^{31}P NMR shifts of neutral bisphosphonates and trisphosphonates and their respective sodium salts.

Due to the evident formation of a trisphosphonate anion and the lack of observable formation of an alkylated trisphosphonate the alkylating agent was

reevaluated. The reactivity of the benzyl group may be limited by steric factors. Allyl bromide retains a similar reactivity as benzyl bromide but has less of a steric impact. Therefore, allyl bromide was added to the NMR tube containing the trisphosphonate anion (**81**), the sample was allowed to remain at room temperature for 4 hours, and then monitored by ^{31}P NMR. There was only one discernible peak in the spectrum and it still occurred at 32 ppm (Figure 35). From these experiments, the ability to alkylate the trisphosphonate anion under standard conditions appeared to be limited. If addition of water to the NMR tube would protonate the anion, it should result in an upfield shift to 14 ppm in the ^{31}P NMR spectrum consistent with the starting material. Unexpectedly, addition of water resulted in no obvious change in the ^{31}P NMR. The anion of the trisphosphonate starting material appears to be extensively stabilized and is able to persist even in aqueous environments. Even addition of a saturated solution of ammonium chloride did not result in the protonation of the anion. Addition of glacial acetic acid to the NMR tube did induce protonation of the anion and a single peak consistent with the trisphosphonate starting material at 14 ppm was observed again in the ^{31}P NMR spectrum.

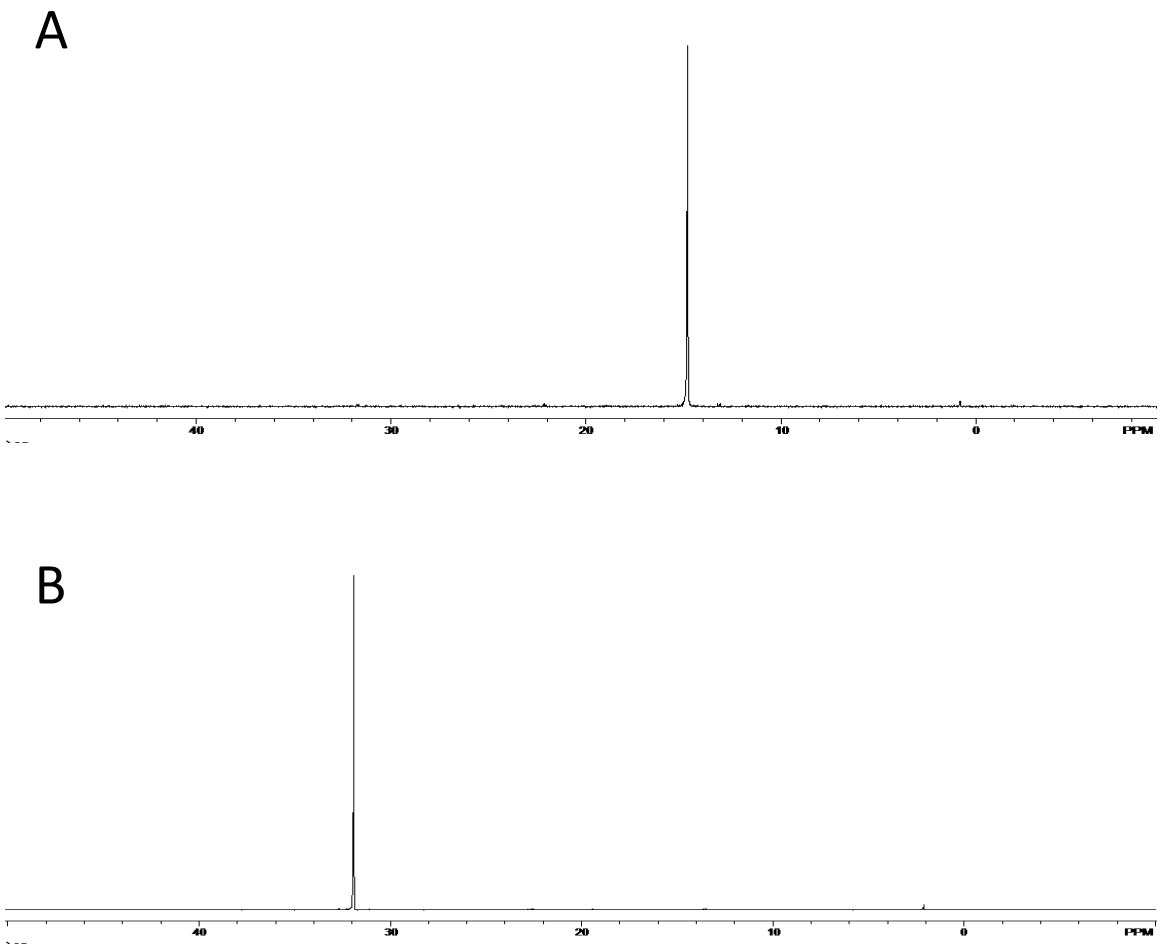


Figure 35. ^{31}P NMR spectra of the trisphosphonate starting material (A) and the corresponding anion (B).

The unanticipated stability of the trisphosphonate anion led to the desire to determine the pK_a of the α-proton of the trisphosphonate ester **75**. All six pK_a's of the trisphosphonic acid prepared from the parent trisphosphonate ester had been reported[79] but there has been no mention in the literature of the pK_a of the methine position of a trisphosphonic ester.

To determine the pK_a, the parent trisphosphonate ester was first dissolved in water and then treated with aqueous sodium hydroxide. The reaction was monitored by ^{31}P NMR to test the applicability of using aqueous sodium hydroxide for a pH titration of the trisphosphonate ester (**75**). There was an observable downfield shift to 32 ppm indicating that the anion had been formed and that sodium hydroxide was a suitable base for titration of this compound.

The parent trisphosphonate then was titrated with sodium hydroxide and the pH of the solution was monitored with a pH electrode to determine the equivalence point. The pH electrode had been calibrated using Log Pro software and the appropriate pH buffers. The trisphosphonate ester (**75**) was first dissolved in water and placed on a stir plate in a flask that was equipped with a pH electrode and then sodium hydroxide was added dropwise. The pH of the solution along with the volume of sodium hydroxide that had been added was measured over a series of increments. The pH of the solution versus the volume of sodium hydroxide was plotted in Excel and fit with an XY scatter line (Figure 36).

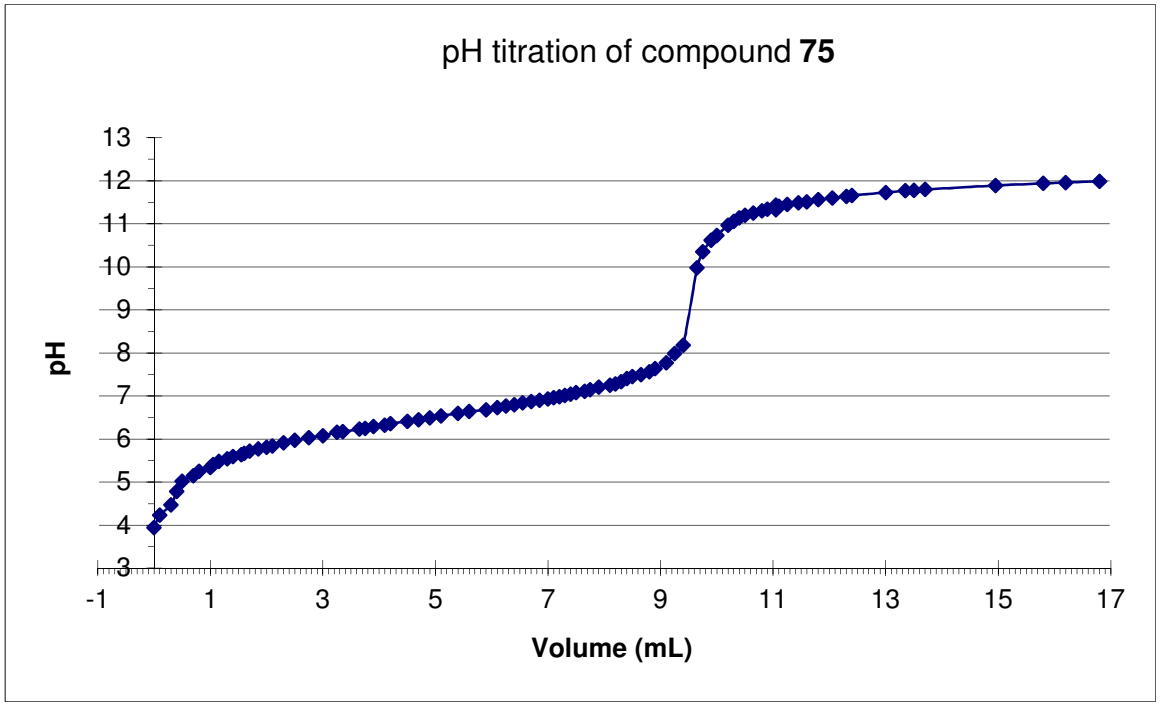


Figure 36. Titration graph of the parent trisphosphonate with NaOH

The Henderson-Hasselbalch equation (eq. 1) was used to derive the pK_a of the α-proton of the trisphosphonate ester **75**. The pH is defined as the pK_a (the acid dissociation constant) plus the log of the concentration of the conjugate base divided by the concentration of the acid species. At one half of the equivalence point the concentration of the conjugate base is equal to the concentration of the acid and the concentrations cancel, so that at one half of the equivalence point the pH of the solution is equivalent to the pK_a of the species in solution. From this experiment the empirical pK_a of the trisphosphonate **75** was determined to be ~6.5, as shown below.

$$pH = pK_a + \log([A^-]/[HA]) \quad \text{(eq.1)}$$

At ½ equivalence point $[A^-] = [HA]$

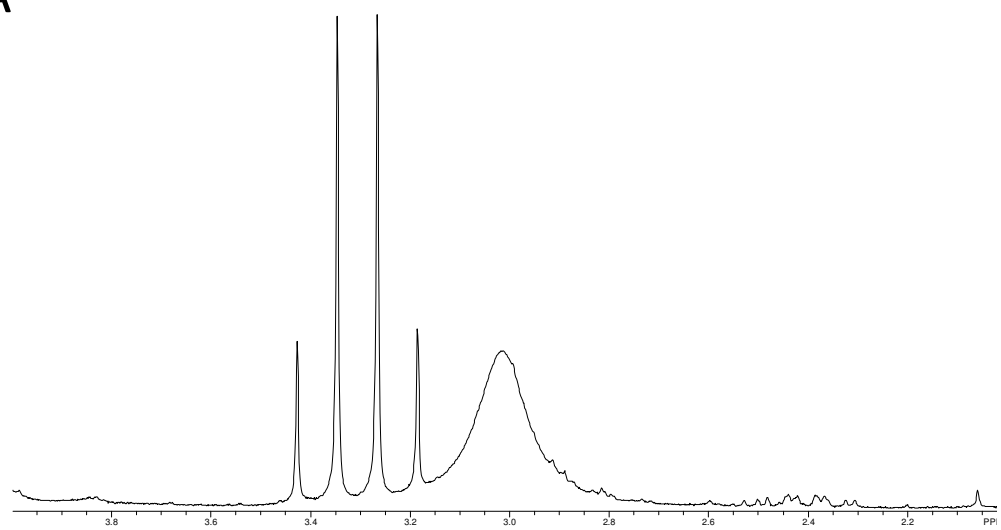
$\log(1) = 0$

$pH = pK_a \approx 6.5$

For an anion to protonate, it must encounter a species that is sufficiently acidic. According to the ^{31}P NMR data, the anion would not protonate upon addition of water (pK_a 15.7) or by addition of saturated NH_4Cl (pK_a 9.24). Upon addition of glacial acetic acid (pK_a 4.76), the anion of (**75**) was entirely protonated. Thus the empirical pK_a of 6.5 is consistent with the observed ^{31}P NMR data.

This ability of the trisphosphonate to act as a strong carbon acid was further confirmed with a deuterium exchange experiment. Addition of D_2O to an NMR sample of the parent trisphosphonate **75** in $CDCl_3$ lead to the disappearance of both the resonance associated with H_2O coordinated to the parent trisphosphonate (3.00 ppm) as well as a significant decrease in intensity of the quartet associated with the α-hydrogen, indicating that the C-H of the parent trisphosphonate is readily exchangeable with D_2O (Figure 37). The ability of the α-proton to exchange with D_2O demonstrates how stabilized the species is, that it has acidic properties similar to acetic acid, and thus should be viewed as a strong carbon acid.

A



B

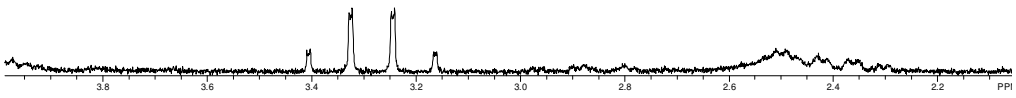


Figure 37. ^{1}H NMR spectrum of the region which contains the C-H resonance for the trisphosphonate (**75**). A: ^{1}H NMR spectrum ($CDCl_3$) **75**. B: ^{1}H NMR spectrum ($CDCl_3$) after addition of D_2O to the sample.

Due to the apparent stability of the trisphosphonate anion and the difficulty encountered in a direct alkylation approach, a new route to synthesize alkyl

trisphosphonates was sought. The first attempt to synthesis alkyl trisphosphonates involved direct phosphonylation. Treatment of benzylbisphosphonate (**82**) with sodium hydride followed by addition of diethyl chlorophosphate proved unsuccessful. Analysis of the ^{31}P NMR spectrum of the reaction mixture did not reveal resonances that could be correlated to an alkyltrisphophonate. Because the benzyl trisphosphonate (**82**) is a more complicated intermediate, the applicability of this methodology was examined on a simplified example in an attempt to produce the parent trisphosphonate **75**. The anion of tetraethyl methylenebisphosphonate (**79**) was formed upon addition of sodium hydride followed by attempted direct phosphonylation using diethyl chlorophosphate (Figure 38). Monitoring the reaction progress by ^{31}P NMR did not show any evidence of a resonance at 14 ppm, which would be an indication of formation of the target trisphosphonate. There have been similar limitations reported during efforts to form the parent trisphosphonate.[76]

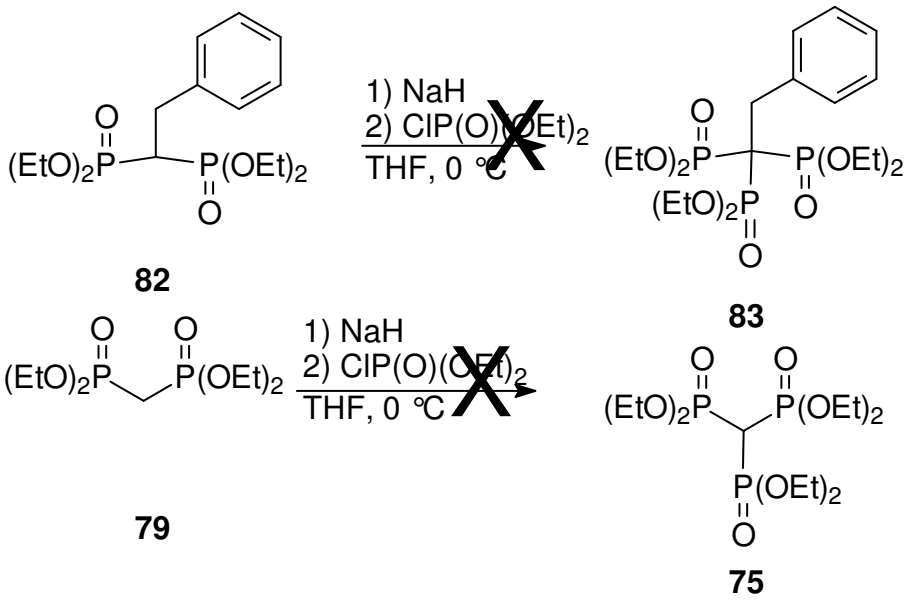


Figure 38. Attempts to synthesize trisphosphonates via direct phosphonylation.

The applicability of a Michaelis-Arbuzov reaction in the synthesis of trisphosphonates also was investigated. It has been reported that dibromomethane (**84**) can undergo reaction with two equivalents of triethyl phosphite resulting in the synthesis of tetraethyl methylenebisphosphonate[82] (Figure 39). It was imagined that treatment of tribromomethane (**85**) with excess triethyl phosphite under reflux conditions may result in the formation of the desired trisphosphonate **75** (Figure 39). Triethyl phosphite was added to bromoform, the reaction was heated at reflux, and the reaction progress was monitored by ^{31}P NMR. After 24 hours there was no observable evidence in the ^{31}P NMR spectrum to indicate the formation of a trisphosphonate **75**. Thus, attempts to utilize this type of phosphorylation reaction for this type of system were found to be unsuccessful in our hands and a new method was pursued.

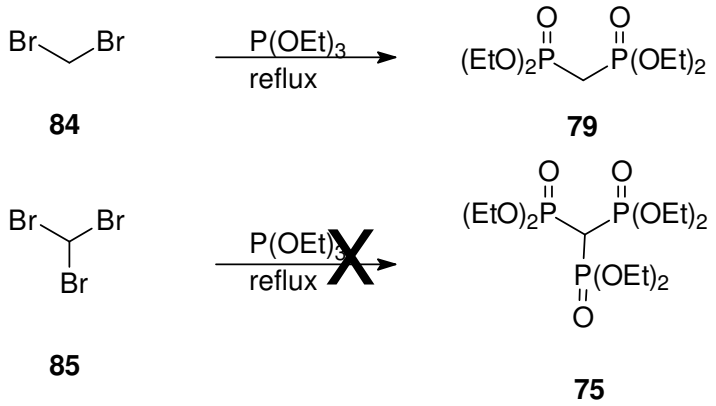


Figure 39. Synthesis of tetraethyl methylenebisphosphonate using dibromomethane and attempted synthesis of trisphosphonate **75** under similar conditions.

A new route that involves a one flask phosphinylation of a bisphosphonate anion followed by oxidation of the newly formed intermediate was examined. This strategy

parallels the methodology previously established (Figure 33). Tetraethyl geranylbisphosphonate (**26**) was deprotonated upon addition of sodium hydride to produce an anion, after which diethyl chlorophosphite was added to the reaction mixture resulting in the formation of a precipitate. The reaction mixture was placed under a stream of air and allowed to oxidize overnight according to the procedures reported for the synthesis of the parent trisphosphonate (**75**).[76] Analysis of the reaction mixture by ^{31}P NMR showed no significant evidence for the formation of the desired alkyltrisphosphonate. Despite the apparent lack of formation of the desired target, mass spectral analysis of the reaction mixture revealed a molecular ion consistent with the formation of geranyltrisphosphonate (**99**). Attempts to use other bases such as LHMDS and KHMDS and alteration of the number of equivalents of base used were unsuccessful. Attempts to increase the formation of the desired alkyl trisphosphonate using an iodine, pyridine, THF and water mixture as the oxidant, an oxidation protocol that was reported successful in the synthesis of analogues of the parent trisphosphonate,[80] were ineffective.

Previously reported investigations on α keto phosphonates that were synthesized through the rapid oxidation of a phosphonite to a phosphonate used hydrogen peroxide.[83,39,84] To begin this sequence, tetraethyl benzylbisphosphonate was deprotonated with NaHMDS. The use of a base such as NaHMDS was preferred due to a greater ability to control carefully the number of equivalents added to the reaction compared to sodium hydride. The anion of tetraethyl benzylbisphosphonate was consumed upon addition of diethyl chlorophosphite, resulting in the formation of a precipitate. Dropwise addition of excess hydrogen peroxide resulted in an exothermic reaction and dissolution of the precipitate. Analysis of the reaction mixture by TLC

provided evidence that a new product was formed. In the ^{31}P NMR spectrum of the reaction mixture, a new peak was observed at 18 ppm. Isolation of the product via column chromatography resulted in a material consistent with the desired benzyltrisphosphonate **83**.

The NMR characteristics of this compound were especially compelling. In the ^{1}H NMR spectrum the benzylic hydrogens appear as a quartet and exhibit dramatic coupling to the adjacent trisphosphonate group (q, J_{PH}= 15.8 Hz). Likewise the ^{13}C NMR spectrum displayed some unique characteristics. The resonances associated with the ethyl groups of the phosphonate esters (63.4 ppm and 16.3 ppm) are split into multiplets, but most notable is the series of distinct quartets observed in the ^{13}C NMR spectrum. The α-carbon appears as a clear quartet with a C-P coupling constant of 118 Hz. There are also observable C-P couplings to the benzylic carbon, which appears as a quartet at 36.1 ppm and has a coupling constant of 5.1 Hz, and the quaternary carbon of the phenyl ring at 136.3 ppm which has a coupling constant of 5.9 Hz. Combustion analysis of the material was consistent with the expected carbon and hydrogen percentages as long as one equivalent of water is added to the molecular formula. The likelihood that the benzyltrisphosphonate **83** coordinates a molecule of water can also be supported by the ^{1}H NMR spectrum. In the ^{1}H NMR spectrum there is a broad signal at ~3.0 ppm which integrates to two hydrogens. Addition of D_2O to the sample causes this peak to disappear, indicating that the hydrogens are readily exchangeable with deuterium.

The inclusion of a coordinated water molecule also has been observed with a number of bisphosphonates.[26] Because no other alkyl trisphosphonates have been synthesized or characterized, the benzyltrisphosphonate product also was analyzed by

high resolution mass spectrometry. The molecular ion found was consistent with the predicted value from the molecular formula. Based on the ^{1}H, ^{13}C, and ^{31}P NMR data along with elemental analysis and HRMS data, the compound was designated as the desired benzyl trisphosphonate **83**.

A series of alkyl bisphosphonates was prepared as precursors to trisphosphonates. Tetraethyl methylenebisphosphonate (**79**) was treated with sodium hydride to produce an anion. Addition of an allylic bromide to the anion resulted in the formation of both mono and dialkylated bisphosphonates. This mixture of mono and dialkylbisphosphonates was separable via flash chromatography. This method resulted in formation of allyl (**88**)[85] and prenyl (**90**)[26] bisphosphonates along with the benzyl (**82**)[33, 86] and geranyl (**26**)[56] bisphosphonates that had been synthesized previously according to the same methodology.

Aliphatic bromides are less reactive as alkylating agents compared to allylic and benzylic bromides. Subsequently bisphosphonates with aliphatic alkyl groups are more difficult to synthesize, and preparation of the representative propylbisphosphonate (**93**) required that the reaction mixture for the alkylation be brought to reflux. The reaction progress was monitored by ^{31}P NMR. After it appeared that propylbisphosphonate (~23 ppm) was no longer being formed the reaction was quenched, worked-up, and purified using the standard procedure resulting in isolation of the desired propylbisphosphonate **93**.[33, 85]

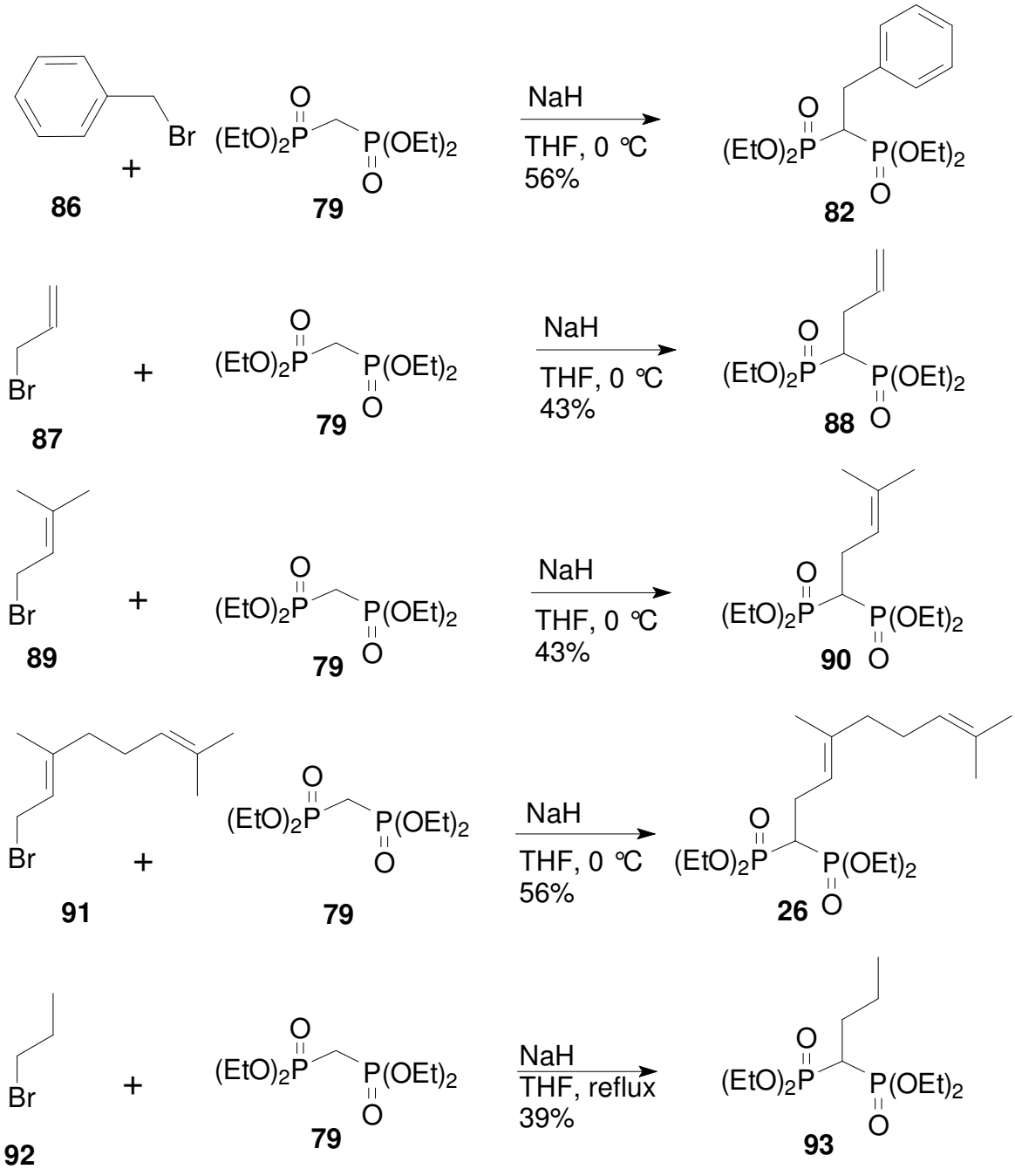


Figure 40. Synthesis of alkylbisphosphonates.

Synthesis of the propargylbisphosphonate (**94**) proved to be more arduous. Utilization of a standard alkylation of tetraethyl methylenebisphosphonate (**79**) with

propargyl bromide resulted in a mixture of mono- (**94**) and dialkylbisphosphonate (**95**) products (Figure 41) that were observed in the ^{31}P NMR of the reaction mixture. Despite extensive attempts to purify this mixture using flash chromatography, the two compounds proved difficult to separate. Similar difficulties in the purification of this mixture have been reported in the literature.[87]

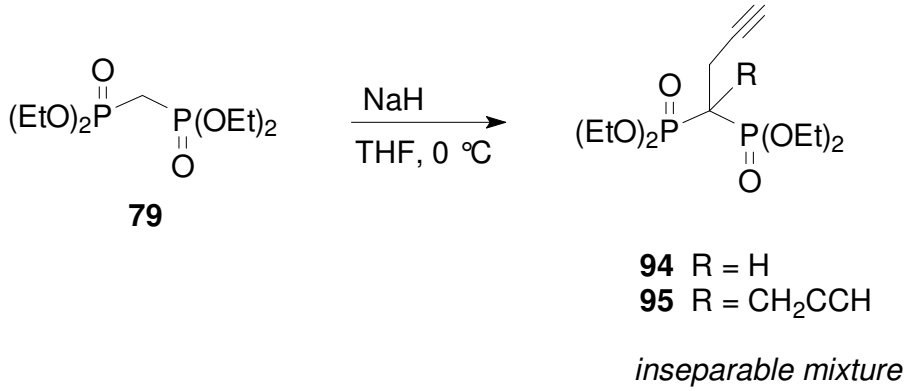


Figure 41. Synthesis of a mixture of propargylic bisphosphonates.

Ultimately this complication was circumvented through use of a Michael addition of sodium acetylide to the vinyl bisphosphonate **96**. Michael addition of a nucleophile to this vinyl bisphosphonate results in a reaction mixture that does not contain any of the dialkylated species, which greatly simplifies purification. The vinyl bisphosphonate **96** was prepared via a Knovenangel condensation of paraformaldehyde with tetraethyl methylenebisphosphonate. Dropwise addition of sodium acetylide to a stirring solution of compound **96** at –15 °C resulted in formation of the desired propargylbisphosphonate **94** which was purified using standard flash chromatographic techniques (Figure 42). The isolated compound had ^{1}H and ^{31}P NMR data consistent with previously reported literature values.[87]

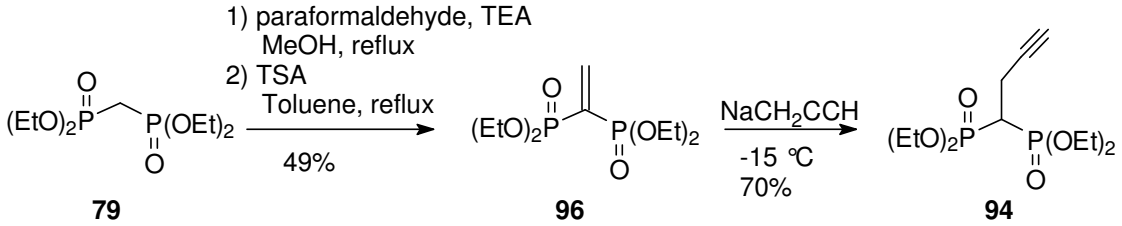


Figure 42. Synthesis of bisphosphonate **94** via Michael addition.

With several mono-alkylated bisphosphonates in hand, the methodology to synthesize alkyltrisphosphonates was applied first to geranyl-(**26**) and allylbisphosphonate (**88**). Treatment of either bisphosphonate with NaHMDS, diethyl chlorophosphite, and hydrogen peroxide according to the standard protocol resulted in a distinguishable new peak in the ^{31}P NMR spectrum. Upon isolation of the newly formed trisphosphonates it was discovered that the NMR characteristics of these two compounds also display the unusual characteristics indicative of an alkyl trisphosphonate. The allylic trisphosphonates displayed notable NMR characteristics including a distinct doublet of quartets for the methylene hydrogens with a phosphorus-hydrogen coupling between approximately 9 and 16 Hz. There are standard hydrogen-hydrogen coupling constants observed in these spectra but the multiplicities of some peaks are increased and many peaks appear as complex multiplets. In the ^{1}H coupled ^{13}C NMR spectrum quartets and multiplets also are observed. The α-carbons appear as distinct quartets with a very low signal-to-noise ratio. This could be expected of a quaternary carbon that is split into a quartet. The coupling constants to the α-carbons are again approximately 120 Hz, and there also are distinguishable couplings to the β (~5.5 Hz) and γ (~ 6.3 Hz) carbons. The combustion analysis of both allyltrisphosphonate **97** and geranyltrisphosphonate **99** match if an equivalent of water is included in the

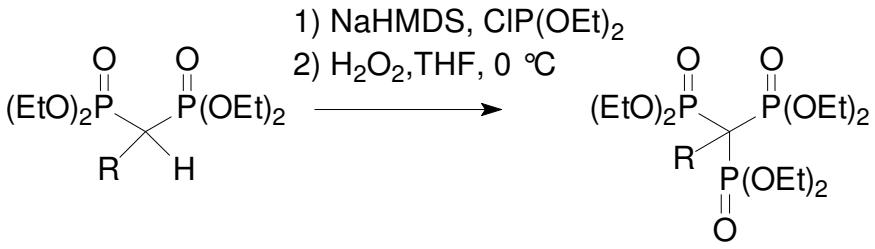
molecular formula, and in both cases a molecular ion was found that was consistent with the designated product.

Initially the isolated yields of the desired trisphosphonate were modest (8-10%), and thus modification to the original procedure was required. One obstacle to modifying the procedure was the purification of the final product to determine the reaction yield. Isolation of the trisphosphonate product proved tedious and time consuming so a more efficient method of screening reaction conditions was sought. It was determined that reaction conversion could be interpreted from the ^{31}P NMR of the reaction mixture. The chemical shifts of the starting materials and the product are distinct. Integration of the area under each of these peaks, and utilization of a 2:3 ratio to correct for the signal intensity of bis- and trisphosphonates, was shown to be applicable by comparison to a calibration curve generated using different concentrations of benzylbisphosphonate (**82**) and benzytrisphosphonate (**83**). This strategy provided a more expedient method to analyze the reaction mixture compared to full purification, and thus allowed faster comparison of reaction conditions.

Despite numerous manipulations to the reaction conditions, including the types and amounts of base used as well as solvent employed, the yields in the formation of trisphosphonates remained modest (~20-30% by ^{31}P NMR). A comparison of the reaction yields of the benzyl (**83**), allyl (**97**), and geranyl trisphosphonates (**99**) revealed a discernible trend. The bisphosphonates that had a higher carbon to phosphorus plus oxygen ratios proceeded to the trisphosphonate in higher yield. Bisphosphonates have been shown to form hydrates upon standing and it was hypothesized that the extent of hydration could be related to the carbon-to-phosphorus ratio. The possibility that water

in the bisphosphonate starting material might complicate the formation of the intermediate became obvious. With this in mind, an extensive drying procedure was implemented. The alkylbisphosphonate starting material was treated with benzene and any residual water was removed through azeotropic distillation. After three such cycles, the bisphosphonate was dissolved in THF and treated with NaHMDS. After 30 minutes diethyl chlorophosphite was added, resulting in formation of a precipitate. This mixture was allowed to stir for an additional thirty minutes and then oxidized by addition of excess hydrogen peroxide.

Use of the extensive drying technique dramatically increased the yields of the trisphosphonates, with conversions now ranging from 64-86% and isolated yields only ~5% less. This methodology was found to be applicable to a variety of mono alkyl bisphosphonates including aliphatic and propargyl bisphosphonates (Figure 43). The extensive drying procedure and use of NaHMDS and hydrogen peroxide also increased the yield of the parent trisphosphonate to 48% verse 32% in the original report.[76] It was later determined that use of excess hydrogen peroxide does not contribute directly to the high yields and that use of as few as two equivalents appears to be sufficient for the oxidation step. However addition of excess hydrogen peroxide was found to greatly simplify the workup procedures, perhaps because of the additional water involved.



R =	Conversion (by ^{31}P NMR) (%)	Isolated Yield (%)
83	67%	61%
97	77%	71%
98	74%	68%
99	69%	63%
100	86%	79%
101	64%	59%

Figure 43. Examples of alkyltrisphosphonates.

One exception that was encountered in the synthesis of alkyltrisphosphonates through this strategy was the conversion of phenylbisphosphonate **103** to the

corresponding trisphosphonate **104**. Tetraethyl phenylbisphosphonate **103** was prepared starting with phosphonylation of the commercial benzylphosphonate **102**. In this case, benzylphosphonate (**102**) was treated with LDA followed by addition of diethylchlorophosphate, resulting in the formation of phenylbisphosphonate **103**. The ^{1}H NMR spectrum of this product matched literature examples.[88] Treatment of compound **103** according to the standard procedure for formation of an alkyltrisphosphonate resulted in no obvious formation of the predicted product (Figure 44). Despite use of extensive drying techniques, there was no evidence for formation of the desired trisphosphonate in the ^{31}P NMR spectrum. Several factors may contribute to the lack of formation of the trisphosphonate product. The phenyl group may introduce some increased steric constraints when compared to its benzyl counterpart. Likewise the phenyl ring may also contribute to the stability of the intermediate anion, resulting in a less reactive intermediate.

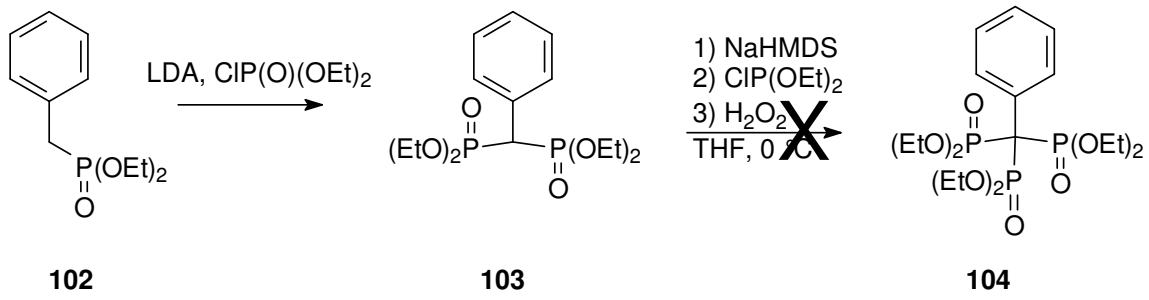
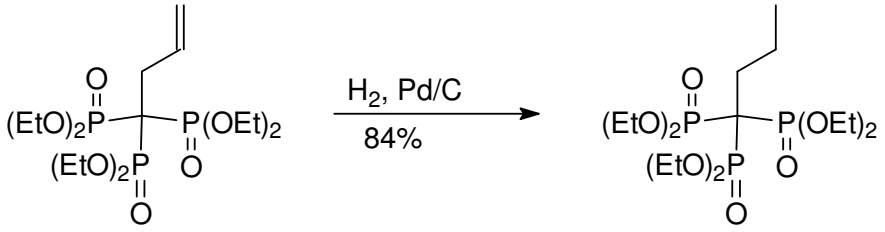


Figure 44. Attempted synthesis of phenytrisphosphonate **104**.

Once a variety of alkyl trisphosphonates had been prepared, the limits to reactivity of this functionality were investigated. Treatment of allyltrisphosphonate **97** with palladium on carbon and hydrogen gas under ~1 atm pressure resulted only in

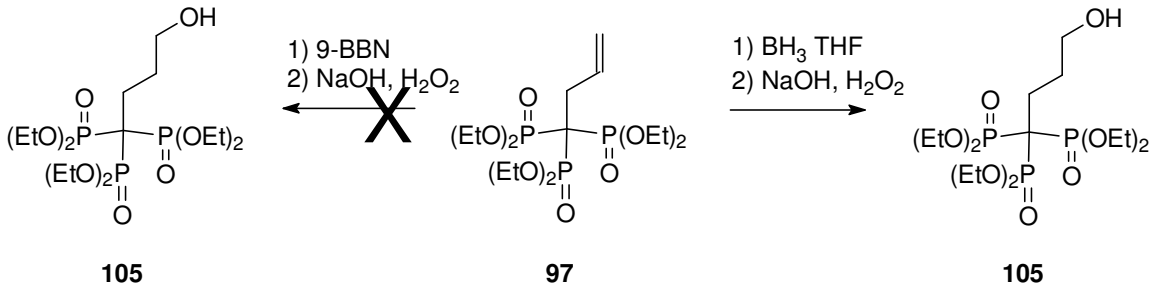
reduction of the olefin (Figure 45). There is no evident decomposition of the trisphosphonate group. The isolated product was identical to the trisphosphonate **100** formed from phosphinylation and oxidation of propylbisphosphonate.



97 **100**

Figure 45. Hydrogenation of allyltrisphosphonate (**97**).

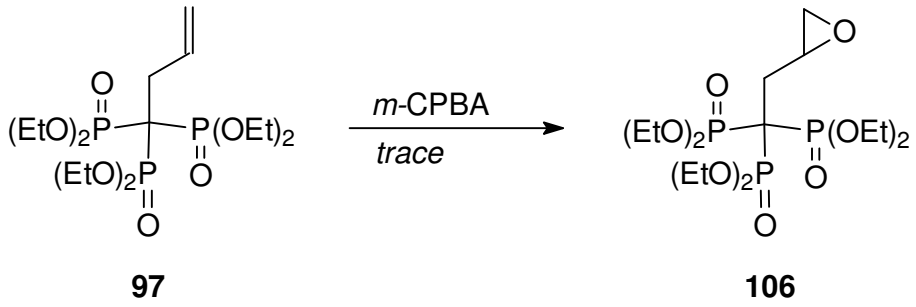
Attempts to perform a hydroboration reaction on the allyltrisphosphonate **97** using 9-BBN as the hydroboration reagent followed by oxidative work-up were unsuccessful. No significant changes were noticed in the ^{13}P NMR spectrum of the reaction mixture and upon attempted isolation it was discovered that only starting material could be found. Despite multiple attempts, hydroboration with 9-BBN was ineffective on allyltrisphosphonate **97**. It was determined that this obstacle could be circumvented by treatment of allyltrisphosphonate with BH_3·THF instead, followed by standard oxidative workup. The expected primary alcohol (**105**) was isolated with a reaction yield (62%) consistent with what is seen for this reaction on bisphosphonates (68% Figure 46).[89] There was no evidence that any of the secondary alcohol was formed and none was isolated from this reaction.



105 **97** **105**

Figure 46. Attempted hydroboration of allyltrisphosphonate (**97**) using different boron reagents.

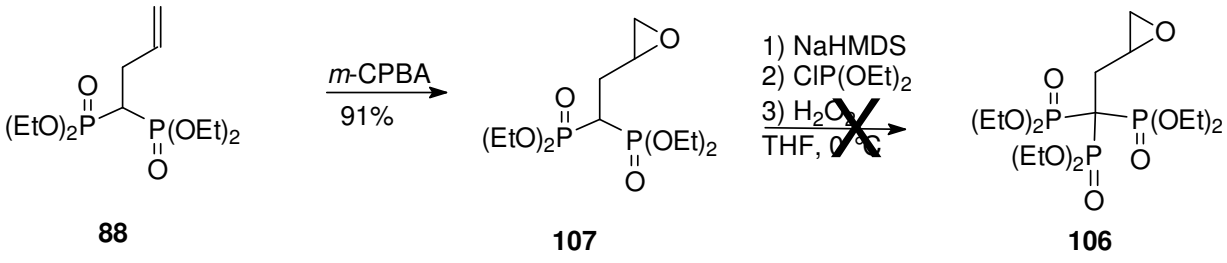
Some oxidative reaction conditions appeared to affect the trisphosphonate group as well. Treatment of allyltrisphosphonate (**97**) with *meta*-chloroperoxybenzoic acid (*m*-CPBA) resulted in significant decomposition of the trisphosphonate (Figure 47). Analysis of the reaction mixture by ^{31}P NMR showed the appearance of multiple phosphorus-containing products. Upon isolation there was some evidence that trace amounts of the desired product were formed. The olefinic hydrogens were no longer observed in the ^{1}H NMR spectrum and a molecular ion consistent with the desired product was detected in the mass spectrum. This compound was obtained as a very minor product of the reaction and enough of the target could not be fully purified to give absolute characterization data.



97 **106**

Figure 47. Epoxidation of allyltrisphosphonate (**97**).

It was imagined that this challenge might be avoided by installing the epoxide functionality prior to formation of the trisphosphonate. Therefore allylbisphosphonate **88** was treated with *m*-CPBA, and the desired epoxide **107** was obtained in good yield (91%).[85] When the epoxide bisphosphonate **107** was treated under standard conditions for the preparation of alkyl trisphosphonates (Figure 48) the ^{31}P NMR spectrum of the reaction mixture exhibited multiple peaks, none of which could be easily associated with the formation of the desired product.

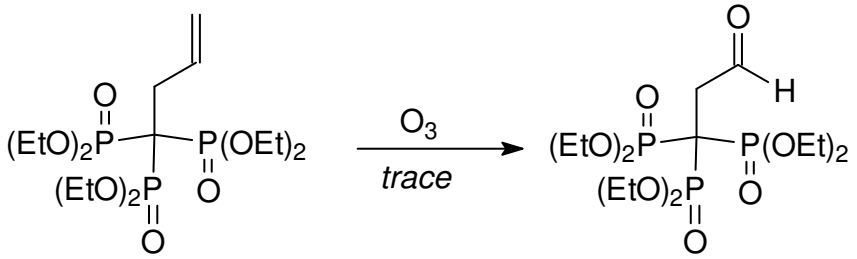


88 **107** **106**

Figure 48. Attempted preparation of trisphosphonate **106**.

Attempts to bring about the oxidative cleavage of the olefin of allyltrisphosphonate to produce an aldehyde trisphosphonate also were unsuccessful.

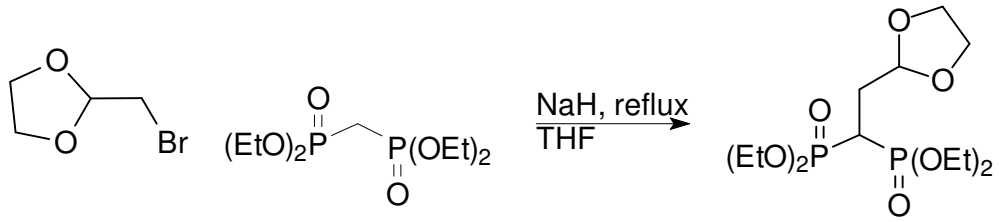
Attempts to cleave the olefin using standard ozonolysis conditions resulted in the formation of multiple products as determined by analysis of the ^{31}P NMR spectrum. It was imagined that excess ozone might be undergoing reaction with the trisphosphonate moiety and that limiting the number of equivalents may result in a more selective oxidation. A Rubin ozonolysis apparatus was assembled, and the process was implemented so that the number of equivalents of ozone could be controlled carefully. A measured volume of methylene chloride was allowed to saturate with ozone.[90] This solution was added to a solution of the allyltrisphosphonate (**97**) in methylene chloride at -78 °C (Figure 49). After fifteen minutes the reaction was purged with air, allowed to warm to room temperature, and then concentrated. The reaction mixture was analyzed using ^{31}P NMR. Even under Rubin ozonolysis conditions, there was observable decomposition of the starting material and many new peaks were observed in the ^{31}P NMR spectrum. Partial purification of the product mixture resulted in the detection of a minor product which had a peak in the aldehyde region of the ^{1}H NMR spectrum as well as a molecular ion consistent with the desired product (**107**). Unfortunately, no appreciable amount of this product could be isolated and attempts at full purification and characterization proved to be challenging. Consequently this route was abandoned.



97 **107**

Figure 49. Attempted preparation of trisphosphonate **107**.

An alternative route was sought in which a protected bisphosphonate was prepared. Treatment of tetraethyl methylenebisphosphonate with base and 2-(bromomethyl)-1,3-dioxolane under reflux condition resulted in formation of a product that was nearly consistent with the expected product **109** (Figure 50). The exception was that the acetal hydrogen was not observed in the ^{1}H NMR spectrum. Due to this impasse the product was not carried further in the sequence.



108 **79** **109**

Figure 50. Attempted preparation of bisphosphonate **109**.

Ultimately some strongly oxidative techniques do not appear to be compatible with the trisphosphonate functionality. This was surprising due to the use of an oxidative step in the preparation of the alkyltrisphosphonates and an oxidative workup in the hydroboration of allyltrisphosphonate, both of which utilize hydrogen peroxide. The desire to synthesize trisphosphonates with an aldehyde or a carboxylic acid moiety may be possible via modification of other functionalities that are more resilient to these reaction conditions.

Unexpected reactivity also was discovered when allyltrisphosphonate **97** was treated with 2-methyl-2-butene and Grubbs second generation catalyst (Grubbs II) under metathesis conditions. Allylphosphonate (**110**) had been shown to undergo reaction with

Grubbs II in the presence of 2-methyl-2-butene to provide prenyl phosphonate (**111,** Figure 51).[91, 92] When allylbisphosphonate (**88**) was treated with Grubbs II and 2-methyl-2-butene at room temperature, conversion to prenyl bisphosphonate (**90**) occurred in high yield (92%), proving that bisphosphonate groups are stable to the reaction conditions and do not appear to react with Grubbs II in a competitive fashion.

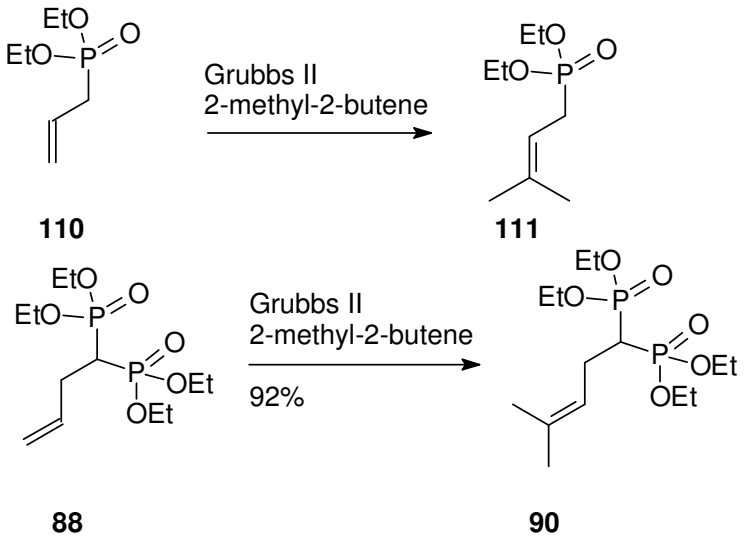


Figure 51. Examples of metathesis of 2-methyl-2-butene with phosphonate compounds.

Similar metathesis reaction conditions with allyltrisphosphonate (**97**) and 2-methyl-2- butene did not bring about a reaction. Treatment of allyltrisphosphonate (**97**) with 2-methyl-2-butene and Grubbs II provided no evidence of new product formation, and only starting material was observed in the ^{31}P and ^{1}H NMR spectra. Although attempted reaction at room temperature did not result in a new product, warming the sealed reaction vial to 40 °C did result in observable new products in the ^{31}P NMR spectrum. There were three new peaks that were distinct in their chemical shifts from

allyltrisphosphonate (**97**, 18.0 ppm), including resonances at 18.6 ppm, 18.5 ppm and 18.3 ppm in a 1:1:4 ratio respectively.

The ternary mixture was not readily separated under standard chromatographic techniques. Comparison of the ^{1}H NMR spectrum of the reaction mixture to a sample of prenyltrisphosphonate (**98**) that had been synthesized independently proved that the major product of the reaction did not match. There were three observable frequencies in the vinylic region and the methyl region contained multiple resonances instead of only two.

It was hypothesized that the reaction may have proceeded to the unexpected 1,2-disubstituted olefin product and that two of the peaks in the ^{31}P NMR spectrum could account for the cis and trans isomers of this product. One of the two minor ^{31}P NMR peaks was consistent with the chemical shift of the prenyl trisphosphonate. High resolution mass spectrometry of the mixture revealed a molecular ion match consistent with a 1,2-disubstituted product. Closer investigation of the mass spectrum also yielded a molecular ion that could have arisen from the trisubstituted olefin product prenyl trisphosphonate (Figure 52).

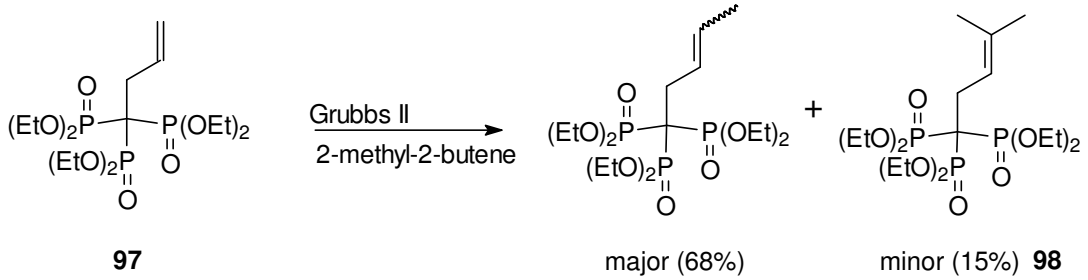


Figure 52. Synthesis of 1,2-disubstituted and trisubstituted olefin products.

Several experiments were initiated to confirm the identity of the 1-2-disubstituted product. The first attempt involved the synthesis of a deuterium labeled 2-methyl-2-butene (**113**). Incorporation of the deuterium labeled portion of the molecule in the isolated metathesis product would confirm that the reaction is occurring to produce the 1,2-disubstituted product. Commercially available prenol (**112**) was converted to prenyl bromide (**89**) by treatment with phosphorous tribromide. The prenyl bromide was then added to a stirring suspension of lithium aluminum deuteride (LAD) in diglyme and the product was immediately distilled. Preparation of a deuterium labeled version of 2-methyl-2-butene was successful but could only be isolated in small amounts and when it was used in the reaction, formation of the metathesis product **114** was not observed (Figure 53). Due to the expense and difficulty in generating the deuterium labeled 2-methyl-2-butene a new strategy to prove the identity of the 1,2-disubstituted product was pursued.

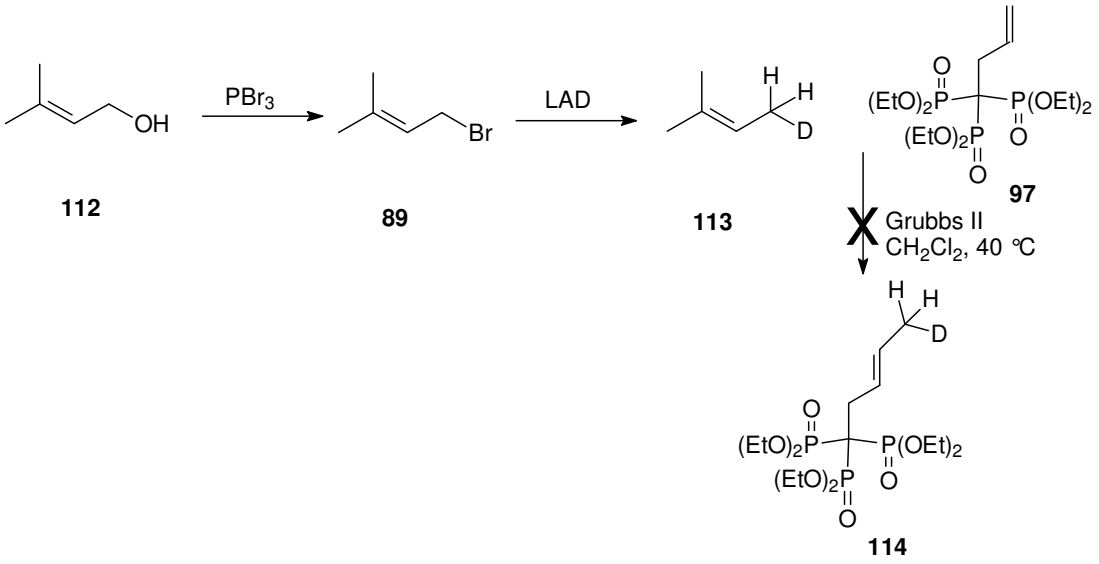
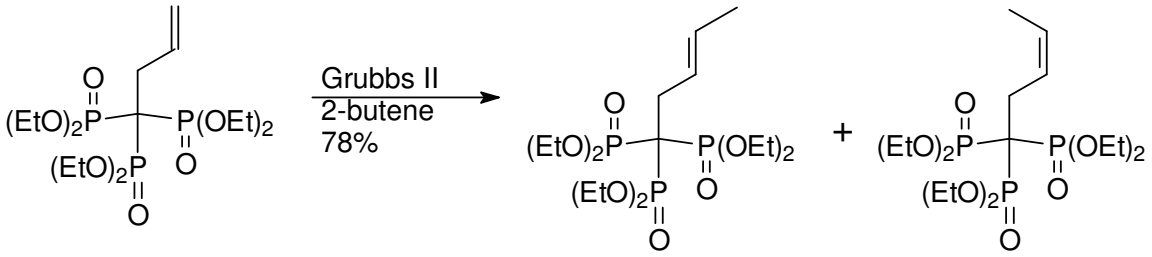


Figure 53. Preparation of deuterium labeled 2-methyl-2-butene (**113**) and attempted metathesis reaction with allyltrisphosphonate **97**.

To conduct an independent synthesis of the 1,2-disubstituted olefin product, allyltrisphosphonate (**97**) and Grubbs II were dissolved in methylene chloride and a balloon filled with 2-butene was added. The vial was then sealed and warmed to 40 °C overnight. These reaction conditions resulted in formation of a product mixture with spectroscopic characteristics that were very similar to the major product in the previous ternary mixture (Figure 54). The ^{31}P NMR spectrum exhibited two new peaks with resonances at 18.4 ppm and 18.3 ppm and an integration ratio of 2.8:1. The frequencies of the major product and one of the minor products in the ternary mixture resulting from reaction with 2-methyl-2-butene were 18.5 ppm and 18.3 ppm respectively. The observation of two distinct peaks in the ^{31}P NMR spectrum could be attributed to formation of cis and trans olefin isomers. Analysis by ^{1}H NMR spectroscopy revealed an unmistakable match to the product formed from metathesis of compound **92** with 2-methyl-2-butene.



97 **115** **116**

Figure 54. Independent synthesis of the 1,2-disubstituted trisphosphonates **115** and **116**.

Because the metathesis product from allyltrisphosphonate **97** and 2-butene resulted in a mixture of cis and trans isomers, it was important define the olefin

stereochemistry of both products. In the ^{1}H NMR spectrum there are discernible features that reinforce the idea that the product is a mixture of cis and trans isomers in a 2.8:1 ratio. There are three distinct sets of vinylic resonances in the spectrum. The resonances observed at 5.85 ppm and 5.94–5.92 ppm each correlate to the vinylic hydrogen located on the γ-carbon of the trans and cis isomers respectively. The ratio of the peaks occurs in approximately a 3:1 ratio. The resonances associated with the methyl hydrogens also are distinct from each other. They appear as two sets of doublets located at approximately 1.60 ppm. The ratio of one set to the other is also ~3:1. The other resonances in the spectrum overlap but integration of the area under those peaks is ~30% more intense than expected for the major isomer, another indication that there are cis and trans isomers present in a 3:1 ratio.

Comparison of the ^{13}C shifts to known literature values of cis and trans isomers also aided in assignment of the olefin stereochemistry (Figure 55). In the example of trisphosphonate **115**, the methyl group appears at 17.9 ppm while the adjacent vinylic carbon is observed at 128.0 ppm. Comparison of the ^{13}C NMR shifts to trans 2-butene (**117**) at 17.6 ppm and 126.0 ppm are consistent with the trans assignment. Likewise the cis isomer **116** has a methyl carbon resonance at 12.9 ppm and an adjacent ^{13}C shift of 125.0 ppm. When compared to the corresponding ^{13}C values of cis 2-butene (118, 12.1 ppm and 124.6 ppm) there is a strong parallel. Although the coupling constant of the cis isomer could not be reliably determined, the coupling constant for the trans isomer was found to be 15 Hz, a value which is consistent with the vinylic coupling in trans olefin stereoisomers.

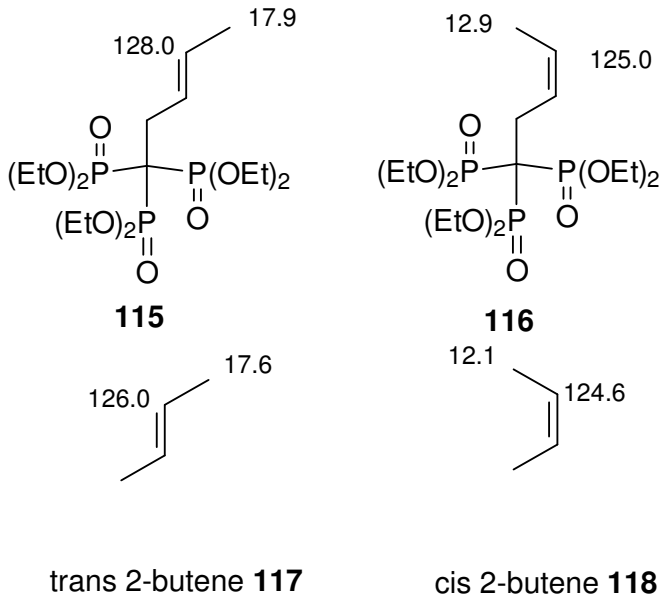


Figure 55. Comparison ^{13}C NMR shifts of cis and trans isomers of the 1,2-disubstituted trisphosphonates.

The trisphosphonate group provides an especially hindered scaffold. The unusual reactivity of allyltrisphosphonate **97** may be due to the proximity of the reactive olefin and the three phosphonate groups. The ability of allyltrisphosphonate to undergo metathesis with 2-methyl-2-butene and provide the 1,2-substituted olefin as the major product is an example of this. This could be due to unexpected reaction routes. If the steric constraints of the trisphosphonate group are greater than those of the vinylic methyl groups of 2-methyl-2-butene, then the 2-methyl-2-butene may undergo metathesis reaction with itself to provide 2-butene and the tetrasubstituted olefin. The 2-butene could then readily react with the allyltrisphosphonate (**97**) to provide the 1,2-disubstituted product. A shift in reaction equilibrium resulting from the consumption of the 2-butene formed could drive this conversion according to Le Chatelier's principle. Alternatively if 2-methyl-2-butene cannot undergo reaction in the familiar fashion due to the steric

encumbrance of the trisphosphonate group, it may add in the reverse orientation resulting in the formation of the observed 1,2-disubstituted product.

To investigate further the steric limitations to the reactivity of allyltrisphosphonate **97** a less sterically hindered analogue was synthesized. Alkylation of tetraethyl methylenebisphosphonate with 6-bromo-1-hexene (**119**) under reflux conditions provided tetraethyl 6-hepten-1,1-bisphosphonate (**120**) which was then subjected to treatment with NaHMDS and diethyl chlorophosphite followed by oxidation with hydrogen peroxide according to the conditions employed to synthesize alkyltrisphosphonates. Isolation of the product yielded the desired trisphosphonate (**121**) in good yield (Figure 56). The ^{1}H and ^{13}C NMR data was consistent with the expected trisphosphonate, including the quartets observed in the ^{13}C spectrum with coupling constants (119.3 Hz, 5.3 Hz and 5.0 Hz) similar to the trisphosphonates that had been synthesized above.

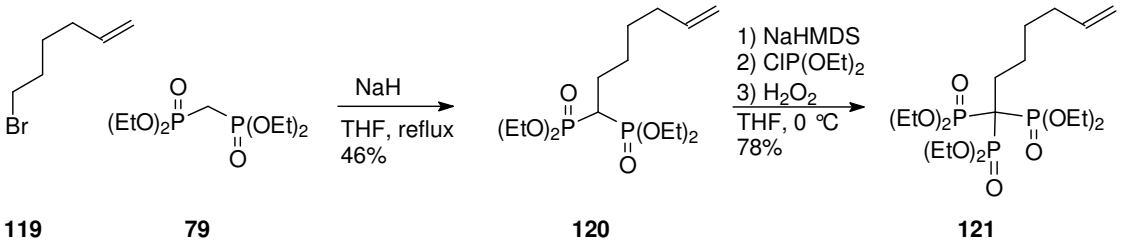


Figure 56. Preparation of 6-hepten-1,1-bisphosphonate (**120**) and trisphosphonate **121**.

Treatment of trisphosphonate **121** with 2-methyl-2-butene and Grubbs II using the previous metathesis conditions resulted in formation of the anticipated trisubstituted olefin **122** in good yield (Figure 57). The trisubstituted olefin was the only product isolated, and there was no evidence for formation of a 1,2-disubstituted product.

Analysis by ^{1}H, ^{13}C and ^{31}P NMR was consistent with the trisubstituted product **122**, as was detection of a molecular ion that would be expected for this product.

A second measure of the reactivity of the more remote olefin was conducted through hydroboration. Treatment of the less sterically hindered olefin **121** with 9-BBN followed by oxidative workup with sodium hydroxide and hydrogen peroxide resulted in formation of the desired primary alcohol **123** (Figure 57).

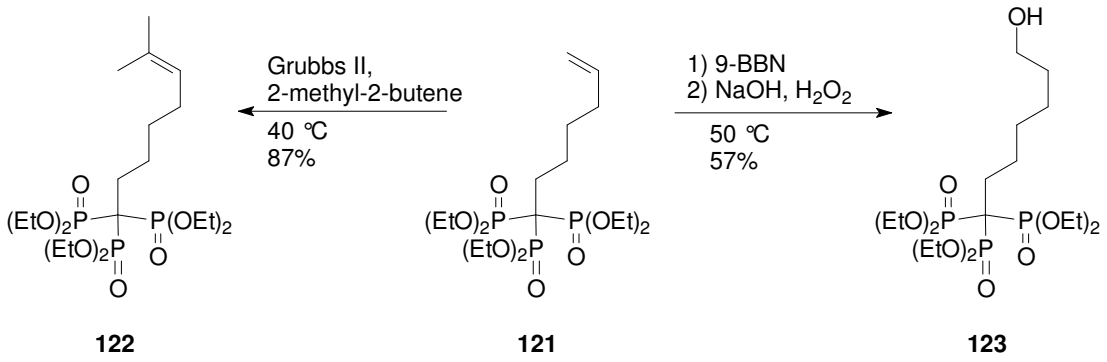


Figure 57. Hydroboration with 9-BBN and metathesis of trisphosphonate **121**.

The formation of the trisubstituted olefin product from metathesis of trisphosphonate **121** provides support to the theory that the trisphosphonate group provides considerable steric hindrance in allyltrisphosphonate **97.** Similarly, attempts to perform hydroboration on the allyltrisphosphonate **97** using 9-BBN were unsuccessful whereas reaction with BH_3·THF provided the desired primary alcohol in good yield. The Grubbs II catalyst is considerably hindered and 9-BBN is a relatively large reagent when compared to BH_3·THF, but both react smoothly with the more remote olefin of compound **121**.

These transformations illustrate that the unexpected reactivity of bulky reagents is not attributed to the reactivity of the trisphosphonate group but rather the steric encumbrance that it provides to reaction at nearby functionalities. Surprisingly the steric limitations do not appear to affect reaction of a propargyltrisphosphonate **101** in a copper-mediated dipolar cycloaddition with an azide, also known as a "click" reaction as described below.[93]

Because organic azides can be explosive, *in situ* formation of the desired azide from the requisite bromide was utilized in this click reaction. Benzyl bromide (**86**) was dissolved in dimethylformamide and treated with sodium azide. Then tBuOH/H_2O was added to the reaction solvent followed by the propargyltrisphosphonate **101**. Addition of a copper sulfate solution and sodium ascorbate facilitate the dipolar cyclization, resulting in the 1,2,3-triazole **124** (Figure 58).

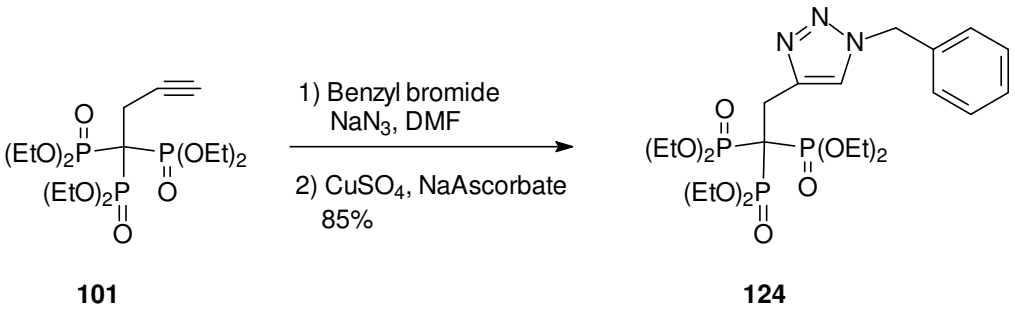


Figure 58. Click reaction with trisphosphonate **101**.

The efficient reaction of benzyl azide with trisphosphonate **101** suggests that other click reactions would also be facile. Numerous organic azides are available commercially including fluorescent azide dyes (**125**), nucleoside analogues (**126**), and biotinylated analogues (**127**),[94] to name a few (Figure 59). The use of click reactions can facilitate the installation of extremely complex functionality. If the one flask protocol

beginning with the corresponding bromide is utilized, the possibilities to modify acetylenic trisphosphonate compounds are virtually limitless.

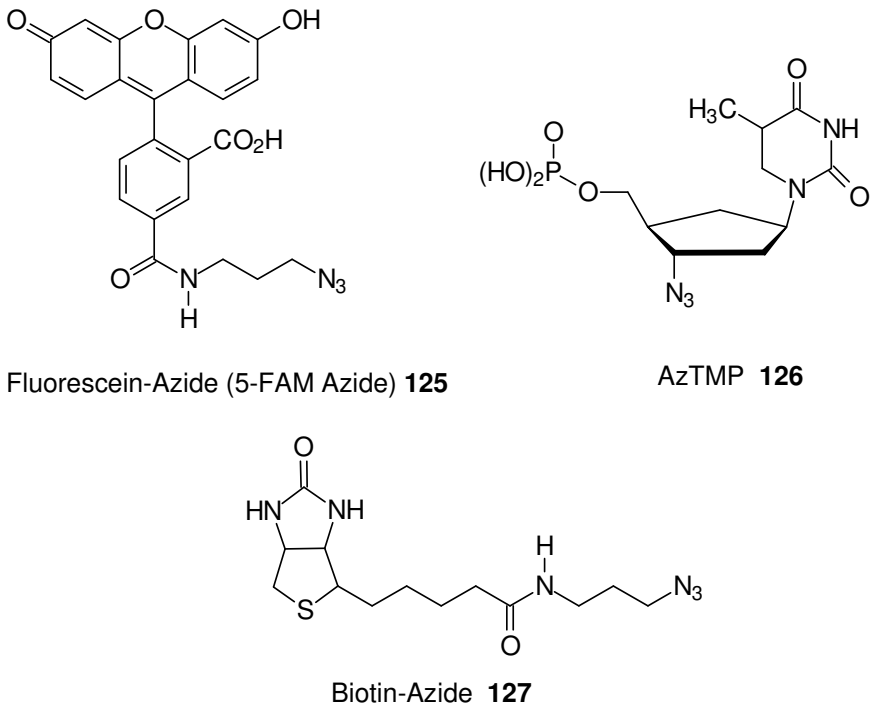
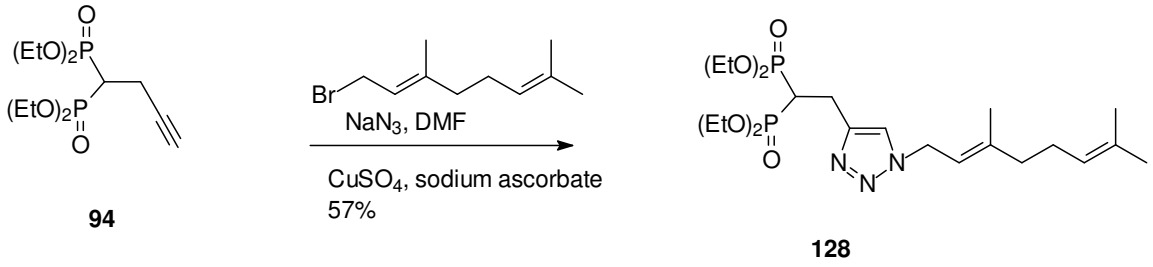


Figure 59. Examples of commercially available azides

The use of a "clicked" bisphosphonate followed by conversion to the trisphosphonate also would furnish compounds for divergent biological studies. An alternative route would provide "clicked" bisphosphonates as intermediates that could be used in biological investigation as well as converted to the desired trisphosphonates. One example in this spirit that was tested was the click reaction of bisphosphonate **94** and geranyl bromide resulting in the bisphosphonate product **128** which proved to be reasonably efficient (Figure 60). Not only can this methodology be used in modifying a

trisphosphonate for biological or structure activity relationship (SAR) investigations, it can also be treated as an alternative way to approach SAR studies of bisphosphonates. As far as we can determine, this concept has not been applied to bisphosphonates, and it may be very useful in probing enzyme activity.

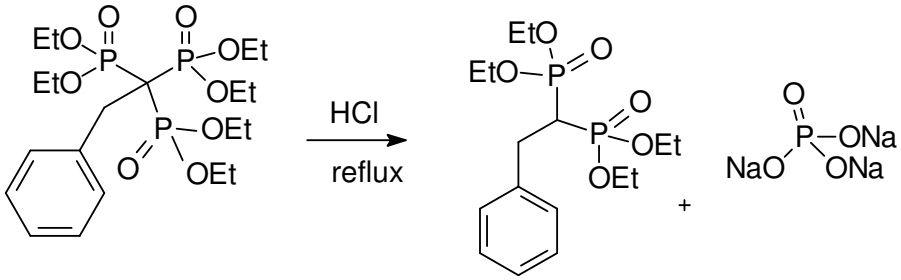


94 **128**

Figure 60. Click reaction with propargylbisphosphonate **94**.

Attempts to convert the trisphosphonate into a biologically testable compound began with the benzyltrisphosphonate **83**. It was hypothesized that the benzyl trisphosphonate **83** might undergo acid-mediated hydrolysis to the hexaacid. There are examples in the literature where this methodology has been shown to work with benzyl bisphosphonate.[86] This method also had been used to hydrolyze phosphonate and carboxylate esters in the preparation of PEPHC, a GGTase II inhibitor.[95] Although the benzyl bisphosphonate has no activity against GGDPS, and it is uncertain whether the benzyl trisphosphonate will have any specific activity, it was selected for this study because of the clear precedent. Furthermore, while geranyltrisphosphonate **99** might yield a more biologically active salt, there is a potential for acid mediated olefin isomizeration in the geranyl substituent.

Benzyltrisphosphonate **83** was treated with hydrochloric acid under reflux conditions. Analysis of the reaction mixture showed that multiple peaks were found within the ^{31}P NMR spectrum. The major peaks corresponded to formation of a bisphosphonate and a phosphate (Figure 61). Dephosphonylation results had been reported for the parent trisphosphonate under similar reaction conditions.[96,76]

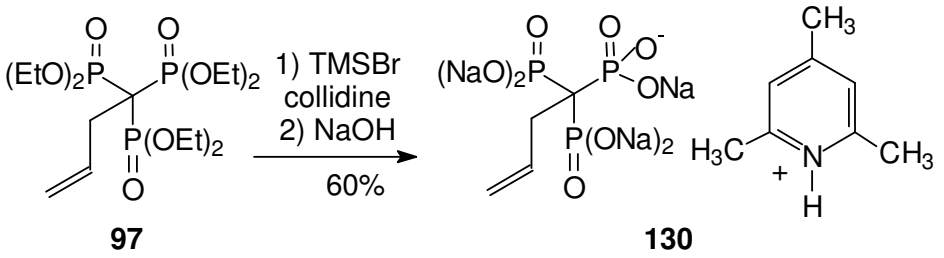


83 **82** **129**

Figure 61. Attempted acid hydrolysis of the trisphosphonate esters.

Bisphosphonate esters often have been cleaved by reaction with bromotrimethylsilane followed by treatment with sodium hydroxide resulting in the formation of the tetrasodium salt.[66] In this reaction 2,4,6-collidine frequently is used to aid in the formation of the TMS ester but it generally not evident in the final product of the reaction sequence. It was imagined that this methodology could be used to produce a hexasodium salt of a trisphosphonate. Therefore a solution of 2,4,6-collidine and bromotrimethylsilane in methylene chloride was prepared. The allyltrisphosphonate **97** was added to the mixture and the reaction was monitored by ^{31}P NMR spectroscopy. An observable upfield shift was noted for formation of the silyl ester followed by a distinct downfield shift after addition of sodium hydroxide. Upon standard workup and isolation

of the product, it appeared that there was still 2,4,6,-collidine in the product. The residue was subjected to extensive washing with methylene chloride and heating under reduced pressure to remove the residual 2,4,6,-collidine (bp = 171 °C), but the intensity of the peaks that correlated to the 2,4,6-collidine did not change. After a closer investigation, it was determined that the salt was actually a pentasodium mono collidinium salt (Figure 62). A comparison of the ^{1}H and ^{13}C NMR shifts of 2,4,6-collidine in D_2O to the isolated trisphosphonate product exhibit several differences. Moreover similar mixed phosphonate salts have been reported.[97] The ^{1}H NMR shifts of these mixed phosphonate salts are consistent with the trisphosphonate salt synthesized. Attempts to hydrolyze the phosphonate ester using only trimethylsilyl bromide were unsuccessful, even under forcing conditions, leading to the determination that 2,4,6-collidine is necessary for this transformation.



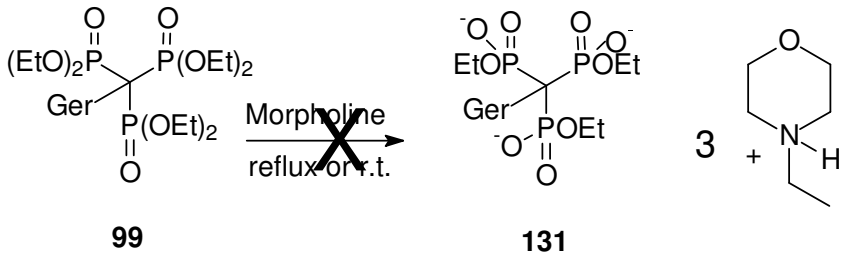
97 **130**

Figure 62. Synthesis of a mixed salt of allyltrisphosphonate (**130**).

Tetraethylbisphosphonates also have been converted to the di-salts using morpholine. Morpholine undergoes *N*-alkylation by one of the ethyl esters of each of the phosphonate groups. In a trisphosphonate this process would only occur once for each of the phosphonate esters resulting in a mixed alkyl trisphosphonate ester and mixed

morpholinium salt for each phosphonate.[98] A process such as this could potentially be utilized in the synthesis of a tri-salt from the trisphosphonate esters.

This methodology was examined briefly in an attempt to produce a tri-salt of geranyl trisphosphonate. Geranyltrisphosphonate (**99**) was dissolved in morpholine and heated at reflux overnight. A ^{31}P NMR analysis of the resulting reaction mixture revealed multiple peaks, leading to the conclusion that the reaction conditions caused significant decomposition (Figure 63). Attempts to perform this reaction at room temperature also were unsuccessful, leading to no change in the starting material even after one week.



99 **131**

Figure 63. Attempted synthesis of a mixed salt of geranyltrisphosphonate (**131**).

Because the trisphosphonate group is so highly stabilized it may be considered a good leaving group. Under forcing conditions it is possible that the morpholine is preferentially attacking at the β-carbon causing the trisphosphonate to act as a leaving group. A possible change in the nucleophilic agent or reaction conditions may more easily facilitate the desired transformation.

CHAPTER V

INVESTIGATIONS OF BISPHOSPHONATE MONOPHOSPHATES

During the course of the synthesis of the parent trisphosphonate **75** an unusual product was detected in moderate yields in the ^{31}P NMR spectrum (Figure 64). This spectrum exhibited obvious formation of compound **75** at ~14 ppm as well as the starting bisphosphonate for this transformation at 19.5 ppm. However, it also revealed another set of peaks that was unexpected. There was a noticeable doublet at 13.4 ppm with a coupling of 13.9 Hz as well as a triplet at -1.3 ppm that has a coupling of 13.9 Hz (Figure 64). The doublet occurs in the same region as the resonance for the trisphosphonate 97 while the triplet was in the region suggestive of a phosphate. These signals led to the hypotheses that an oxidized product may have formed, one in which two phosphonate and one phosphate groups are attached to the central carbon atom (**132**). After workup, the unexpected product was analyzed using high resolution mass spectrometry and the observed molecular ion was consistent with the hypothesized product.

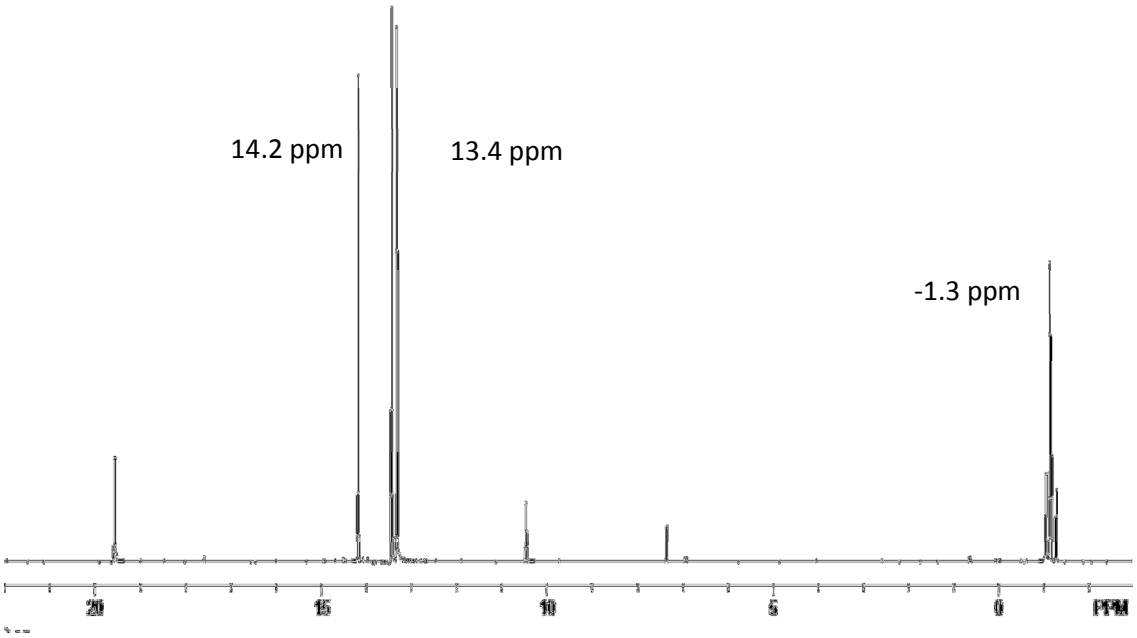


Figure 64. ^{31}P NMR spectrum of the trisphosphonate reaction mixture

There have been only two previous reports of this type of phosphorus-containing species and the 31P NMR analysis compares well with the reported compounds.76,99 The in situ formation of this type of compound from a phosphonylation and subsequent oxidation is unprecedented and the bisphosphonate phosphate moiety may be an interesting scaffold to elaborate.

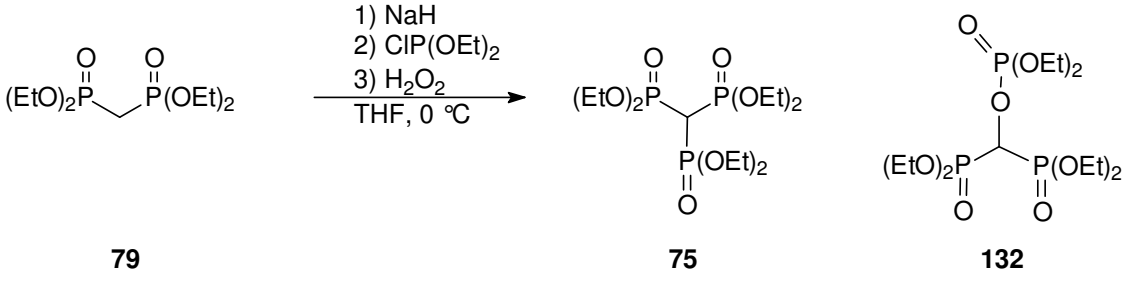


Figure 65. Synthesis of trisphosphonate **75** and bisphosphonate phosphate **132**.

In an attempt to understand the formation of this unusual product a series of experiments was conducted. The first reactions involved the possibility of a reaction where tetraethyl methylenebisphosphonate undergoes oxidation and the newly formed intermediate is subsequently phosphorylated. The prospect of this occurring as the result of an oxidative insertion similar to the Bayer-Villager reaction[100] was investigated. Treatment of tetraethyl methylenebisphosphonate with *m*-CPBA did not result in any detectable formation of this oxidized product and only starting material was observed in the ^{31}P NMR spectrum.

The possibility that compound **132** may result through formation of peroxide radicals also was examined. The radical-initiating reagent AIBN was added to a solution of hydrogen peroxide and tetraethyl methylenebisphosphonate, and the reaction progress was monitored by ^{31}P NMR. Even after several hours the tetraethyl methylenebisphosphonate starting material was the only species observed in the ^{31}P NMR spectrum. The possibility that hydrogen peroxide may oxidize tetraethyl methylenebisphosphonate to provide an α-hydroxylated species that could undergo phosphorylation was examined as well (Figure 66). However treatment of compound **79** with hydrogen peroxide resulted in no noticeable change in the ^{13}P NMR spectrum of the reaction mixture.

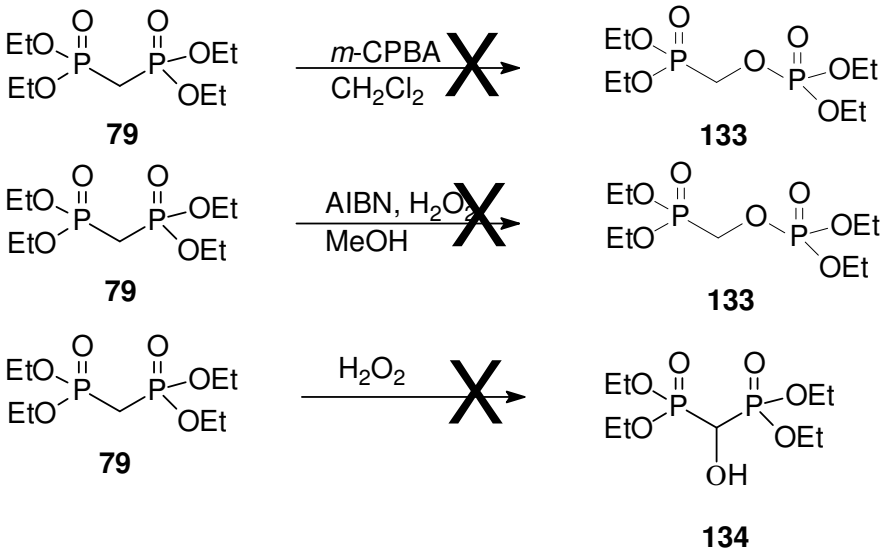


Figure 66. Attempts to form oxidized reaction intermediates.

Next a one-flask phosphonylation procedure was examined starting with the α-hydroxy phosphonate **135**. Treatment of compound **135** with excess strong base followed by addition of diethylchlorophosphate did not result in any obvious formation of the target compound according to ^{31}P NMR analysis, nor did a process paralleling the formation of trisphosphonate **75** using diethyl chlorophosphite followed by oxidation with hydrogen peroxide (Figure 67).

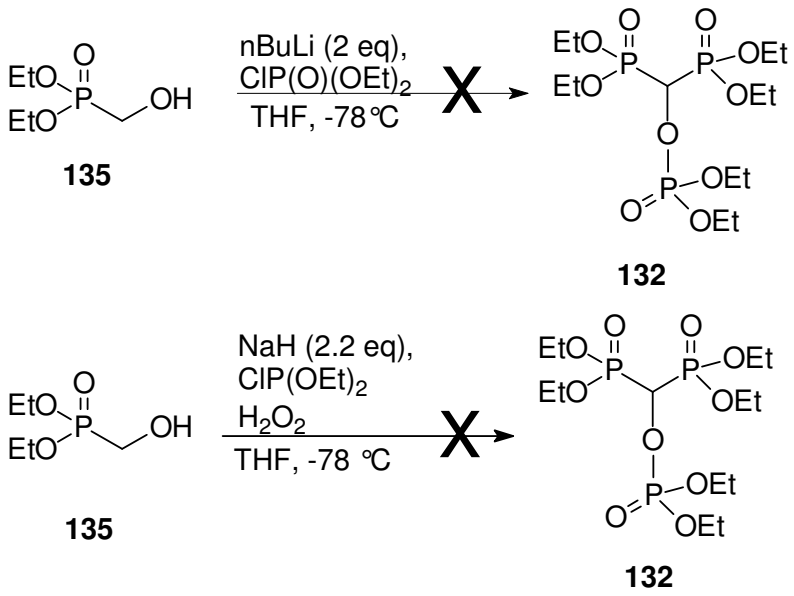


Figure 67. Attempts to form bisphosphonate phosphate **132** using a one-flask protocol.

The methodology used to make alkyl trisphosphonates was tested to make alkyl bisphosphonate phosphates using the phenyl phosphonate phosphate **136** previously prepared in house.[34] Reaction conditions using different bases and additives to attempt the preparation of compound **137** were unsuccessful in this case (Figure 68).

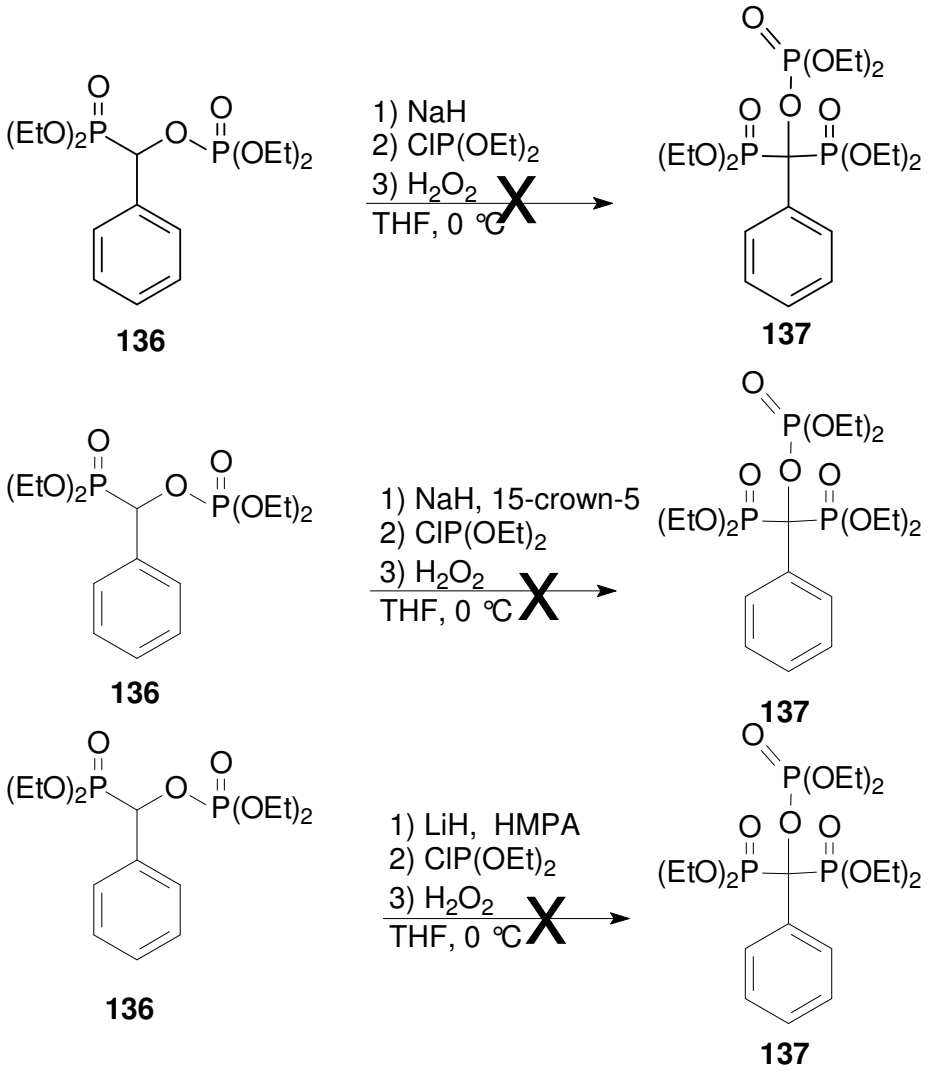


Figure 68. Attempts to synthesize an alkylbisphosphonate phosphate product **137**.

Ultimately the phenyl compound **136** may be a poor model to test these procedures. For example, in the attempted synthesis of the phenyltrisphosphonate **104** using phenyl bisphosphonate **103** there was no reaction product observed (Figure 44). It was hypothesized that conjugation with the aromatic ring may contribute to the stability of the anion intermediate leaving it insufficiently reactive. Similarly the phenyl ring may contribute to the stability of the anionic intermediate derived by deprotonation of

compound **136**, making it less reactive. The use of a phosphonate phosphate with a different alkyl substituent may be more productive in testing these reaction conditions.

Because of the limited success of phosphonylation on compound **136**, a reinvestigation of how the original bisphosphonate monophosphate **132** was formed was commenced. Treatment of phosphonate **135** with base and diethyl chlorophosphate resulted in the desired phosphate monophosphonate **133**. There was an obvious doublet of doublets in the ^{31}P NMR spectrum which matched the literature data.[101] Unfortunately, addition of base to this intermediate followed by diethyl chlorophosphite and hydrogen peroxide did not result in the desired product **132** (Figure 69) according to the ^{31}P NMR spectrum.

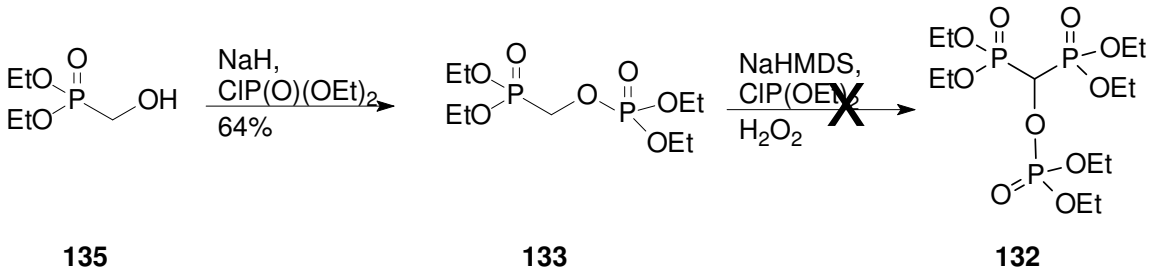


Figure 69. Attempts to form phosphonate **132** via phosphonate phosphate **133**.

Attempted oxidation of the trisphosphonate **75** with hydrogen peroxide did not form the desired compound **132**. There was no observable change in the ^{31}P NMR spectrum of the starting material when compared to the reaction mixture. A re-examination of the conditions in the original reaction showed that an excess of sodium hydride had been used. It has been shown that water in the starting material may complicate reaction conditions and it was hypothesized that the use of excess base in the

presence of starting material that has not been thoroughly dried might have resulted in the formation of the compound **132** in the original reaction.

This hypothesis was tested by addition of excess base (~5 eq) to compound **79** that had not been dried, followed by addition of diethylchlorophosphite and oxidation with hydrogen peroxide (Figure 70). According to the ^{31}P NMR spectrum of the reaction mixture these reactions conditions did result in formation of the bisphosphonate phosphate product **132**. This reaction was reproducible and the reaction conditions could be related to those used when compound **132** was first observed. Attempted isolation of the phosphonate phosphate **132** by distillation was unsuccessful and ultimately full purification may require extensive chromatography. Isolation of this target may represent an important step forward in the formation of alkyl bisphosphonate monophosphates, especially if it could be extended to alkylated bisphosphonate substrates.

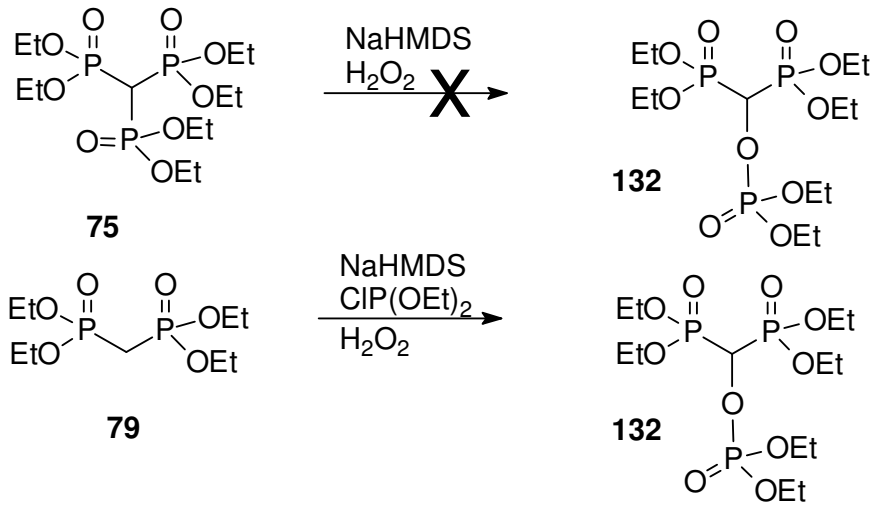


Figure 70. Synthesis of the bisphosphonate phosphate product **132**.

CHAPTER VI

SUMMARY AND FUTURE DIRECTIONS

The tetrasodium salt bisphosphonate **25** was identified as an inhibitor of the enzyme SQS though *in vitro* enzyme assays. Compound **25** originally was synthesized through a copper-mediated displacement of the THP-protected alcohol of 9-hydroxygeranylbisphosphonate (**29**). Attempts to utilize this method to re-synthesize compound **30** along with other analogues (**42** and **43**) were unsuccessful, possibly due to formation of a product resulting from either an Ullman coupled or Wurtz-Fittig homo-coupling. Difficulties encountered with the original method led to a new synthetic strategy in which compound **25** along with the analogues (**23** and **24**) were prepared through formation of the desired alkyl chains followed by alkylation of tetraethyl methylenebisphosphonate. The bisphosphonate analogues **23** and **24** also show inhibitory activity against SQS, with compound **24** having the greatest impact. It was predicted that the addition of the biphenyl moiety would aid in the selectivity for SQS. Treatment of HepG2 cells with **25** alone dramatically decreased cholesterol levels and co-treatment with both compound **25** and lovastatin (**18**) decreased cholesterol levels to an even greater extent while reducing the downstream impact on the biosynthesis of other isoprenoids relative to treatment with lovastatin alone. Likewise, co-treatment with the FDPS inhibitor zolendronate and compound **25** had similar effects. Ultimately the dual inhibition of both HMGCR (lovastatin) and SQS (**25**), or use of a FDPS inhibitor along with an SQS inhibitor, could provide a means of reducing cholesterol while decreasing off-target effects that are the result of depletion of nonsterol isoprenoids.

In addition to the bisphosphonates synthesized as SQS inhibitors a series of stilbenebisphosphonates (**45**, **46**, and **47**) were prepared as potential inhibitors of FDDPS or GGDPS. Despite the limited activity found for these compounds as inhibitors of these two enzymes, a screen against decaprenyl diphosphate synthase revealed the modest activity of the mono-stilbenebisphosphonate **46**. An electron-withdrawing nitro analogue **59** was synthesized and was found to have enhanced inhibitory activity for inhibition of decaprenyl diphosphate synthase. The synthesis of other electron-withdrawing stilbenebisphosphonate analogues was attempted multiple times, but full purification and characterization of the final targets was not achieved. The inability to separate these compounds from their reaction byproducts may be alleviated by utilizing an alternate synthetic strategy as shown in Figure 71. Synthesis of a stilbene aldehyde could be achieved through Horner-Wadsworth-Emmons olefination and the alcohol could be oxidized to the requiste aldehyde (**139** and **140**). With the aldehyde in hand, a Lewis acid-mediated Michaelis-Arbuzov reaction could provide a homologue of the desired bisphosphonates (Figure 71).[30] This procedure has been shown to be compatible with the synthesis of bisphosphonates from benzaldehydes and α-hydroxy phosphonates from heteroarylmethyl alcohols at room temperature but has not been demonstrated in the formation of allylic or benzylic bisphosphonates from homo-allylic or homo-benzylic aldehydes.[31] Alternatively the aldehyde precursors **144** and **145** could be synthesized and Lewis acid mediated Michaelis-Arbuzov reaction could be attempted to produce the desired bisphosphonates **70** and **73**. Not only would this process allow for the formation of the desired bisphosphonates, but it may also expand on the methodology that has been established to synthesize bisphosphonates.

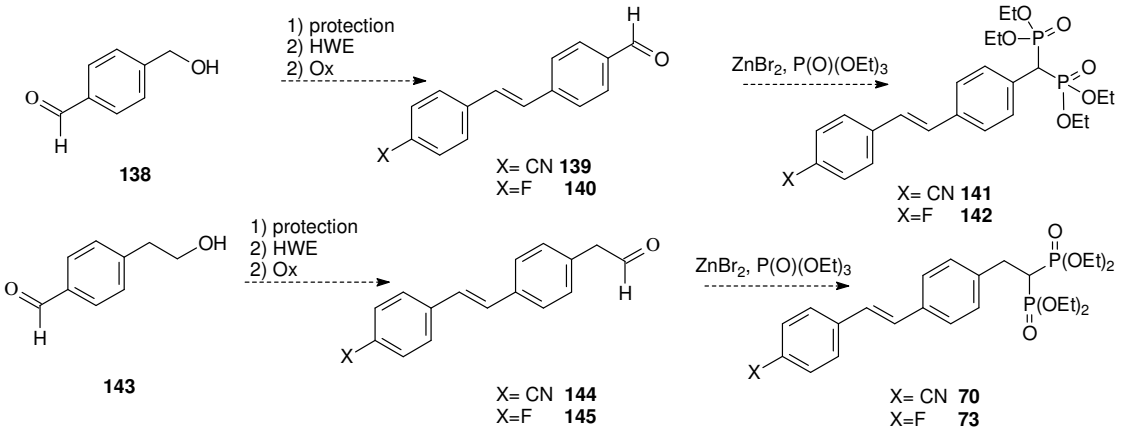


Figure 71. Alternate synthetic strategy for the synthesis of stilbene bisphosphonates.

A new class of compounds designated as alkyl-1,1,1-trisphosphonates has been established using a general strategy for their preparation from the corresponding alkyl-1,1-bisphosphonates through phosphinylation and oxidation with hydrogen peroxide. The methodology used to synthesize alkyl-1,1,1-trisphosphonates also has been applied to the parent compound **75** resulting in a higher yield than in the original report.[76] It has been determined through titration that the anion of compound **75** is highly stabilized, and should be viewed as a strong carbon acid. The trisphosphonate functionality was found to be stable to a variety of reaction conditions allowing for a number of modifications of the alkyl group (Figure 72).

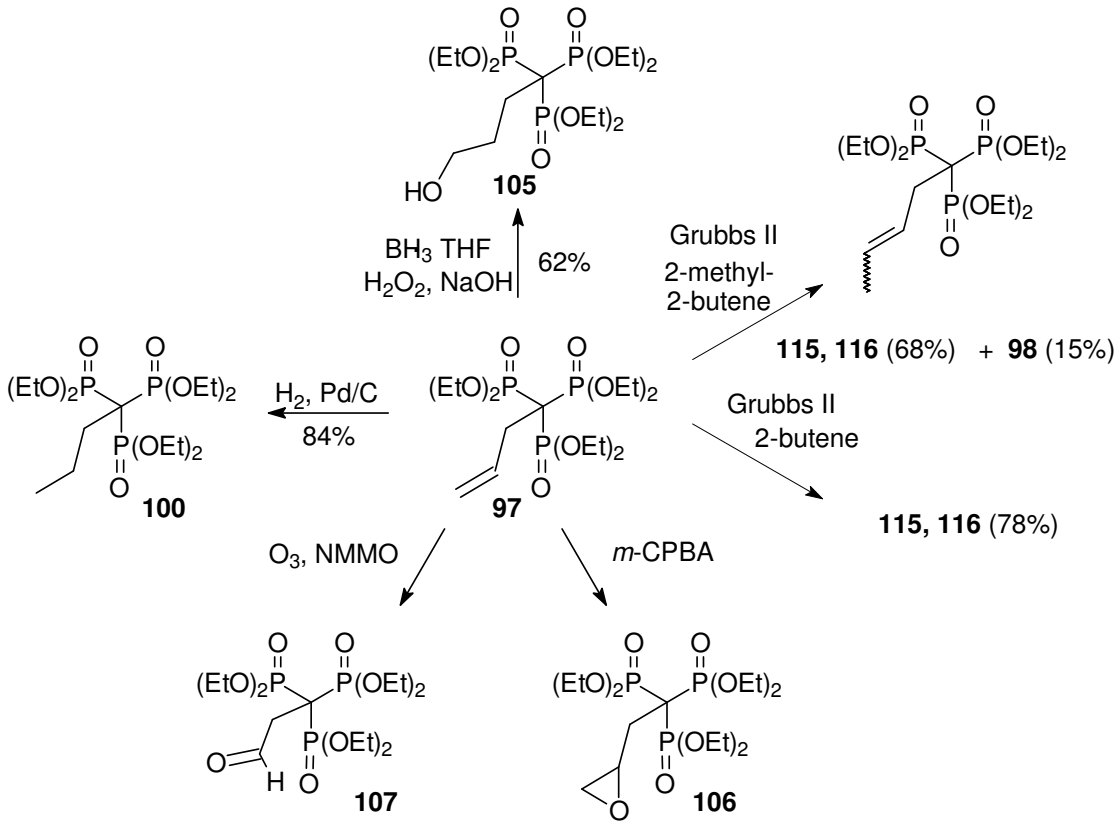


Figure 72. Reactivity of the allyltrisphosphonate **97**.

As long as steric factors from the bulky trisphosphonate group are considered, alkyl-1,1,1-trisphosphonates can undergo a variety of functional group transformations. Furthermore, steric factors already have led to an interesting variation on the Grubbs metathesis where a disubstituted olefin was observed as the major product from metathesis with 2-methyl-2-butene rather than the expected trisubstituted alkene.[46] It also was discovered that alkyl-1,1,1-trisphosphonates are sensitive to some oxidative conditions and attempts to synthesize an aldehyde analogue of **107** through ozonolyisis were not successful. Despite the limited success with oxidation of allyltriphosphonate **97** to aldehydes, there may be an opportunity to synthesize similar aldehydes from the

proparglytrisphosphonate **101**. Acetylenes are known to undergo hydroboration reactions resulting in the enol,[102,103] which can then tautomerize to give the desired aldehyde **146** (Figure 73). It has been demonstrated that the trisphosphonate functionality is stable to hydroboration and thus this may be a viable method to obtain trisphosphonate aldehydes. Likewise treatment of propargyltrisphosphonate **101** with base could facilitate the formation of an anion at the terminal end of the acetylene. Nucleophilic attack of this anion on DMF could yield an aldehyde (**147**), which could be subjected to different reduction conditions to provide either the saturated aldehyde **148** or the α,β-unsaturated aldehyde **149** (Figure 73).

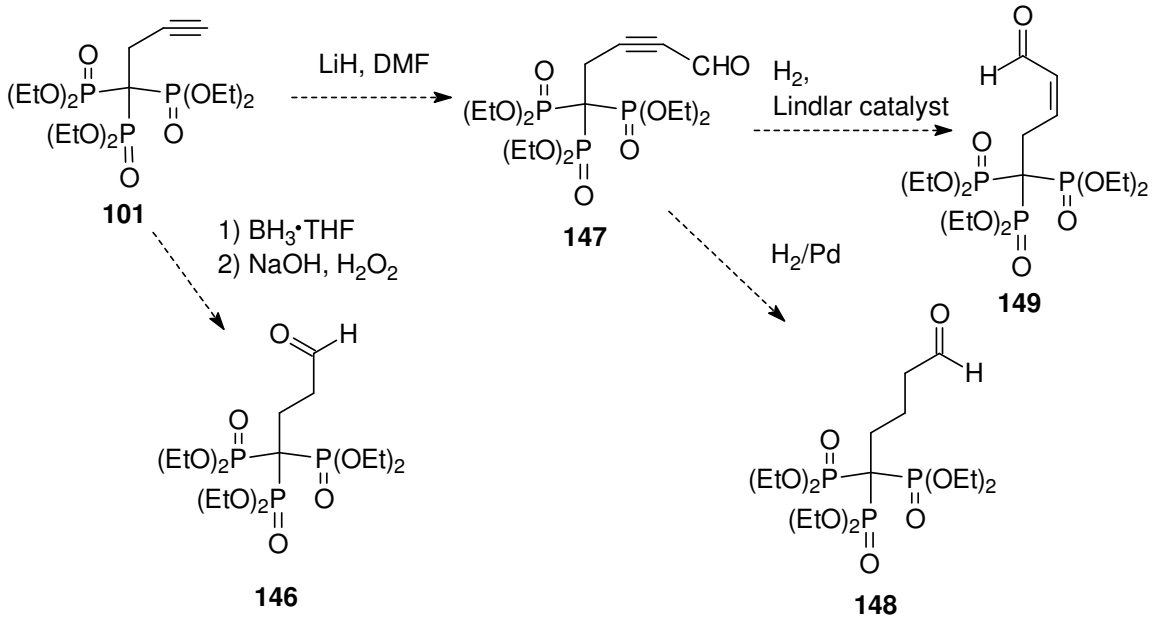


Figure 73. Strategies to synthesize aldehyde trisphosphonates.

The ability of an acetylene trisphosphonate **101** to undergo click chemistry suggests that libraries of trisphosphonates should be readily available. Examples of some

interesting commercially available azides that could be used in a click reaction with a trisphosphonate are shown in Chapter 4.

Although the hexa-salt of a trisphosphonate was prepared, the tri-salts of these species remain elusive. Attempts to form the tri-morpholinium salt of a trisphosphonate through nucleophilic attack of morpholine resulted in decomposition of one starting material, compound **99**, and attempts to perform this transformation at room temperature resulted in no reaction. Eventually formation of the tri-salt may be achieved by reaction with an alternative nucleophilic agent (Figure 74) under reaction conditions which may be considered as more mild. Selective symmetrical cleavage of tetraethyl bisphosphonate esters has been realized using piperidine, *N*-methylpiperazine, and *N*-ethylpiperazine but these conditions have not been investigated on trisphosphonates.[98] It may also be prudent to consider the use of pyrrolidine for this transformation as well.

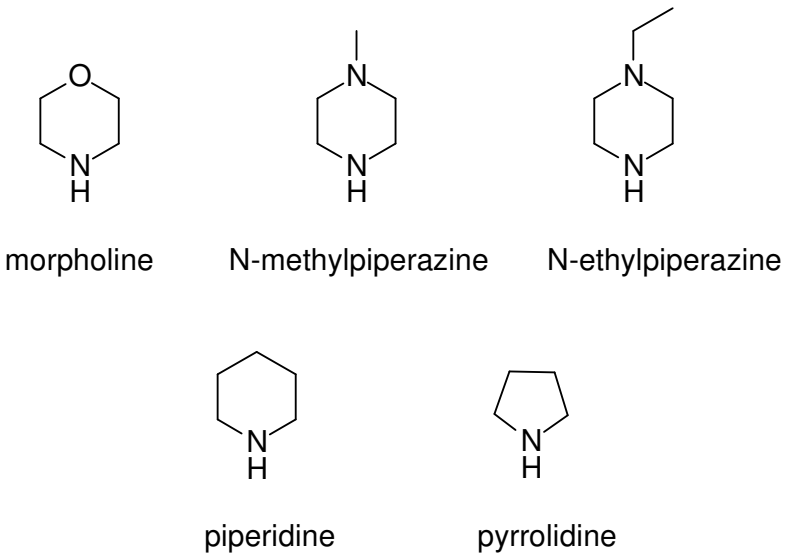


Figure 74. Alternative nucleophiles for cleavage of phosphonate esters.

An interesting bisphosphonate phosphate (**132**) was discovered as a byproduct of the formation of the parent trisphosphonate. Reaction conditions that lead to the formation of this species have been clarified, which may yield a new scaffold for the synthesis of a class of alkylbisphosphonate phosphate compounds. There have been few reports on these types of compounds and there are no literature examples of their alkyl counterparts.[76, 99] Formation of the anion from this product may result in a species that can undergo alkylation. Unlike the anion derived from trisphosphonate **75**, an anion from compound **132** would be expected to be less stabilized and more reactive. If a direct alkylation approach is unsuccessful, then these types of compounds might be produced in a manner similar to the alkyl trisphosphonates through formation of an anion followed by phosphinylation and oxidation.

There is abundant literature available on the O-P rearrangement of α-hydroxy bisphosphonates resulting in the formation of phosphate phosphonates.[34, 35] There have been no reports on the hydrolysis of these esters and their potential medicinal properties. As a result, this rearrangement is often viewed as an inconvenience and an undesired byproduct in the formation of α-hydroxy bisphosphonates. The ability to form of O-P rearranged α-hydroxy bisphosphonates may also represent an opportunity. Treatment of an alkyl phosphate phosphonate with strong base could facilitate formation of an anion which could then undergo phosphonylation and oxidation to yield a new class of bisphosphonate phosphate compounds (Figure 75). Exploration of this strategy would continue the tradition of developing new organo-phosphorus compounds in the Wiemer research group, a tradition which has been encouraged through preparation of the

biologically active bisphosphonates and the chemically intriguing trisphosphonates reported here.

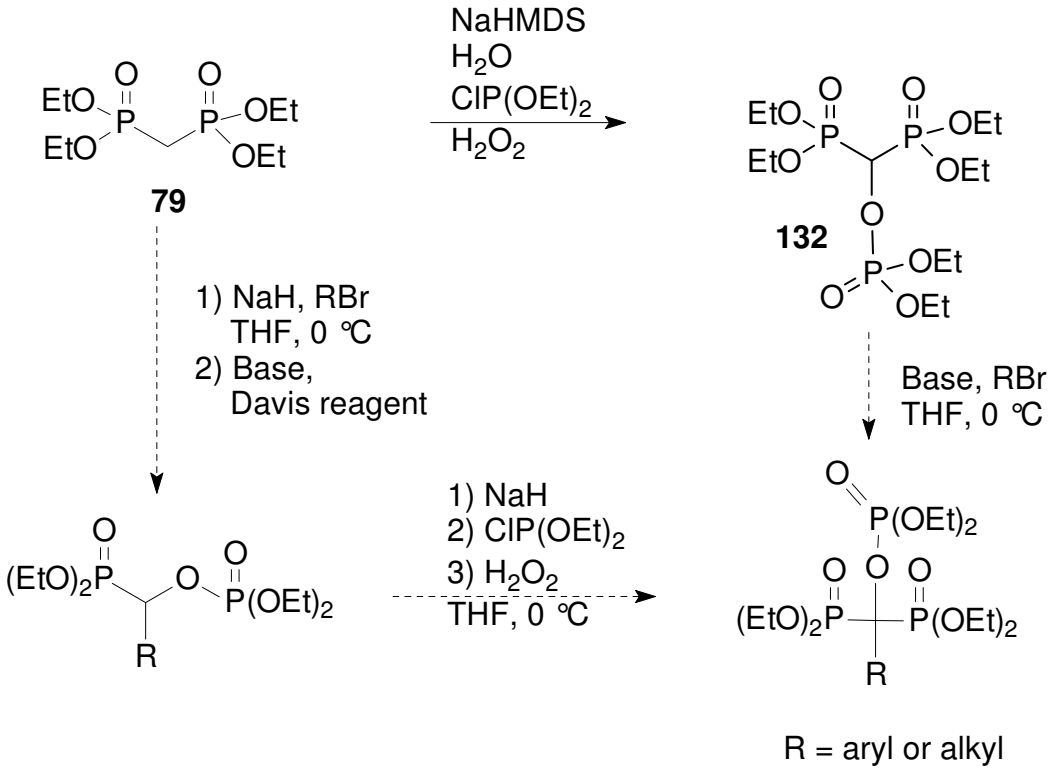


Figure 75. Possible routes for the synthesis of alkylbisphosphonate phosphates.

CHAPTER VII

EXPERIMENTAL PROCEEDURES

General Experimental Conditions

Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium and benzophenone immediately prior to use. All non-aqueous reactions were performed with either oven-dried or flame-dried glassware under an argon atmosphere. Flash chromatography was performed using silica gel with an average particle size of 40-63 μm. The ^{1}H NMR spectra were recorded on either a 300 MHz (75 MHz for ^{13}C), 400 MHz, or 500 MHz Bruker NMR spectrometer with $CDCl_3$ as solvent and $(CH_3)_4Si$ as internal standard unless otherwise noted. The NMR spectra that were recorded in D_2O used residual H_2O (4.80 ppm) as an internal reference standard. For ^{13}C NMR spectra recorded in D_2O, 1,4-dioxane (67.0 ppm) was added as an internal reference. Chemical shifts of ^{31}P NMR spectra are reported in ppm relative to H_3PO_4 (external standard). High resolution mass spectra (HRMS) were obtained at the University of Iowa Mass Spectrometry Facility. Mass analysis was performed with a quadrupole time of flight hybrid mass spectrometer with the capacity for positive and negative ionization modes. Electrospray ionization was employed with acetonitrile or aqueous solutions. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA).

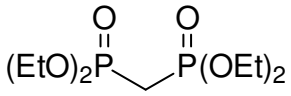
General Procedure for the Preparation of Bisphosphonate Tetrasodium Salts.

After 2,4,6-collidine (10 eq) was dissolved in CH_2Cl_2 and added to a reaction flask, TMSBr (12 eq) was added to the reaction mixture. The flask was equipped with and oil bubbler and placed into an ice bath then allowed to stir. After 20 min, the

requisite bisphosphonate ester was added to the flask and the reaction was allowed to stir for 12 h. The reaction was diluted with toluene, and the solvent was removed *in vacuo*. After aqueous sodium hydroxide (5 eq) was added to the flask, the mixture was allowed to stir overnight. The reaction was then lyophilized, dissolved in a minimum amount of water, and then added to cold acetone and the mixture was stored at 4 °C overnight. The precipitate was removed by filtration and washed with cold acetone. The filtrate was discarded. The residue was dissolved in hot water and filtered. The filtrate was retained and lyophilized to yield a floculant residue.

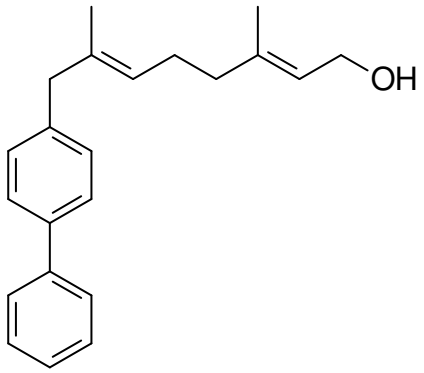
General Procedure for the Alkylation of Tetraethyl Methylenebisphosphonate with Allylic or Benzylic Bromides.

Tetraethyl methylenebisphosphonate (1 eq) was dissolved in THF and placed into a reaction vessel and cooled to 0 °C, and either KHMDS (0.5 M in toluene, 1 eq) or NaH (1 eq), was added. The mixture was allowed to stir under argon for 40 min after which the vessel was equipped with a pressure equalized dropping funnel loaded with the desired allylic bromide (1.1 eq) dissolved in a minimum amount of THF. The allylic bromide solution was added slowly to the reaction mixture. After the reaction mixture was allowed to stir overnight and warm to room temperature, the reaction was quenched by addition of saturated NH_4Cl and extracted with Et_2O. The organic portions were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo*, and the resulting residue was purified via flash chromatography.



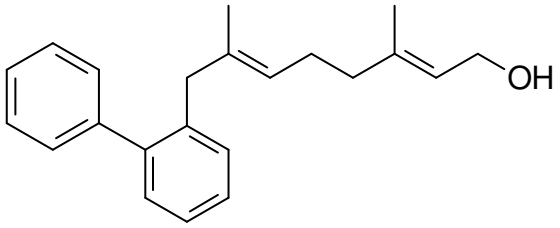
Tetraethyl methylenebisphosphonate (79). [104]

Sodium metal (6.19 g, 27.2 mmol) was slowly added to a reaction vessel containing ethanol (90 mL) that had been cooled to 0 °C. After the solid sodium metal was no longer visible, diethyl phosphite (37.6 g, 27.2 mmol) was added and the mixture was allowed to stir at room temperature overnight. After the solvent was removed *in vacuo*, the resulting residue was dissolved in CH_2Cl_2 (300 mL) and allowed to stir at room temperature. Reaction progress was periodically monitored by ^{31}P NMR spectroscopy. After 2 days the reaction was washed with water and the organic and aqueous phases were separated. The aqueous portion was extracted with CH_2Cl_2. The organic portions were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The resulting clear liquid was purified via distillation (0.1 torr, 140 °C). The desired product was isolated as a clear oil (39.2 g, 53%). Comparison of the ^{31}P, ^{1}H, and ^{13}C NMR data for the sample matched the reported values.



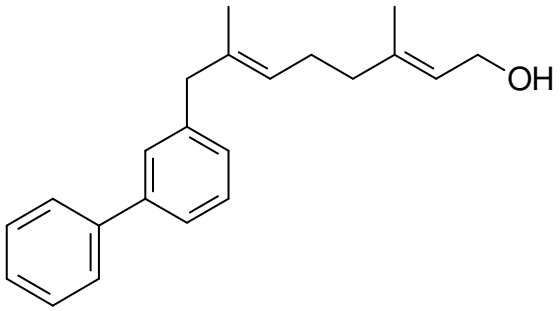
(2*E*,6*E*)-2,6-Octadien-1-ol, 3,7-dimethyl-8-[1,1'-biphenyl]-4-yl (38).

According to the general procedure for Grignard displacement of a THP ether, 4-bromobiphenyl (6.44 g, 27.5 mmol) was treated with Mg (1.00 g, 41.2 mmol), CuI (785 mg, 4.13 mmol) and (2*E*,6*E*)-3-methyl-7-((tetrahydro-2H-pyran-2-yloxy)methyl)octa-2,6-dien-1-ol (611 mg, 2.75 mmol). After standard workup, the target compound was purified via flash chromatography (silica gel, 20% EtOAc in hexanes), and the desired product was isolated as a clear oil (468 mg, 64% yield): ^{1}H NMR δ 7.60–7.57 (m, 2H), 7.53–7.49 (m, 2H), 7.46–7.39 (m, 2H), 7.35–7.30 (m, 1H), 7.29–7.22 (m, 2H), 5.43 (dt, *J* = 6.9 Hz, 1H), 5.27 (t, *J* = 6.3 Hz, 1H), 4.16 (d, *J* = 6.9 Hz, 2H), 3.31 (s, 2H), 2.22–2.05 (m, 4H), 1.66 (s, 3H), 1.54 (s, 3H), 1.33 (bs, 1H); ^{13}C NMR δ 141.0, 139.6, 139.5, 138.9, 134.7, 129.2 (2C), 128.7 (2C), 127.0 (4C), 126.9, 126.0, 123.5, 59.4, 45.8, 39.4, 26.2, 16.2, 15.9; HRMS calcd for $C_{22}H_{26}O$ $(M+H)^+$, 307.2017, found 307.2014.



(2*E*,6*E*)-2,6-Octadien-1-ol, 3,7-dimethyl-8-[1,1'-Biphenyl]-2-yl (36).

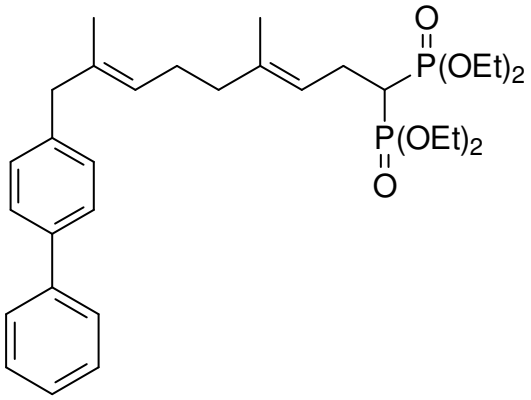
According to the general procedure for Grignard displacement of a THP ether, 2-bromobiphenyl (1.00 g, 4.29 mmol) was treated with Mg (302 mg, 10.7 mmol), CuI (612 mg, 3.21 mmol), and (2*E*,6*E*)-3-methyl-7-((tetrahydro-2H-pyran-2-yloxy)methyl)octa-2,6-dien-1-ol (546 mg, 2.14 mmol). After standard workup and purification via flash chromatograpy (silica gel, 20% EtOAc in hexanes) the desired product was isolated as an oil in 87% yield (380 mg): ^{1}H NMR δ 7.40–7.21 (m, 9H), 5.37 (t, *J* = 6.9 Hz, 1H), 4.93 (t, *J* = 6.6 Hz, 1H), 4.11 (d, *J* = 7.2 Hz, 2H), 3.29 (s, 2H), 2.13–1.96 (m, 4H), 1.64 (s, 3H), 1.48 (bs, 1H), 1.45 (s, 3H); ^{13}C NMR δ 142.2, 141.7, 139.3, 137.2, 134.8, 129.9, 129.8, 129.1 (2C), 127.8, (2C) 127.0, 126.7, 125.9, 125.8, 123.4, 59.2, 42.6, 39.3, 26.1, 16.2, 16.1; HRMS calcd for $C_{22}H_{26}O$ $(M+H)^+$, 307.2017, found 307.2018.



(2*E*,6*E*)-2,6-Octadien-1-ol, 3,7-dimethyl-8-[1,1'-Biphenyl]-3-yl (37).

According to the general procedure for Grignard displacement of a THP ether, 3-bromobiphenyl (1.71 g, 7.34 mmol), was treated with Mg (208 mg, 8.56 mmol), CuI (698 mg, 3.67 mmol) and (2*E*,6*E*)-3-methyl-7-((tetrahydro-2H-pyran-2-yloxy)methyl)octa-

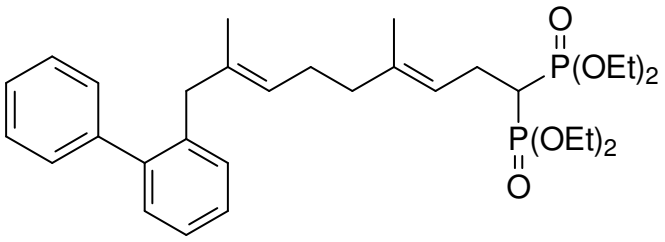
2,6-dien-1-ol (621 mg, 2.44 mmol). After standard workup and purification via flash chromatography (silica gel, 20% EtOAc in hexanes), the desired product was isolated as an oil (408 mg, 55%): ^{1}H NMR δ 7.61–7.58, (m, 2H), 7.46–7.31(m, 6H) 7.21–7.14 (m, 1H), 5.42 (t, *J* = 6.8 Hz, 1H), 5.27 (t, *J* = 6.8 Hz, 1H), 4.12 (d, *J* = 6.6 Hz, 2H), 3.34 (s, 2H), 2.19–2.04 (m, 4H), 1.68 (s, 3H) 1.57 (s, 3H) 1.15 (bs, 1H); ^{13}C NMR δ 141.3, 141.1, 140.8, 139.5, 134.7, 134.8, 128.7 (2C), 128.6, 127.8 (2C), 127.7, 127.1, 126.0, 124.8, 123.5, 59.3, 46.2, 39.4, 26.2, 16.2, 15.9; HRMS calcd for $C_{22}H_{26}O$ $(M+H)^{+}$, 307.2017, found 307.2015.



Tetraethyl 4,8-dimethyl-3,7-nonadienyl-9-[1,1'-biphenyl]-4-yl-1,1-bisphosphonate (30)[57]

Alcohol **38** (468 mg, 1.53 mmol) was dissolved in Et_2O (5 mL) and placed into an ice bath. To the solution PBr_3 (0.16 mL, 1.68 mmol) was added. The mixture was allowed to stir for 3 h after which it was poured into ice water. The organic and the aqueous layers were separated. The aqueous portion was extracted with Et_2O. The organic extracts were combined, dried ($NaSO_4$), filtered through celite and basic alumina, and concentrated *in vacuo*. The resulting oil was used without further purification in the subsequent step.

According to the general procedure, tetraethyl methylenebisphosphonate (330 mg, 1.15 mmol) was treated with KHMDS (2.3 mL, 1.15 mmol) and the corresponding allylic bromide (423 mg, 1.15 mmol). After standard workup and purification by flash chromatography (silica gel, 10% EtOH in hexanes), the desired product was isolated in 14% yield (90 mg). Both ^{31}P and ^{1}H NMR data were consistent with previously reported values.

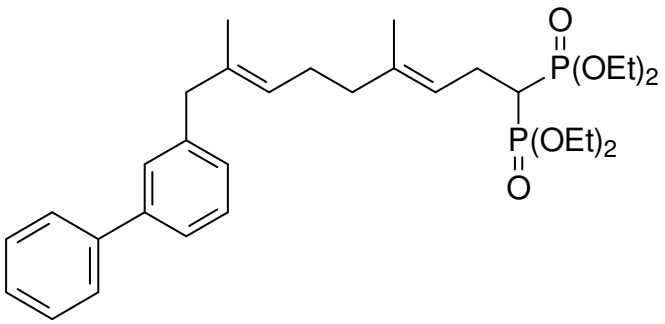


Tetraethyl 4,8-dimethyl-3,7-nonadienyl-9-[1,1'-biphenyl]-2-yl-1,1-bisphosphonate (42).

Alcohol **36** (379 mg, 1.24 mmol) was dissolved in Et_2O (5 mL) and placed into an ice bath. To the solution PBr_3 (0.13 mL, 1.36 mmol) was added. The mixture was allowed to stir for 3 h after which it was poured into ice water. The organic and the aqueous layers were separated, and the aqueous portion was extracted with Et_2O. The organic extracts were combined, dried ($NaSO_4$), filtered through celite and basic alumina, and concentrated *in vacuo*. The resulting oil was used without further purification in the subsequent step.

According to the general procedure tetraethyl methylenebisphosphonate (403 mg, 1.36 mmol) was treated with KHMDS (2.7 mL, 1.36 mmol) and allylic bromide **14** (337 mg, 1.24 mmol). After standard workup and purification by flash chromatography (silica gel, 10% EtOH in hexanes), the desired product was isolated in 20% yield (162 mg): ^{1}H NMR δ 7.38–7.23 (m, 9H), 5.29 (t, J = 7.2 Hz, 1H), 4.94 (t, J = 6.6 Hz, 1H), 4.21–4.12

(m, 8H), 3.23 (s, 2H), 2.71–2.55 (m, 2H), 2.30 (tt, J_{HP} = 24.0 Hz, J = 6.0 Hz, 1H) 2.08–1.92 (m, 4H), 1.62 (s, 3H), 1.44 (s, 3H), 1.36–1.29 (m, 12H); ^{13}C NMR δ 142.3, 141.7, 137.3, 136.7, 134.6, 129.9 (2C), 129.8 (2C), 129.2, 127.9, 127.1 126.7, 126.3, 125.8, 121.8, 62.4 (t, J_{CP} = 7.3 Hz, 4C), 42.7, 39.6, 37.4 (t, J_{CP} = 133 Hz), 26.5, 24.0, 16.4 (d, J_{CP} = 6.5 Hz, 4C), 16.2, 16.0; ^{31}P NMR +23.7 ppm. Anal. Calcd for $C_{31}H_{46}O_6P_2 \cdot 0.5\ H_2O$: C, 63.58; H, 8.09 Found C, 63.61; H, 8.05.

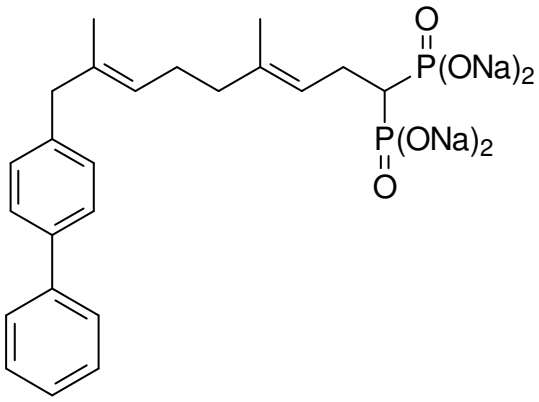


Tetraethyl 4,8-dimethyl-3,7-nonadienyl-9-[1,1'-biphenyl]-3-yl-1,1-bisphosphonate (43).

Alcohol **37** (408 mg, 1.33 mmol) was dissolved in Et_2O (5 mL), placed into an ice bath and PBr_3 (0.14 mL, 1.47 mmol) was added via syringe. The mixture was allowed to stir for 3 h after which it was poured into ice water. The organic and the aqueous layers were separated. The aqueous portion was extracted with Et_2O. The organic extracts were combined, dried ($NaSO_4$), filtered through celite and basic alumina, and concentrated *in vacuo*. The resulting oil was used without further purification in the subsequent step.

According to the general procedure tetraethyl methylenebisphosphonate, (3 eq, 403 mg, 1.36 mmol) was treated with KHMDS (2.7 mL, 1.36 mmol) and allylic bromide **15** (337 mg, 1.24 mmol). After standard workup and purification by flash

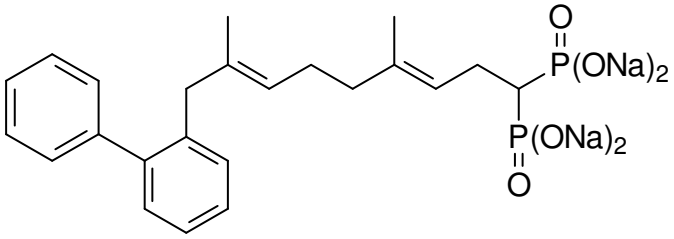
chromatography (silica gel, 10% EtOH in hexanes), the desired target was isolated in 84% yield (169 mg): ^{1}H NMR δ 7.61–7.58 (m, 2H), 7.46–7.39 (m, 6H), 7.17–7.14 (m, 1H), 5.34 (t, J = 6.9 Hz, 1H), 5.29 (t, J = 6.6 Hz, 1H), 4.22–4.13 (m, 8H), 3.34 (s, 2H), 2.73–2.57(m, 2H), 2.32 (tt, J_{HP} = 24.0 Hz, J = 6.0 Hz, 1H), 2.18–2.02 (m, 4H), 1.66 (s, 3H), 1.56 (s, 3H), 1.36–1.31(m, 12H); ^{13}C NMR δ 141.4, 141.1, 140.9, 136.6, 134.3, 128.7, 128.6, 127.7 (2C), 127.1 (2C) 126.4, 124.8, 122.1, 122.0, 121.9, 62.4 (t, J_{CP} = 7.3 Hz, 4C), 46.3, 39.6, 37.5 (t, J_{CP} = 133 Hz) 26.6, 24.1, (t, J_{CP} = 5.1 Hz, 4C), 16.4 (d J_{CP} = 9.6 Hz), 16.1, 15.8; ^{31}P NMR +23.7 ppm. Anal. Calcd for $C_{31}H_{46}O_6P_2$; C, 64.57; H, 8.04. Found: C, 64.31; H, 8.10.



Tetrsodium 4,8-dimethyl-3,7-nonadienyl-9-[1,1'-biphenyl]-4-yl-1,1-bisphosphonate (25). [26]

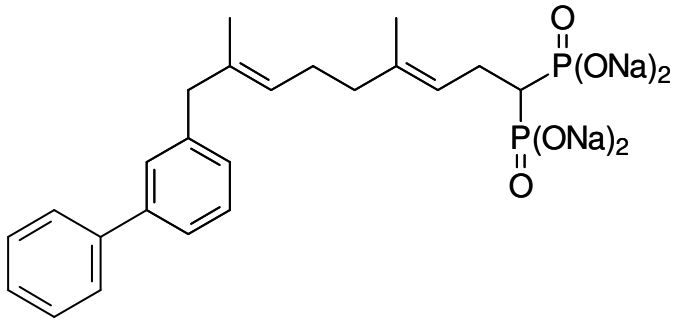
According to the general procedure 2,4,6-collidine (189 mg, 1.55 mmol), and TMSBr (285 mg, 1.87 mmol) were combined and treated with bisphosphonate ester **30** (90 mg, 0.16 mmol) and then 6 equivalents of 1N NaOH were added. After workup according to the standard procedure, the desired product was isolated as a white powder (71 mg, 82%): ^{1}H NMR (D_2O) δ 7.67–7.32 (m, 9H), 5.56 (t, J = 6.3 Hz, 1H), 5.39 (t, J =

6.4 Hz, 1H), 3.32 (s, 2H), 2.55–2.45 (m, 2H), 2.17–2.07 (m, 4H), 1.72 (tt, J_{HP} = 21.6 Hz, J = 6.3 Hz, 1H) 1.66 (s, 3H), 1.56 (s, 3H); ^{13}C NMR δ 143.2, 143.1, 141.0, 138.2, 137.8, 132.3 (2C), 131.9 (2C), 130.2, 129.6 (2C), 129.5 (2C), 129.1 (t, J_{CP} = 7.5 Hz), 124.5, 47.7, 43.7 (t, J_{CP} = 114.9 Hz), 41.9, 29.1, 28.0, 18.2, 17.8; ^{31}P NMR +21.1 ppm; HRMS (neg. ion ESI) calcd for $C_{23}H_{29}P_2O_6$ $(M–H)^-$, 463.1439; found, 463.1468.



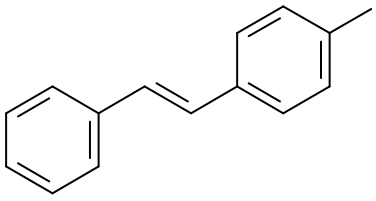
Tetrasodium 4,8-dimethyl-3,7-nonadienyl-9-[1,1'-biphenyl]-2-yl-1,1-bisphosphonate (23)

According to the general procedure bisphosphonate ester **42** (103 mg, 0.18 mmol) was treated with 2,4,6-collidine (218 mg, 1.79 mmol) and TMSBr (329 mg, 2.15 mmol), and then with 1N NaOH (6 eq). After workup according to the standard procedure, the desired product was isolated as a white powder (89 mg, 89%): ^{1}H NMR δ 7.49–7.26 (m, 9H), 5.47 (t, J = 6.8 Hz, 1H) (overlap with H_2O peak, 1H), 3.28 (s, 2H), 2.52–2.38 (m, 2H), 2.04–1.91 (m, 4H), 1.71 (tt, J_{HP} = 21.0 Hz, J = 6.8 Hz, 1H) 1.61 (s, 3H) 1.43 (s, 3H); ^{13}C NMR δ 143.1, 142.3, 138.5, 136.6, 131.3, 131.0, 130.0, 129.9 (2C), 129.1 (2C), 128.5, 128.0, 127.7, 127.3, 125.7, (t, J_{CP} = 8.2 Hz), 43.0, 41.0 (t, J_{CP} = 112.3 Hz), 39.5, 27.1, 25. 2, 16.4, 16.2; ^{31}P NMR +20.7 pp; HRMS (neg. ion ESI) calcd for $C_{23}H_{29}P_2O_6$ $(M–H)^-$, 463.1439, found, 463.1440.



Tetrasodium 4,8-dimethyl-3,7-nonadienyl-9-[1,1'-biphenyl]-3-yl-1,1-bisphosphonate (24)

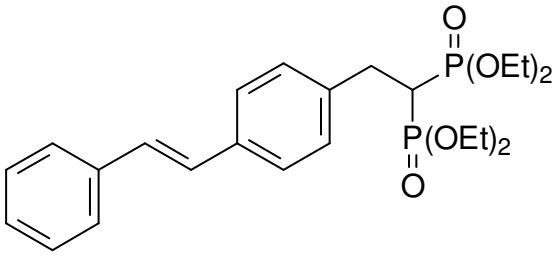
According to the general procedure the bisphosphonate ester **43** (46 mg, 0.08 mmol) was treated with 2,4,6-collidine (96 mg, 0.79 mmol), TMSBr (103 mg, 0.68 mmol), and then 1N NaOH (6 eq). After standard workup, the desired target was isolated as a white powder (28 mg, 65%): ^{1}H NMR δ 7.51–7.15 (m, 9H), 5.51 (t, J = 6.8 Hz, 1H), 5.30 (t, J = 6.0 Hz, 1H), 3.20 (s, 2H), 2.57–2.46 (m, 2H), 2.09–2.00 (m, 4H), 1.81 (tt, J_{HP} = 21.0 Hz, J = 6.8 Hz, 1H), 1.65 (s, 3H), 1.47 (s, 3H); ^{13}C NMR δ 142.6, 141.6, 141.4, 136.6, 136.3, 130.1, 130.0, 129.0 (2 CH), 128.5 (2 CH), 128.3, 127.8 127.7, 125.6, 125.5, 46.2, 40.9 (t, J_{CP} = 112.4 Hz), 40.0, 27.1, 25.0, 16.3, 15.8; ^{31}P NMR +20.3 ppm; HRMS (neg. ion ESI) calcd for $C_{23}H_{29}P_2O_6$ $(M–H)^-$, 463.1439; found, 463.1439.



4-Methyl stilbene (50). [105]

Benzyl phosphonate (2.00 g, 8.8 mmol) was placed into a reaction flask and THF (10 mL) was added. The reaction vessel was placed into an ice-bath, NaH (423 mg, 10.5

mmol) was added, and the solution was allowed to stir. After 45 minutes *p*-methylbenzaldehyde (1.30 mL, 10.5 mmol) was added and the mixture was allowed to stir overnight. The reaction was quenched by addition of saturated NH_4Cl, and extracted with Et_2O. The organic portions were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was purified via flash chromatography (silica gel, 5% EtOAc in hexanes) to provide the desired stilbene (1.52 g, 89%). Comparison of the ^1H NMR data for the sample matched the reported values.

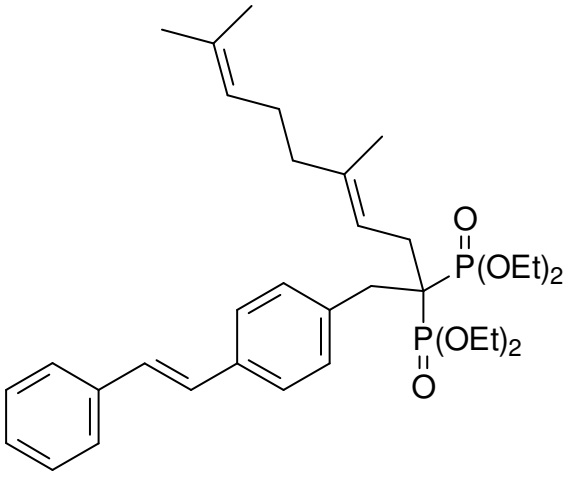


Tetraethyl methylidene stilbenebisphosphonate (52).

4-Methylstilbene (1.31g, 6.70 mmol) was added to a reaction vessel and dissolved in CCl_4 (17 mL). To the flask NBS (1.20 g, 6.70 mmol) and benzoyl peroxide (33 mg, 0.12 mmol) were added and the reaction mixture was brought to reflux. After 12 h the mixture was cooled and washed with water. The organic portion and the aqueous layer were separated and the aqueous portion was extracted with CH_2Cl_2. The organic portions were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting solid was used without further purification.

Tetraethyl methylenebisphosphonate (1.49 g, 5.21 mmol) was added dropwise to a stirring suspension of NaH (208 mg, 5.21 mmol) in THF (10 mL) at 0 °C. After 45 min bromide **51** (1.26 g, 4.60 mmol) was added dropwise and the mixture was allowed to stir.

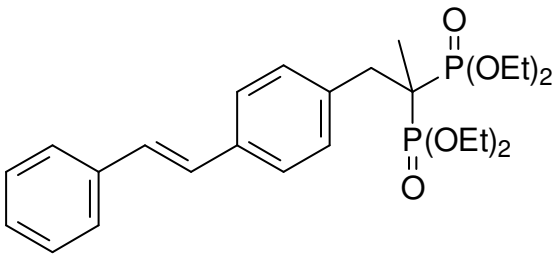
After 12 h the reaction was quenched by addition of saturated NH_4Cl. The organic and the aqueous layers were separated and the aqueous portion was extracted with CH_2Cl_2. The organic extracts were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting oil was purified via flash chromatography (silica gel; EtOH: CH_2Cl_2: hexanes, 5:5:90) to provide the desired target (1.31g, 60%): ^{1}H NMR δ 7.52–7.24 (m, 9H), 7.08 (s, 2H), 4.20–4.07 (m, 8H), 3.26 (td, J_{PH} = 16.5 Hz, J = 6.0 Hz, 2H), 2.67 (tt, J_{PH} = 24 Hz, J = 6.3 Hz, 1H), 1.31–1.22 (m, 12H); ^{13}C NMR δ 139.0 (t, J_{PC} = 7.5 Hz), 137.2, 136.0, 129.2, 128.6 (2C), 128.3, 128.2, 127.5(2C), 126.4 (2C), 126.3 (2C), 62.7–62.4 (m, 4C), 38.9 (t, J_{PC} = 131.6 Hz), 30.9 (t, J_{PC} = 4.7 Hz), 16.3–16.2 (m, 4C); ^{31}P NMR +22.9 ppm; HRMS calcd for $C_{24}H_{35}O_6P_2$ $(M+H)^+$, 481.1909, found 481.1911



Tetraethyl geranyl (methylidene) stilbenebisphosphonate (53).

Bisphosphonate **52** (158 mg, 0.30 mmol) was dissolved in THF (5 mL) and placed into an ice bath. To the reaction mixture NaH (16 mg, 0.40 mmol) was added and the mixture was allowed to stir. After 30 min, geranyl bromide (93 mg, 0.42 mmol) was

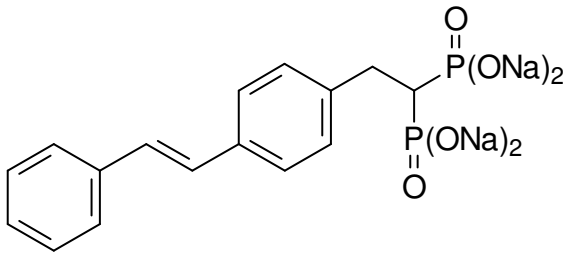
added. After 12 h, the reaction was quenched by addition of saturated NH_4Cl, the organic layer was retained and the aqueous portion was extracted with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resulting oil was purified via flash chromatography (silica gel; EtOH: CH_2Cl_2: hexanes, 5:5:90) to provide the desired target **53** (150 mg, 74%): ^{1}H NMR δ 7.53–7.23 (m, 9H), 7.08 (s, 2H), 5.64 (t, J = 6.3 Hz, 1H), 5.15 (t, J = 6.3 Hz, 1H), 4.18–4.04 (m, 8H), 3.35–3.25 (m, 2H), 2.64 (td, J_{PH} = 15.6 Hz, J = 6.0 Hz, 2H), 2.16–2.07 (m, 4H), 1.79 (s, 3H), 1.68–1.62 (m, 6H), 1.28–1.18 (m, 12H); ^{13}C NMR δ 137.4, 137.0, 136.1–135.9 (m), 135.5, 131.8, 131.4 (2C), 128.6, 128.5, 127.9 (2C), 127.4, 126.3 (2C), 125.5 (2C), 124.3, 119.2–119.0 (m), 62.5–62.3 (m, 4C), 47.5 (t, J_{PC} = 129.8 Hz), 39.9, 35.1–35.0 (m), 28.5–28.4 (m), 26.5, 25.7, 17.6 , 16.4, 16.3–16.3 (m, 4C); ^{31}P NMR +25.5 ppm; HRMS calcd for $C_{34}H_{51}O_6P_2$ $(M+H)^+$, 617.3161, found 617.3159.



Tetraethyl methyl (methylidyne) stilbenebisphosphonate (54).

Tetraethyl methylidene stilbenebisphosphonate (**52**) (296 mg, 0.60 mmol) was dissolved in THF (10 mL) and the solution was placed into an ice bath. To the stirring solution, NaH (41 mg, 0.90 mmol) was added and the mixture was allowed to stir for 30 min, after which MeI (133 mg, 0.93 mmol) was added. The mixture was allowed to stir overnight and then it was quenched by addition of saturated NH_4Cl. The organic layer

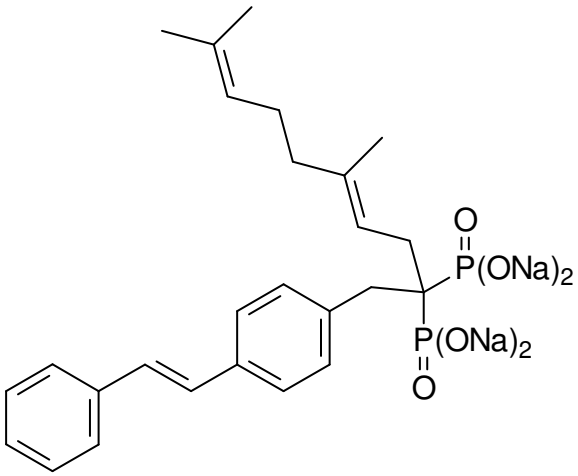
was retained and the aqueous portion was extracted with CH_2Cl_2. The organic extracts were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting oil was purified via flash chromatography (silica gel; EtOH: CH_2Cl_2: hexanes, 5:5:90) to provide the desired bisphosphonate **54** (237 mg, 78%): ^{1}H NMR δ 7.53–7.23 (m, 9H), 7.09 (s, 2H), 4.21–4.09 (m, 8H), 3.15 (m, 2H), 1.45 (t, J_{PH} = 16.5 Hz, 3H), 1.32–1.22 (m, 12H); ^{13}C NMR δ 137.4, 135.7 (t, J_{PC} = 8.3 Hz), 135.6, 131.9, 128.6, 128.4, 128.1 (2C), 127.5 (2C), 126.4 (2C), 125.5 (2C), 62.8–62.4 (m, 4C), 42.3 (t, J_{PC} = 127.1 Hz), 37.4 (t, J_{CP} = 4.3 Hz), 16.4–16.3 (m, 4C), 16.1 (t, J_{CP} = 5.7 Hz); ^{31}P NMR +26.3 ppm. Anal. Calcd for $C_{25}H_{36}O_6P_2 \bullet H_2O$; C, 58.39; H, 7.66. Found: C, 58.59; H, 7.47.



Tetrasodium methylidyne stilbenebisphosphonate (45).

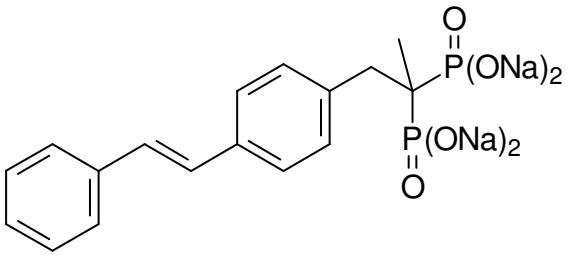
Tetraethyl methylidyne stilbenebisphosphonate (**52**) was prepared according to the general procedure for the preparation of the tetrasodium salt using 2,4,6-collidine (249 mg, 2.05 mmol), TMSBr (330 mg, 2.50 mmol) and bisphosphonate ester **52** (98 mg, 0.21 mmol). In the second step, 1N NaOH (1.02 mmol) was added to the reaction. Following standard work-up and purification by precipitation and filtration the target compound was isolated in 89% yield (77 mg): ^{1}H NMR δ 7.61–7.28 (m, 9H), 7.22 (s, 2H), 3.14 (dt, J_{PH} = 16.2 Hz, J = 6.0 Hz, 2H), 2.20 (tt, J_{PH} = 21.0 Hz, J = 6.0 Hz, 1H); ^{13}C NMR δ 143.8–143.6 (m, 1C), 137.8, 134.9, 130.0, 129.4, 129.0, 128.2, 128.0, 126.8,

126.7, 42.2 (t, J_{CP} = 111.8 Hz), 32.4–32.3 (m, 1C); ^{31}P NMR +23.7 ppm; HRMS calcd for $C_{16}H_{17}O_6P_2$ $(M-H)^-$, 367.0500, found 367.0513.



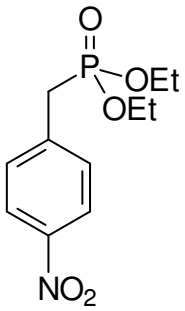
Tetrasodium geranyl (methylidene stilbene) bisphosphonate (47)

According to the general procedure for the preparation of the tetrasodium salt using 2,4,6- collidine (205 mg, 1.7 mmol), TMSBr (0.27 mL, 2.0 mmol) and the corresponding bisphosphonate ester (**43**) (104 mg, 0.2 mmol). Aqueous NaOH 1N (0.9 mmol) was added to the reaction in the second step. Following standard work-up the target was isolated in 57% yield (57 mg):^{31}P NMR +23.0 ppm; HRMS calcd for $C_{26}H_{33}O_6P_2$ $(M-4Na+3H)^-$, 503.1552, found 503.1751.



Tetrasodium methyl (methylidyne stilbene) bisphosphonate (46).

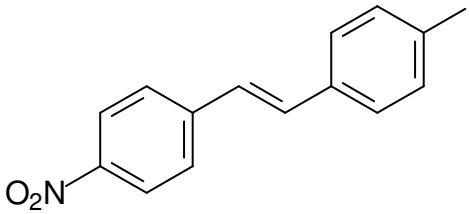
According to the general procedure for the preparation of the tetrasodium salt bisphosphonate ester **54** (73 mg, 0.20 mmol) was treated with 2,4,6-collidine (0.19 mL, 1.53 mmol), and TMSBr (0.23 mL, 1.80 mmol). Aqueous NaOH 1N (0.90 mmol) was added to the reaction in the second step. Following standard work- up and purification by precipitation and filtration comound **54** was isolated in 87% yield (80 mg): ^{1}H NMR δ 7.66–7.27 (m, 10H), 3.19 (t, J_{CP} = 13.8 Hz, 2H), 1.24 (t, J_{CP} = 15.0 Hz, 3H); ^{13}C NMR δ 140.1–140.0 (m), 137.8, 135.1, 132.5, 129.4, 129.1, 128.2 (2C), 126.9, 126.1, 41.5 (t, J_{CP} = 111.8 Hz), 37.8, 18.6; ^{31}P NMR +23.8 ppm; HRMS calcd for $C_{17}H_{19}O_6P_2$ $(M-H)^-$, 381.0672, found 381.0657



Phosphonic acid, P-[(4-nitrophenyl)methyl]-, diethyl ester (62). [106]

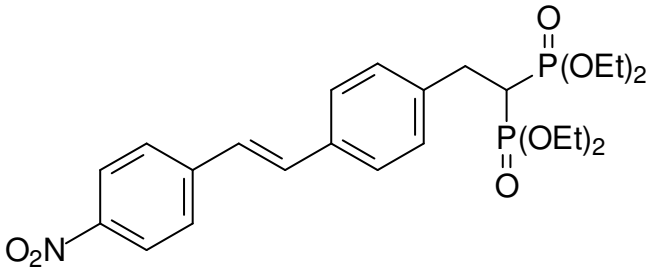
To a reaction vessel was added *p*-nitro benzyl bromide (2.01 g, 9.31 mmol) along with $P(OEt)_3$ (1.75 mL, 10.2 mmol), and the reaction mixture was heated to reflux. After 12 h the excess $P(OEt)_3$ was removed *in vacuo* and the resulting oil was purified via flash

chromatography (silica, EtOAc:Hex, 85:15) providing the desired phosphonate (**62**) (1.98 g, 78%). The ^{1}H and ^{31}P NMR spectra were consistent with literature values.



4-Methyl-4'-nitro-*trans*-stilbene (64). [107]

Phosphonate **62** (609 mg, 2.20 mmol) was placed into a reaction flask and THF (10 mL) was added. The reaction vessel was placed into an ice bath, NaH (703 mg, 17.6 mmol) was added, and the solution was allowed to stir. After 45 min *p*-methylbenzaldehyde (317 mg, 2.60 mmol) was added, and the mixture was allowed to stir overnight. The reaction was quenched by addition of saturated NH_4Cl, and extracted with CH_2Cl_2. The organic portions were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was purified via flash chromatography (silica gel; 18% EtOAc in hexanes) to provide the desired stilbene **64** (507 mg, 95%). The ^{1}H NMR data of the product was consistent with previously reported literature values.

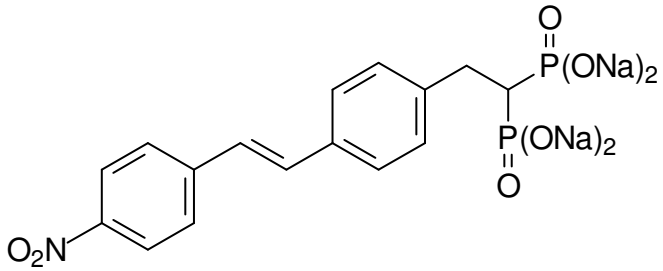


Tetraethyl methylidyne 4'-nitro-*trans*-stilbenebisphosphonate (65).

Stilbene **64** (508 mg, 2.1 mmol) was added to a reaction vessel and dissolved in CCl_4 (15 mL). To the flask, NBS (378 mg, 2.1 mmol) and benzoyl peroxide (10 mg, 0.04 mmol) were added and the reaction mixture was brought to reflux. After 12 h the mixture was allowed to cool and washed with water. The organic and the aqueous layers were separated and the aqueous portion was extracted with CH_2Cl_2. The organic extracts were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resulting solid bromide was used without further purification.

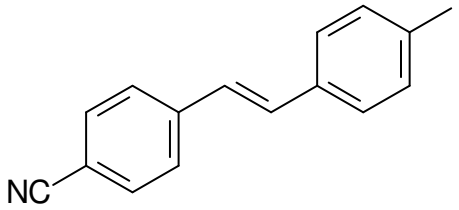
Tetraethyl methylenebisphosphonate (1.21 g, 4.2 mmol) was added dropwise to a stirring suspension of NaH (56 mg, 1.4 mmol) in THF (10 mL) at 0 °C. After 45 min, the bromide (445 mg, 1.4 mmol) was added dropwise and the mixture was allowed to stir. After 12 h the reaction was quenched by addition of saturated NH_4Cl. The organic and the aqueous layers were separated and the aqueous portion was extracted with CH_2Cl_2. The organic extracts were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resulting oil was purified via flash chromatography (silica gel; EtOH: CH_2Cl_2: hexanes, 5:5:90) to provide compound **65** (336 mg, 46%): ^{1}H NMR 8.23–8.20 (m, 2H), 7.64–7.61 (m,2H), 7.49–7.47 (m, 2H) 7.33–7.08 (m, 4H), 4.21–4.06 (m, 8H), 3.28 (td, J_{PH} = 16.5 Hz, J = 6.0 Hz, 2H), 2.66 (tt, J_{PH} = 23.7 Hz, J = 6.3 Hz, 1H), 2.0 (acetone), 1.41–1.23 (m, 12H); ^{13}C NMR δ 146.6, 143.8, 140.5 (t, J_{CP} = 7.4 Hz), 134.4, 132.9,

129.4, 126.8, 126.7, 125.7, 124.1, 62.6–62.4 (m 6C), 38.9 (t, J_{CP} = 132.0 Hz), 31.0 (t, J_{CP} = 4.6 Hz) 16.3–16.2; ^{31}P NMR +22.7 ppm; HRMS calcd for $C_{24}H_{34}NO_8P_2$ $(M+H)^+$, 526.1760, found 526.1764.



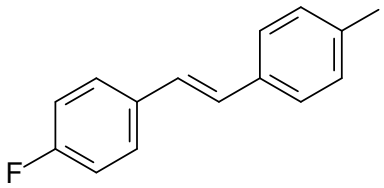
Tetrasodium methylidyne 4'-nitro-*trans*-stilbene bisphosphonate (59).

According to the general procedure 2,4,6-collidine (142 mg, 1.23 mmol) and TMSBr (214 mg, 1.42 mmol) were added to the reaction vessel followed by bisphosphonate (65) (61 mg, 0.1 mmol). The mixture was allowed to stir overnight after which toluene was added to the reaction vessel and then removed under reduced pressure. NaOH (1M, 0.72 mmol) was added. After standard workup the desired product was isolated as a yellow powder 63% (37 mg). ^{1}H NMR δ 8.25–8.22 (m, 2H), 7.76–7.24 (m,8H), 3.20 (td, J_{PH} =15.6 Hz, J = 6.6 Hz), 2.48–2.40 (m, 1H); ^{13}C NMR δ 146.4, 144.9, 142.8 (t, J_{CP} = 7.8 Hz), 134.5, 133.3 (2C), 129.8 (2C), 127.3, 127.2, 126.1 (2C), 124.5 (2C), 41.5 (t, J_{CP} = 115.7 Hz), 31.5 (t, J_{CP} =3.9 Hz) ;^{31}P NMR +19.4 ppm; HRMS calcd for $C_{16}H_{16}NO_8P_2$ $(M-4Na+3H)^-$, 412.0351 found 412.0354.



***E*-4-(4-Methylstyryl)benzonitrile (69).** [108]

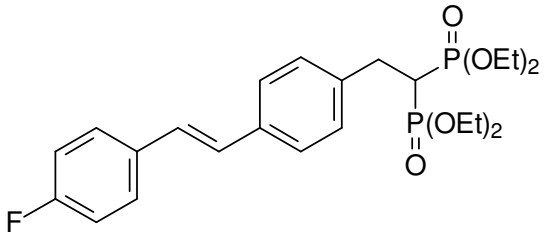
Phosphonate **68** (609 mg, 2.20 mmol) was placed into a reaction flask and THF (10 mL) was added. The reaction vessel was placed into an ice bath, NaH (703 mg, 17.6 mmol) was added, and the solution was allowed to stir. After 45 min *p*-methylbenzaldehyde (317 mg, 2.60 mmol) was added and the mixture was allowed to stir overnight. The reaction was quenched by addition of saturated NH_4Cl, and extracted with CH_2Cl_2. The organic portions were concentrated, dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was purified via flash chromatography (silica gel; 15% EtOAc in hexanes) to provide the stilbene **69** (507 mg, 50%). The ^1H NMR data of the product was consistent with previously reported literature values.



Benzene, 1-fluoro-4-[(1*E*)-2-(4-methylphenyl)ethenyl]- (72). [109]

Phosphonate **71** (1.00 g, 3.8 mmol) was placed into a reaction flask and THF (10 mL) was added. The reaction vessel was placed into an ice-bath, NaH (561 mg, 13.8 mmol) was added, and the solution was allowed to stir. After 45 min *p*-methylbenzaldehyde (413 mg, 3.40 mmol) was added and the mixture was allowed to stir overnight. The reaction was quenched by addition of saturated NH_4Cl, and extracted

with CH_2Cl_2. The organic extracts were combined, dried ($MgSO_4$), and concentrated *in vacuo*. The resulting residue was purified via flash chromatography (silica gel; CH_2Cl_2) to provide the desired stilbene **72** (554 mg, 76%). The ^1H NMR data of the product was consistent with reported literature values.

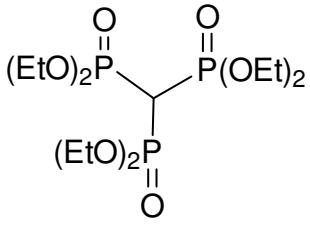


Attempted synthesis of tetraethyl methylidyne 4'-flouro-*trans*-stilbene bisphosphonate (73).

Stilbene **72** (554 mg, 2.6 mmol) was added to a reaction vessel and dissolved in CCl_4 (10 mL). To the flask, NBS (511 mg, 2.9 mmol) and benzoyl peroxide (126 mg, 0.5 mmol) were added and the reaction mixture was brought to reflux. After 12 h the mixture was cooled and washed with water. The organic portion and the aqueous layers were separated and the aqueous portion was extracted with CH_2Cl_2. The organic extracts were combined, dried ($MgSO_4$), filtered and concentrated i*n vacuo*. The resulting solid was used without further purification.

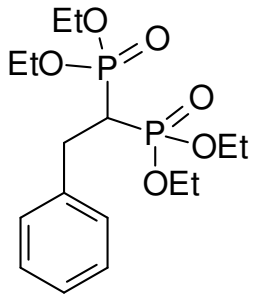
Tetraethyl methylenebisphosphonate (1.45 g, 5.0 mmol) was added dropwise to a stirring suspension of NaH (112 mg, 2.5 mmol) in THF (10 mL) at 0 °C. After 45 min the corresponding bromide (732 mg, 2.5 mmol) was added dropwise and the mixture was allowed to stir. After 12 h the reaction was quenched by addition of saturated NH_4Cl. The organic portion and the aqueous layers were separated and the aqueous portion was extracted with CH_2Cl_2. The organic extracts were combined, dried ($MgSO_4$), filtered and

concentrated *in vacuo*. ^{1}H NMR δ 7.49–7.40 (m, 4H), 7.28–7.25 (m, 2H), 7.07–7.00 (m,4H), 4.33–4.26 (m, 3H), 4.17–4.07 (m, 8H), 3.25, (td, J_{CP} = 16.5 Hz, J = 6.0 Hz, 2H), 2.65 (tt, J_{CP} =24.3 Hz, J = 6.3 Hz, 1H), 1.70 (s, 3H), 1.41–1.35 (m, 4H), 1.31–1.25 (m, 12H); ^{31}P NMR +22.9 ppm: +13.3 ppm; HRMS calcd for $C_{24}H_{34}NO_8P_2$ $(M+H)^+$, 526.1760, found 526.1764



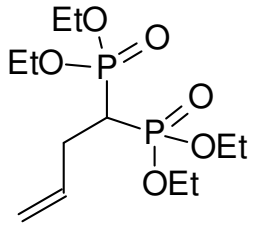
Methylidynetrisphosphonic acid, hexaethyl ester (75).[75, 76]

Tetraethyl methylenebisphosphonate (251 mg, 0.87 mmol) was dissolved in benzene (5 mL) and then concentrated *in vacuo* to remove traces of water. After three such cycles, the residue was dissolved in THF (10 mL) and cooled to 0 °C in an ice bath. A solution of NaHMDS in THF (1.0 M, 1.3 mL, 1.3 mmol) was added, and the mixture was allowed to stir at 0 °C for 30 min after which $ClP(OEt)_2$ (340 mg, 2.17 mmol) was added. After an additional 30 min, H_2O_2 (2.0 mL, 17.6 mmol) was very slowly added dropwise to the vessel. The reaction mixture was allowed to stir for one h, then diluted with brine, and extracted with CH_2Cl_2. The organic portions were combined, dried ($MgSO_4$), and filtered, and the filtrate was concentrated *in vacuo*. Final purification via flash chromatography (silica gel, 0 to 50% EtOH in EtOAc) gave the desired trisphosphonate **7** as a light yellow oil (176 mg, 48%). Both ^{31}P and ^{1}H NMR data were consistent with previously reported values.



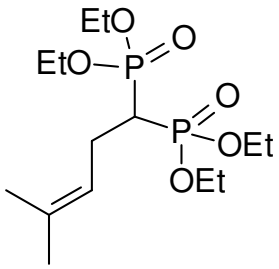
Phosphonic acid, *P,P′*-(2-phenylethylidene)bis-, *P,P,P′,P′*-tetraethyl ester (82). [86]

According to the procedure for alkylation of tetraethyl methylenebisphosphonate NaH (768 mg, 19.1 mmol), tetraethyl methylenebisphosphonate (5.00 g, 17.3 mmol) and benzyl bromide (3.30 mL, 19.1 mmol) were allowed to react. After standard work-up and purification by flash chromatography (silica gel, 10% EtOH in hexanes). The product was isolated in 56% yield (3.70 g). The ^{31}P and ^{1}H NMR spectra were consistent with previously reported literature values.



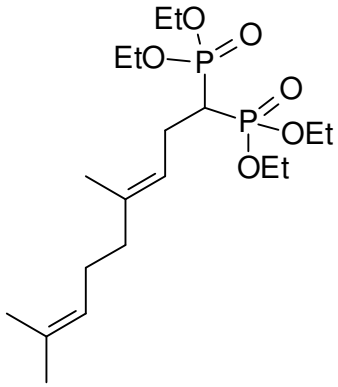
Phosphonic acid, P,P'-(3-butenylidene)bis-, P,P,P',P'-tetraethyl ester (88). [85]

According to the procedure for alkylation of tetraethyl methylenebisphosphonate NaH (1.07 g, 26.7 mmol), tetraethyl methylenebisphosphonate (7.01 g, 24.3 mmol) and allyl bromide (2.94 g, 24.3 mmol) were allowed to react. After standard work-up and purification by flash chromatography (silica gel, 10% EtOH in hexanes). The product was isolated in 43% yield (3.46 g). The ^{31}P and ^{1}H NMR spectra were consistent with previously reported literature values.



Tetraethyl 4-methyl-3-pentenyl-1,1-bisphosphonate (90). [26]

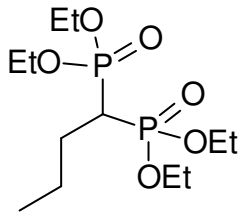
According to the procedure for alkylation of tetraethyl methylenebisphosphonate NaH (1.13g, 28.2 mmol), tetraethyl methylenebisphosphonate (8.13 g, 28.2 mmol) and prenyl bromide (4.67g, 31.1 mmol) were allowed to react. After standard work-up and purification by flash chromatography (silica gel, 10% EtOH in hexanes). The product was isolated in 43% yield (4.30 g). The ^{31}P and ^{1}H NMR spectra were consistent with previously reported literature values.



Tetraethyl 4,8-dimethyl-3,7-nonadienyl-1,1-bisphosphonate (26). [56]

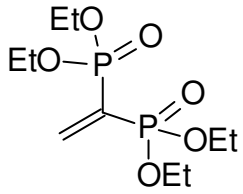
According to the procedure for alkylation of tetraethyl methylenebisphosphonate NaH (4.08 g, 102 mmol), tetraethyl methylenebisphosphonate (28.5 g, 100 mmol) and benzyl bromide (21.8mL, 100 mmol) were allowed to react. After standard work-up and purification by flash chromatography (silica gel, 10% EtOH in hexanes). The product

was isolated in 56% yield (23.4 g). The ^{31}P and ^{1}H NMR spectrawere consistent with previously reported literature values.



Tetraethyl butylidene-1,1-bisphosphonate (93). [33, 110]

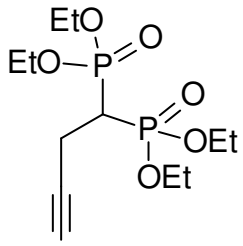
According to the procedure for alkylation of tetraethyl methylenebisphosphonate with the alteration that the reaction was brought to reflux after addition of the bromide; NaH (1.07g, 26.8 mmol), tetraethyl methylenebisphosphonate (7.03 g, 14.0 mmol) and proply bromide (3.00 g, 24.4 mmol) were allowed to react. After standard work-up and purification by flash chromatography (silica gel,10% EtOH in hexanes). The product was isolated in 48% yield (3.87g). The ^{31}P and ^{1}H NMR spectra were consistent with previously reported literature values.



Phosphonic acid, *P,P′*-ethenylidenebis-, *P,P,P′,P′*-tetraethyl ester (96). [111]

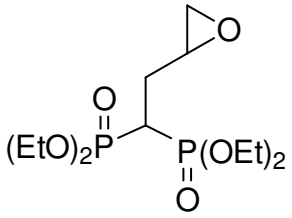
Para formaldehyde (5.21 g) and triethylamine (3.6 mL, 49.0 mmol) were added to MeOH (100 mL) and and the mixture was warmed to 60 °C until the parafomaldehyde had dissolved. Tetraethyl methylenebisphosphonate (10.0 g, 34.6 mmol) was added and the mixture was allowed to reflux overnight. After the reaction had heated overnight, the

solvent was removed *in vacuo*. The resulting residue was then dissolved in toluene and *p*-toulenesulfonic acid (255 mg, 1.30 mmol) was added. The reaction was heated and the methanol formed was removed with a Dean Stark trap. The resulting oil was distilled to provide the desired target in 49% yield (5.12 g). The ^{31}P and ^{1}H NMR spectra were consistent with previously reported literature values.



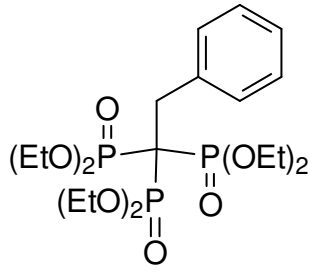
Tetraethyl-(3-butyn-1-ylidene)-1,1-bisphosphonate (94). [87]

Bisphosphonate **96** (3.0 g, 10.0 mmol) was dissolved in THF and the solution was cooled to −15 °C. To the stirring solution, NaCCH (4.5 mL, 15.0 mmol) was added and the mixture was allowed to react overnight. The reaction was quenched by addition of 1N HCl and the aqueous and the organic layers were separated. The aqueous portion was placed into a continuous liquid-liquid extractor and extracted for 12 h with EtOAc. The organic extract was dried ($MgSO_4$), filtered and concentrated *in vacuo.* The resulting oil was purified via flash chromatography (silica gel; 10% EtOH in hexanes) to provide the desired target in 70% yield (2.30 g). Comparison of the ^{31}P and ^{1}H data matched the reported values.



Tetraethyl (2-oxiranylethylidene)-1,1-bisphosphonate (107). [85]

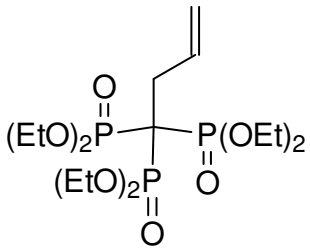
Allylbisphosphonate (**88**) (459 mg, 1.40 mmol) was added to a flask along with CH_2Cl_2 (7 mL) and *m*-CPBA (712 mg, 3.20 mmol). The reaction was allowed to stir at room temperature. Reaction progress was monitored by TLC. After 24 h the reaction was quenched by addition of saturated $NaHCO_3$ solution. The aqueous portion and the organic portion were separated and the aqueous portion was extracted with CH_2Cl_2. The organic portions were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting oil was purified via flash chromatography (silica gel, 10% EtOH in hexanes) to provide the desired product **107** (439 mg, 91%). The ^{31}P and ^{1}H NMR spectra were consistent with reported literature values.



2-Phenylethylidynetrisphosphonic acid, hexaethyl ester (83).

General procedure for the synthesis of alkyl trisphosphonates. A sample of tetraethyl benzylbisphosphonate (**82**)[86, 33] (518 mg, 1.37 mmol) was dissolved in benzene (5 mL) and then concentrated *in vacuo*. After three such cycles, the residue was dissolved in THF (6.4 mL) and cooled to 0 °C in an ice bath. A solution of NaHMDS in

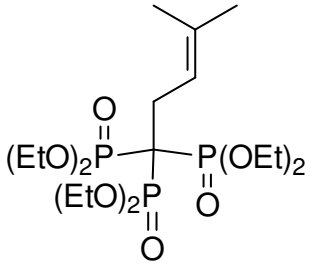
THF (1.0 M, 2.1 mL, 2.1 mmol) was added, and the mixture was allowed to stir at 0 °C for 30 min, after which $ClP(OEt)_2$ (437 mg, 2.74 mmol) was added. After an additional 30 min, excess H_2O_2 (2.0 mL, ~30% by titration) was slowly added dropwise (5 – 10 min) to the vessel. The reaction mixture was allowed to stir for one h, then diluted with brine, and extracted with CH_2Cl_2. The organic portions were combined, dried ($MgSO_4$), and filtered, and the filtrate was concentrated *in vacuo*. Final purification via flash chromatography (silica gel, 0 to 30% EtOH in EtOAc) gave the desired trisphosphonate **83** as a clear oil (432 mg, 61%): ^{1}H NMR δ 7.60–7.57 (m, 2H), 7.23–7.20 (m, 3H), 4.24–4.13 (m, 12H), 3.59 (q, J_{PH} = 15.8 Hz, 2H), 1.27 (t, J = 8.9 Hz, 18H); ^{13}C NMR (100 MHz) δ 136.3 (q, J_{PC} = 5.9 Hz), 132.5 (2C), 126.9 (2C), 126.6, 63.4 (m, 6C), 52.8 (q, J_{PC} = 117.8 Hz), 36.1 (q, J_{PC} = 5.1 Hz), 16.3 (m, 6C); ^{31}P NMR (121 MHz, $CDCl_3$) +17.7 ppm; HRMS calcd for $C_{20}H_{37}O_9NaP_3$ $(M+Na)^+$, 537.1548, found 537.1553. Anal. Calcd for $C_{20}H_{37}O_9P_3 \cdot H_2O$: C, 45.12; H, 7.38. Found: C, 45.26; H, 7.57.



3-Butenylidynetrisphosphonic acid, hexaethyl ester (97).

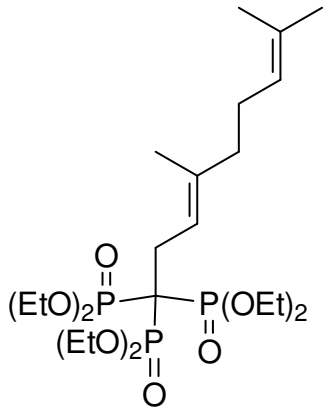
According to the general procedure for synthesis of alkylated trisphosphonates, allylbisphosphonate (**88**) [85] (985 mg, 3.0 mmol) was treated with NaHMDS (4.5 mL, 4.5 mmol) and $ClP(OEt)_2$ (1.29 g, 8.2 mmol), and then after 30 min H_2O_2 (3.50 mL, 31 mmol) was added to the reaction mixture. After standard workup the product was

purified via column chromatography on silica gel (0 to 40% EtOH in EtOAc) and compound **97** was isolated as a clear oil (987 mg, 71%): ^{1}H NMR δ 6.32–6.18 (m, 1H), 5.15–5.05 (m, 2H), 4.34–4.14 (m, 12H), 2.92 (qd, J_{PH} = 9.0 Hz, J = 6.6 Hz, 2H), 1.34 (t, J = 7.2 Hz, 18H); ^{13}C NMR δ 134.4 (q, J_{PC} = 6.3 Hz), 117.1, 63.4 (m, 6C), 50.5 (q, J_{PC} = 119.8 Hz), 35.0 (q, J_{PC} = 5.5 Hz), 16.5 (m, 6C); ^{31}P NMR +18.0 ppm; HRMS calcd for $C_{16}H_{35}O_9NaP_3$ $(M+Na)^+$, 487.1392, found 487.1407. Anal. Calcd for $C_{16}H_{35}O_9P_3 \cdot H_2O$: C, 39.84; H, 7.73. Found: C, 40.19; H, 7.83.



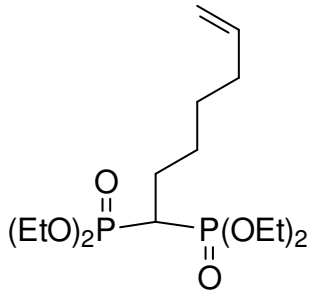
4-Methyl-3-pentenylidynetrisphosphonic acid, hexaethyl ester (98).

According to the general procedure for synthesis of alkylated trisphosphonates, prenylbisphosphonate (**90**) [26] (494 mg, 1.39 mmol) was treated with NaHMDS (2.10 mL, 2.1 mmol) and $ClP(OEt)_2$ (472 mg, 2.77 mmol). After 30 min H_2O_2 (2.00 mL, 17.6 mmol) was added to the reaction mixture. The product was purified by column chromatography on silica gel (0 to 30% EtOH in EtOAc) and was isolated as a faint yellow oil (461 mg, 68%): ^{1}H NMR δ 5.67 (t, J = 6.6 Hz, 1H), 4.29–4.17 (m, 12H), 2.86 (qd, J_{PH} = 15.9 Hz, J = 6.6 Hz, 2H), 1.72 (s, 3H), 1.64 (s, 3H), 1.36–1.30 (m, 18H); ^{13}C NMR δ 132.5, 120.2 (q, J_{PC} = 6.1 Hz), 63.4–63.3 (m, 6C), 50.4 (q, J_{PC} = 117.6 Hz), 29.5, (q, J_{PC} = 5.4 Hz), 26.0, 17.9, 16.5–16.3 (m, 6C); ^{31}P NMR (121 MHz, $CDCl_3$) +18.6 ppm; HRMS calcd for $C_{18}H_{40}O_9P_3$ $(M+H)^+$, 493.1885, found 493.1883.



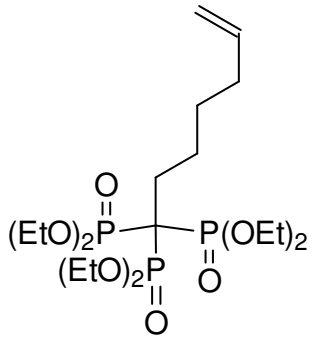
(3E)-4,9-Dimethyl-3-nonadienylidynetrisphosphonic acid, hexaethyl ester (99).

According to the general procedure for synthesis of alkylated trisphosphonates, geranylbisphosphonate (**22**) [56] (249 mg, 0.6 mmol) was treated with NaHMDS (1.00 mL, 1.0 mmol) and $ClP(OEt)_2$ (125 mg, 0.8mmol) and then after 30 min H_2O_2 (1.00 mL, 8.8 mmol) was added to the reaction mixture. After standard workup, the product **99** was purified by column chromatography on silica gel (0 to 30% EtOH in EtOAc) and was isolated as a faintly yellow oil (208 mg, 63%): ^{1}H NMR δ 5.72 (t, J = 6.3 Hz, 1H), 5.13 (t, J = 6.2 Hz, 1H), 4.25 (q, J = 7.1 Hz, 12H), 2.87 (qd, J_{PH} = 16.2, J = 7.1 Hz, 2H), 2.09–2.03 (m, 4H), 1.68 (s, 3H), 1.63 (s, 3H), 1.60 (s, 3H), 1.33 (t, J = 6.6 Hz, 18H); ^{13}C NMR δ 135.8, 131.1, 124.1, 119.8 (q, J_{PC} = 6.2 Hz), 63.1 (m, 6C), 50.2 (q, J_{PC} = 119.6 Hz), 39.9, 29.2 (q, J_{PC} = 5.6 Hz), 26.5, 25.5, 17.4, 16.3–16.2 (m, 6C), 16.1; ^{31}P NMR +18.6 ppm; HRMS calcd for $C_{23}H_{48}O_9P_3$ $(M+H)^+$, 561.2511, found 561.2529. Anal. Calcd for $C_{23}H_{47}O_9P_3 \cdot H_2O$: C, 47.75; H, 8.54. Found: C, 47.98; H, 8.44.



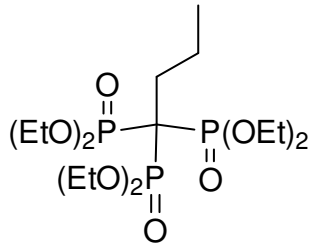
Tetraethyl 6-hepten-1,1-bisphosphonate (120).

Tetraethyl methylenebisphosphonate (5.31 g, 18.4 mmol) was added dropwise to a stirring suspension of NaH (810 mg, 20.2 mmol) in THF (10 mL). After 30 min, 6-bromo-1-hexene (3.00 g, 18.4 mmol) was added and the mixture was heated at reflux overnight. After the reaction mixture had cooled to room temperature, saturated NH_4Cl was added and the organic and aqueous portions were separated. The aqueous portion was extracted with Et_2O and the organic layers were combined, dried ($MgSO_4$), and concentrated *in vacuo*. The resulting oil was purified via flash chromatography (silica gel, 10% EtOH in hexanes) and the desired bisphosphonate was isolated in 46% yield (3.10 g): ^{1}H NMR δ 5.84–5.73 (m, 1H), 5.03–4.92 (m, 2H), 4.23–4.12 (m, 8H), 2.27 (tt, J_{PH} = 24.3 Hz, J = 6.3 Hz, 1H), 2.01–1.83 (m, 4H) 1.64–1.54 (m, 2H), 1.45–1.32 (m, 14H); ^{13}C NMR δ 138.6, 114.3, 62.4–62.1 (m, 4C) 36.6 (t, J_{PC} = 132.5 Hz), 28.5 (2C), 25.3, 16.3–16.2 (m, 4C); ^{31}P NMR +23.9 ppm; HRMS calcd for $C_{15}H_{33}O_6P_2$ $(M+H)^+$, 371.1752, found 371.1745.



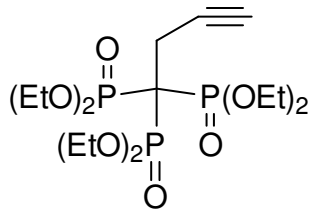
6-Heptenylidynetrisphosphonic acid, hexaethyl ester (121).

According to the general procedure for synthesis of alkylated trisphosphonates, tetraethyl 6-hepten-1,1-bisphosphonate (**120**) (508 mg, 1.37 mmol) was treated with NaHMDS (2.06 mL, 2.06 mmol) and $ClP(OEt)_2$ (438 mg, 2.75 mmol), and then H_2O_2 (2.00 mL, 17.6 mmol) was added. After standard workup the product **121** was purified by column chromatography on silica gel (0 to 30% EtOH in EtOAc) and was isolated as a faintly yellow oil (435 mg, 78%): ^{1}H NMR ($CDCl_3$) δ 5.89–5.75 (m, 1H), 5.04–4.92 (m, 2H), 4.29–4.19 (m, 12H), 2.11–2.00 (m, 4H; 2 exchange with D_2O) 1.91–1.80, (m, 2H) 1.43–1.13 (m, 22H); ^{13}C NMR ($CDCl_3$) δ 138.8, 114.2, 63.4–63.1 (m, 6C), 50.6 (q, J_{PC} = 119.3 Hz), 33.4, 30.8 (q, J_{PC} = 5.3 Hz), 29.3, 25.2 (q, J_{PC} = 5.0 Hz), 16.5–16.2 (m, 6C); ^{31}P NMR (121 MHz, $CDCl_3$) +18.7 ppm; HRMS calcd for $C_{19}H_{41}O_9NaP_3$ $(M+Na)^+$, 529.1861, found 529.1867.



Butylidynetrisphosphonic acid, hexaethyl ester (100).

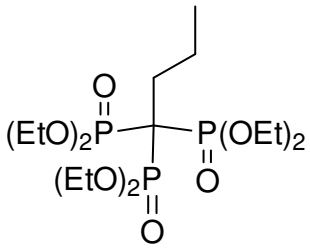
According to the general procedure for synthesis of alkylated trisphosphonates, propylbisphosphonate (**93**) [33, 110] (292 mg, 0.9 mmol) was treated with NaHMDS (1.30 mL, 1.3 mmol) and $ClP(OEt)_2$ (346 mg, 2.2 mmol). After 30 min H_2O_2 (2.00 mL, 17.6 mmol) was added to the reaction mixture. Standard workup and purification via column chromatography on silica gel (0 to 35% EtOH in EtOAc) gave compound **100** as a clear oil (324 mg, 79%). Both the ^{31}P and ^{1}H NMR spectra are consistent with material prepared via hydrogenation of compound **11** (*vide infra*).



3-Butyn-1-ylidynetrisphosphonic acid, hexaethyl ester (101).

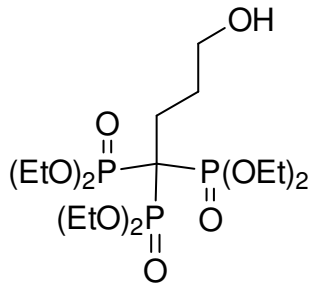
According to the general procedure for synthesis of alkylated trisphosphonates, propargylbisphosphonate (**94**) [87] (291 mg, 0.9 mmol) was treated with NaHMDS (0.9 mL, 0.9 mmol) and $ClP(OEt)_2$ (280 mg, 1.8 mmol) and then after 30 min H_2O_2 (2.00 mL, 17.6 mmol) was added to the reaction mixture. After standard workup the product was purified via column chromatography on silica gel (0 to 30% EtOH in EtOAc) and compound **101** was isolated as a clear oil (243 mg, 59%): ^{1}H NMR δ 4.33–4.21 (m,

12H), 3.03 (qd, J_{PH} = 14.7 Hz, J = 3.3 Hz 2H), 2.09–2.07 (m, 1H), 1.34–1.32 (m, 18H); ^{13}C NMR δ 79.9 (q, J_{PC} = 9.1 Hz), 70.5, 63.9–63.6 (m, 6C), 49.7 (q, J_{PC} = 120.0 Hz), 21.0 (q, J_{PC} = 5.6 Hz), 16.4–16.3 (m, 6C); ^{31}P NMR +16.7 ppm; HRMS calcd for $C_{16}H_{34}O_9P_3$ $(M+H)^+$, 463.1416, found 463.1420.



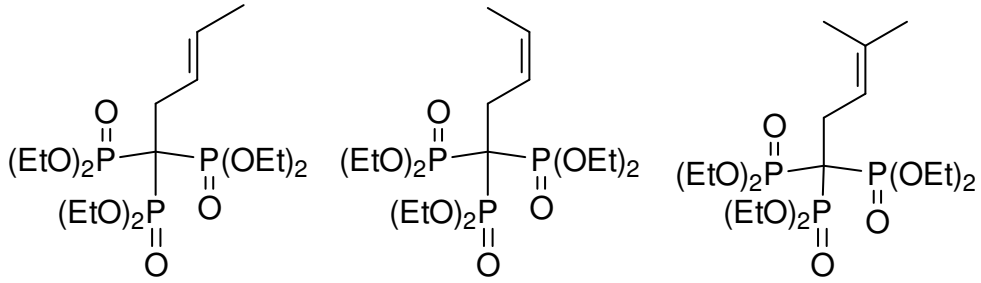
Trisphosphonate 100 via catalytic hydrogenation of compound 97.

Trisphosphonate **97** (96 mg, 0.2 mmol) in EtOH (5 mL) was treated with Pd/C (23 mg, 0.2 mmol) under an H_2 atmosphere. After 12 h the reaction mixture was filtered through celite, and the filtrate was collected and concentrated *in vacuo*. The resulting oil was purified using flash chromatography (silica gel, 0 to 25% EtOH in EtOAc) to obtain compound **100** as a clear oil (81 mg, 84%): ^{1}H NMR δ 4.30–4.18 (m, 12H), 2.18–1.77 (m, 4H), 1.34 (t, J = 6.6 Hz, 18H), 0.91 (t, J = 6.9 Hz, 3H); ^{13}C NMR δ 63.4 (m, 6C), 51.6 (q, J_{PC} = 119.8 Hz), 33.0 (q, J_{PC} = 5.5 Hz), 19.2 (q, J_{PC} = 5.2 Hz) 16.4 (m, 6C), 15.0; ^{31}P NMR +18.8 ppm; HRMS calcd for $C_{16}H_{37}O_9NaP_3$ $(M+Na)^+$, 489.1548, found 489.1564. Anal. Calcd for $C_{16}H_{37}O_9P_3 \cdot H_2O$: C, 39.67; H, 8.11. Found: C, 39.66; H, 8.02.



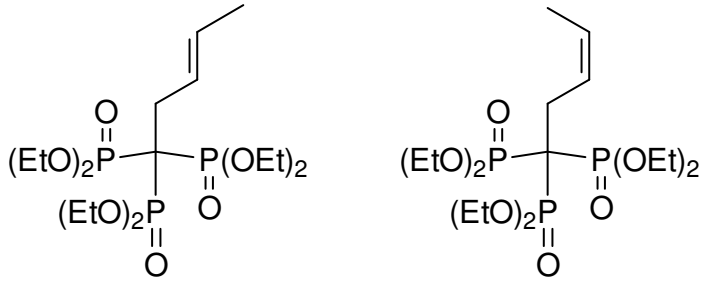
4-Hydroxybutylidynetrisphosphonic acid, hexaethyl ester (105).

Trisphosphonate **97** (102 mg, 0.22 mmol) was dried under vacuum in the presence of P_2O_5 overnight. The remaining oil was dissolved in THF (5 mL) and placed into an ice bath. To the reaction flask, BH_3·THF (1M in THF, 0.45 mL, 0.45 mmol) was added and the mixture was allowed to stir. After 1.5 h, MeOH (2 mL) was added to the flask, followed by NaOH (3M, 0.5 mL, 1.5 mmol) and then H_2O_2 (0.3 mL, 2.7 mmol), and the resulting mixture was heated at 50 °C for 1 h. The reaction mixture was washed with saturated NaCl and the aqueous portions were retained and extracted with CH_2Cl_2. The organic portions were combined, dried ($MgSO_4$), and concentrated *in vacuo*. The resulting oil was purified via flash chromatography (silica gel, 0 to 45% EtOH in EtOAc) to obtain compound **19** as a clear oil (66 mg, 62%): ^{1}H NMR δ 4.33–4.20 (m, 12H), 3.64 (t, J = 5.7 Hz, 2H), 2.30–2.05 (m, 7H; 2 exchange with D_2O) 1.35 (t, J = 6.6 Hz, 18H); ^{13}C NMR δ 63.5–63.4 (m, 6C), 63.0, 51.6 (q, J_{PC} = 119.8 Hz), 33.0 (q, J_{PC} = 5.5 Hz), 19.2 (q, J_{PC} = 5.2 Hz) 16.4 (m, 6C); ^{31}P NMR +18.8 ppm; HRMS calcd for $C_{16}H_{37}O_{10}NaP_3$ $(M+Na)^+$, 505.1497, found 505.1503.



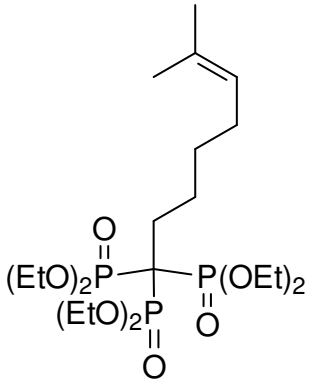
3-Pentenylidynetrisphosphonic acid, hexaethyl ester (115 and 116) and compound 98.

Grubbs second generation catalyst (4.9 mg, 3 mol %) was dissolved in 2-methyl-2-butene (1 mL) and placed in a 1-dram vial. The trisphosphonate **97** (88.3 mg, 0.2 mmol) was added to this mixture, along with an additional 1 mL of 2-methyl 2-butene. The vial was sealed and the reaction was allowed to stir at 40 °C overnight. After the solvent was removed *in vacuo*, the resulting oil was purified via flash chromatography (silica gel, 0 to 30% EtOH in EtOAc). The reaction products (76 mg, 83% total) were isolated as an inseparable mixture of cis (**116**) and trans (**115**) isomers. (68%, 1.2:4.3 isomer ratio) and prenyl trisphosphonate **98** (15%, 1:5.5 ratio with respect to olefins **115 and 116**). The ^{31}P, ^{1}H and ^{13}C NMR spectra were consistent with a mixture of compounds **115, 116** and **98**, both of which had been prepared independently.



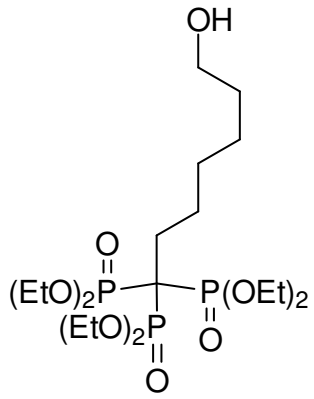
Compounds 115 and 116 via metathesis with 2-butene.

Grubbs second generation catalyst (2.3 mg, 6 mol %) was dissolved in CH_2Cl_2 (0.5 mL) and placed in a 1-dram vial, and trisphosphonate **97** (24 mg, 0.1 mmol) was added to this mixture. After 2-butene was added to the vessel via balloon, the vessel was sealed and the reaction was allowed to stir at 40 °C overnight. The volatile materials were removed *in vacuo* and the resulting oil was purified via flash chromatography (silica gel, 0 to 30% EtOH in EtOAc). The olefin compounds were isolated as a mixture of trans (**115)** and cis (**116**) isomers (19 mg, 78%) in a 2.8:1 ratio. For the trans isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.85 (dt, J = 14.0, 7.0 Hz, 1H), 5.57–5.50 (m, 1H), 4.29–4.18 (m, 12H), 2.89–2.84 (m, 2H), 1.67 (dd, J = 7.0, 1.5 Hz, 3H), 1.35–1.32 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 128.0, 126.7 (q, J_{PC} = 6.3 Hz), 63.4–63.3 (6C), 50.7 (q, J_{PC} = 119.5 Hz), 33.9 (q, J_{PC} = 5.3 Hz), 17.9, 16.5–16.3 (6C); ^{31}P NMR (121 MHz, $CDCl_3$), +18.4 ppm. For the cis isomer: ^{1}H NMR (500 MHz, $CDCl_3$), δ 5.94–5.92 (m, 1H), 5.57–5.50 (m, 1H), 4.29–4.18 (m, 12H), 2.89–2.84 (m, 2H), 1.64 (dd, J = 7.0, 1.0 Hz, 3H), 1.35–1.32 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) 126.1 (q, J_{PC} = 6.0 Hz), 125.0, 63.5–63.4 (6C), 50.1 (q, J_{PC} = 119.6 Hz), 28.3 (q, J_{PC} = 8.0 Hz), 16.5–16.3 (6C), 12.9; ^{31}P NMR (121 MHz, $CDCl_3$) +18.5 ppm; HRMS calcd for $C_{17}H_{37}O_9NaP_3$ $(M+Na)^+$, 501.1548, found 501.1554. Anal. Calcd for $C_{17}H_{37}O_9P_3 \cdot H_2O$: C, 41.13; H, 7.92. Found: C, 41.35; H, 7.91.



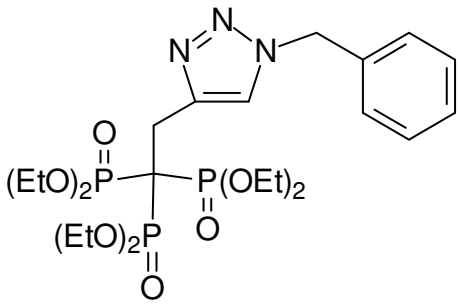
7-Methyl-6-octenylidynetrisphosphonic acid, hexaethyl ester (122).

Grubbs second generation catalyst (3.1 mg, 3 mol %) was dissolved in 2-methyl-2-butene, placed in a 1-dram vial, and trisphosphonate **121** (62 mg, 0.12 mmol) was added along with an additional 1 mL of 2-methyl-2-butene. The vial was sealed and the reaction was allowed to stir at 40 °C overnight. After concentration *in vacuo,* the resulting oil was purified via flash chromatography (silica gel, 0 to 30% EtOH in EtOAc), and the desired product 1**22** was isolated as an oil (57 mg, 87%): ^{1}H NMR δ 5.12 (t, J = 6.0 Hz, 1H), 4.19–4.31 (m, 12H), 1.96–2.24 (m, 6H), 1.77–1.88 (m, 2H), 1.68 (3H), 1.57 (3H), 1.34 (t, J = 6.6 Hz, 18H); ^{13}C NMR δ 131.2, 124.7, 63.5–63.2 (m, 6C), 50.7 (q, J_{PC} = 119.4 Hz), 31.0–30.9 (m), 30.9, 27.8, 25.7, 25.4 (q, J_{PC} = 5.2 Hz) 17.6, 16.4–16.2 (m, 6C); ^{31}P NMR +18.8 ppm; HRMS calcd for $C_{21}H_{46}O_9P_3$ $(M+H)^+$, 535.2355, found 535.2357.



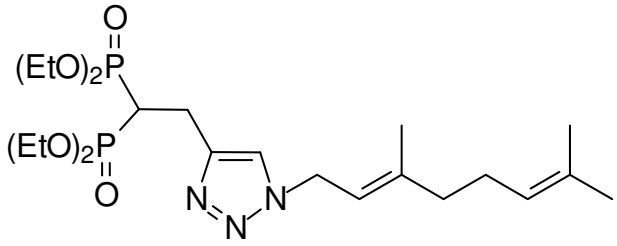
7-Hydroxyheptylidynetrisphosphonic acid, hexaethyl ester (123).

Trisphosphonate **121** (109 mg, 0.2 mmol) was dried overnight under vacuum in the presence of P_2O_5. The remaining oil was dissolved in THF (5 mL) and placed into an ice bath. To the reaction flask, 9-BBN (0.5 M in THF, 1.0 mL, 0.5 mmol) was added and the mixture was allowed to stir. After 1.5 h, MeOH (2 mL) was added to the flask, followed by NaOH (3 M, 0.5 mL, 1.5 mmol) and then H_2O_2 (0.5 mL, 4.4 mmol), and the resulting mixture was heated at 50 °C for 1 h. The reaction mixture was washed with saturated NaCl and the aqueous portions were retained and extracted with CH_2Cl_2. The organic portions were combined, dried ($MgSO_4$), and concentrated *in vacuo*. The resulting oil was purified via flash chromatography (silica gel, 0 to 40% EtOH in EtOAc) to obtain compound **123** as a clear oil (64 mg, 57%): ^{1}H NMR δ 4.30–4.17 (m, 12H), 3.63 (t, J = 6.3 Hz, 2H), 2.18–2.03 (m, 3H), 1.90–1.82 (m, 2H), 1.60–1.53 (m, 2H), 1.44–1.28 (m, 22); ^{13}C NMR δ 63.5–63.2 (m, 6C), 62.8, 50.6 (q, J_{PC} = 119.5 Hz), 32.7, 30.8 (q, J_{PC} = 5.3 Hz), 30.3, 25.5 (q, J_{PC} = 5.3 Hz), 25.3, 16.5–16.2 (6C); ^{31}P NMR +18.8 ppm; HRMS calcd for $C_{19}H_{43}O_{10}NaP_3$ $(M+Na)^+$, 547.1967, found 547.1991.



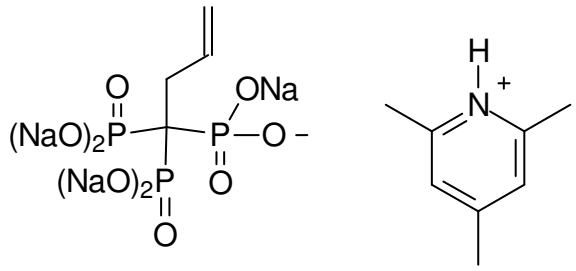
1-Benzyl-4-[2,2,2-tris(diethyoxyphosphinyl)ethyl-1H-1,2,3-triazole (124).

Benzyl bromide (182 mg, 1.1 mmol) was added to a suspension of sodium azide (83 mg, 1.3 mmol) in DMF (5 mL) and the resulting mixture was allowed to stir. After 10 min, trisphosphonate **101** (164 mg, 0.4 mmol) was added along with 0.1 mL $CuSO_4$ (5M), sodium ascorbate (43 mg, 0.2 mmol), and a solution of tBuOH in water (1:4 ratio, 5 mL), and the reaction mixture was allowed to stir at room temperature. After 24 h EDTA and 1M NH_4OH were added, the resulting solution was placed in a continuous liquid-liquid extractor and extracted for 4 h with EtOAc. The organic portion was retained and concentrated *in vacuo*. The resulting oil was purified via flash chromatography (silica gel, 0 to 50% EtOH in EtOAc) to provide the desired triazole **124** (179 mg, 85%): ^{1}H NMR δ 7.95 (s, 1H), 7.35–7.31 (m, 5H), 5.47 (s, 2H), 4.22–4.07 (m, 12H), 3.66 (q, J_{PH} = 15.9 Hz, 2H), 1.26–1.21 (m, 18H); ^{13}C NMR δ 143.3 (q, J_{PC} = 7.4 Hz), 135.2, 128.9 (2C), 128.4, 128.0 (2C), 124.7, 63.7–63.4 (m, 6C), 53.9, 50.6 (q, J_{PC} = 119.2 Hz), 27.9, (q, J_{PC} = 5.5 Hz), 16.4–16.1 (m, 6C); ^{31}P NMR +17.6 ppm; HRMS calcd for $C_{23}H_{40}N_3O_9NaP_3$ $(M+Na)^+$, 618.1875, found 618.1893.



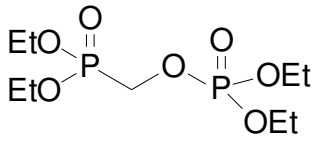
1-Geranyl-4-[2,2-bis(diethyoxyphosphinyl)ethyl-1H-1,2,3-triazole (128).

Geranyl bromide (455 mg, 2.10 mmol) was dissolved in DMF (5 mL) and NaN_3 (133 mg, 2.10 mmol) was added to the solution. The mixture was allowed to stir for 15 min after which a solution of tBuOH/H_2O (5 mL) was added along with tetraethyl-(3-butyn-1-ylidene)-1,1-bisphosphonate (**94**) (228 mg, 0.72 mmol). The solution was allowed to stir while $CuSO_4$ (5M, 0.10 mL) and sodium ascorbate (8.3 mg, 0.43 mmol) were added. The mixture was allowed to stir overnight, then EDTA and 1M NH_4OH were added. Then the resulting solution was placed in a continuous liquid-liquid extractor and extracted for 24 h with EtOAc. The organic portion was retained and concentrated *in vacuo*. The resulting oil was purified via flash chromatography (silica gel, 10% EtOH in hexanes) to provide the desired triazole **128** (188 mg, 53%): ^{1}H NMR δ 7.42 (s, 1H), 5.40 (t, J = 7.5 Hz, 1H), 5.09–5.05 (m, 1H), 4.94–4.89 (m, 2H), 4.26–4.06 (m, 8H), 3.39–3.25 (td, J_{PH} = 16.2 Hz, J = 6.9 Hz, 2H), 3.01–2.91 (m, 1H), 2.19–2.00 (m, 4H), 1.78 (s, 3H), 1.69 (s, 3H), 1.60 (s, 3H), 1.38–1.24 (m, 12H); ^{13}C NMR δ 145.0, 142.8, 123.4, 121.5, 118.0, 117.1, 62.6 (dd, J = 24.7 Hz, J = 6.5 Hz, 4C), 47.8, 39.4, 36.6 (t, J_{CP} = 129.5 Hz), 32.1, 22.1 (t, J = 4.8 Hz), 17.6, 16.4 (2C), 16.3–16.2 (m, 4C); ^{31}P NMR +22.5 ppm; $(M+H)^+$ $C_{22}H_{42}N_3O_6P_2$, 506.2549, found 506.2568.



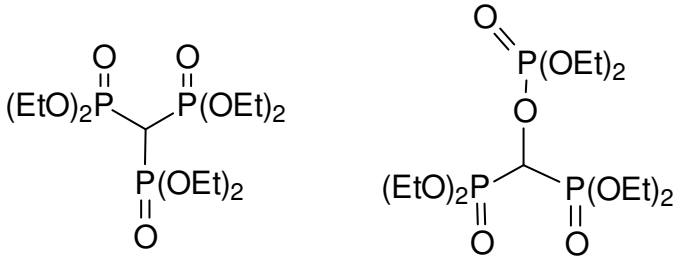
3-Butenylidynetrisphosphonic acid, pentasodium, 2,4,6-trimethylpyridinium salt (130).

A solution of 2,4,6-collidine (524 mg, 4.3 mmol) and TMSBr (568 mg, 4.3 mmol) was allowed to stir in an ice bath. After 20 min, trisphosphonate **97** (75 mg, 0.2 mmol) was added and the reaction was allowed to stir for 24 h with periodic monitoring by ^{31}P NMR spectroscopy. Once the reaction was complete, it was quenched by addition of toluene, the solvent was removed *in vacuo*, and aqueous sodium hydroxide (1.5 mmol, 9 eq) was added. The mixture was allowed to stir overnight and again was monitored by ^{31}P NMR. The reaction mixture then was lyophilized, the resulting solid was dissolved in a minimum amount of water, then slowly poured into cold acetone and kept at 40 °C overnight. The resulting precipitate was filtered and washed with cold acetone. The remaining residue was dissolved in water and lyophilized to afford compound **130** as a flocculent white residue (51 mg, 60%): ^{1}H NMR (D_2O) δ 7.43 (s, 2H), 6.19–6.10 (m, 1H), 5.23–5.05 (m, 2H), 2.92–2.85 (m, 2H), 2.93 (s, 6H), 2.50 (s, 3H); ^{13}C NMR (D_2O) δ 164.1 (2C), 155.8, 139.1–139.0 (m), 129.2, 121.6 (2C), 52.3 (q, J_{PC} = 103.6 Hz), 38.6. 25.3 (2C), 22.5; ^{31}P NMR (121 MHz, D_2O) +17.5 ppm; HRMS calcd for $C_4H_{10}O_9P_3$ $(M-H)^-$, 294.9538, found 294.9542.



Diethoxyphosphinylmethyl diethyl phosphate (133).[101]

A suspension of NaH (270 mg, 6.54 mmol) in THF (5mL) was prepared and place into an ice bath. To the stirring mixture diethyl hydroxymethylphosphonate (0.90 mL, 5.95 mmol) was added and the resulting mixture was allowed to stir for 15 min after which diethyl chlorophosphate (0.94 mL, 6.54 mmol) was added. The reaction mixture was allowed to stir overnight then saturated NH_4Cl was added. The aqueous layer and the organic layer were separated, and the aqueous portion was retained and extracted with CH_2Cl_2. The organic extracts were combined, dried ($MgSO_4$), and concentrated *in vacuo*. The resulting oil was purified via distillation (1.04g, 64%). The ^1H and ^{31}P NMR data of the isolated product matched the reported values.



Compounds 75 and 132.[75, 76, 99]

Tetraethyl methylenebisphosphonate (206 mg, 0.69 mmol) was dissolved in THF (5 mL) and cooled to 0 °C in an ice bath. A solution of NaHMDS in THF (1.0 M, 1.3 mL, 2.4 mmol) was added, and the mixture was allowed to stir at 0 °C for 30 min after which $ClP(OEt)_2$ (272 mg, 1.74 mmol) was added Dropwise to the reaction mixture. After an additional 30 min, H_2O_2 (2.0 mL, 17.6 mmol) was very slowly added dropwise

to the vessel. After 1 hour the reaction was monitored by ^{31}P NMR and the spectral data was used to determine reaction yields. Compound **75** accounted for 29% while compound **132** was formed in 39%. The ^{31}P NMR data for compounds **75** and **132** were consistent with previously reported values.

APPENDIX

SELECTED NMR SPECTRA

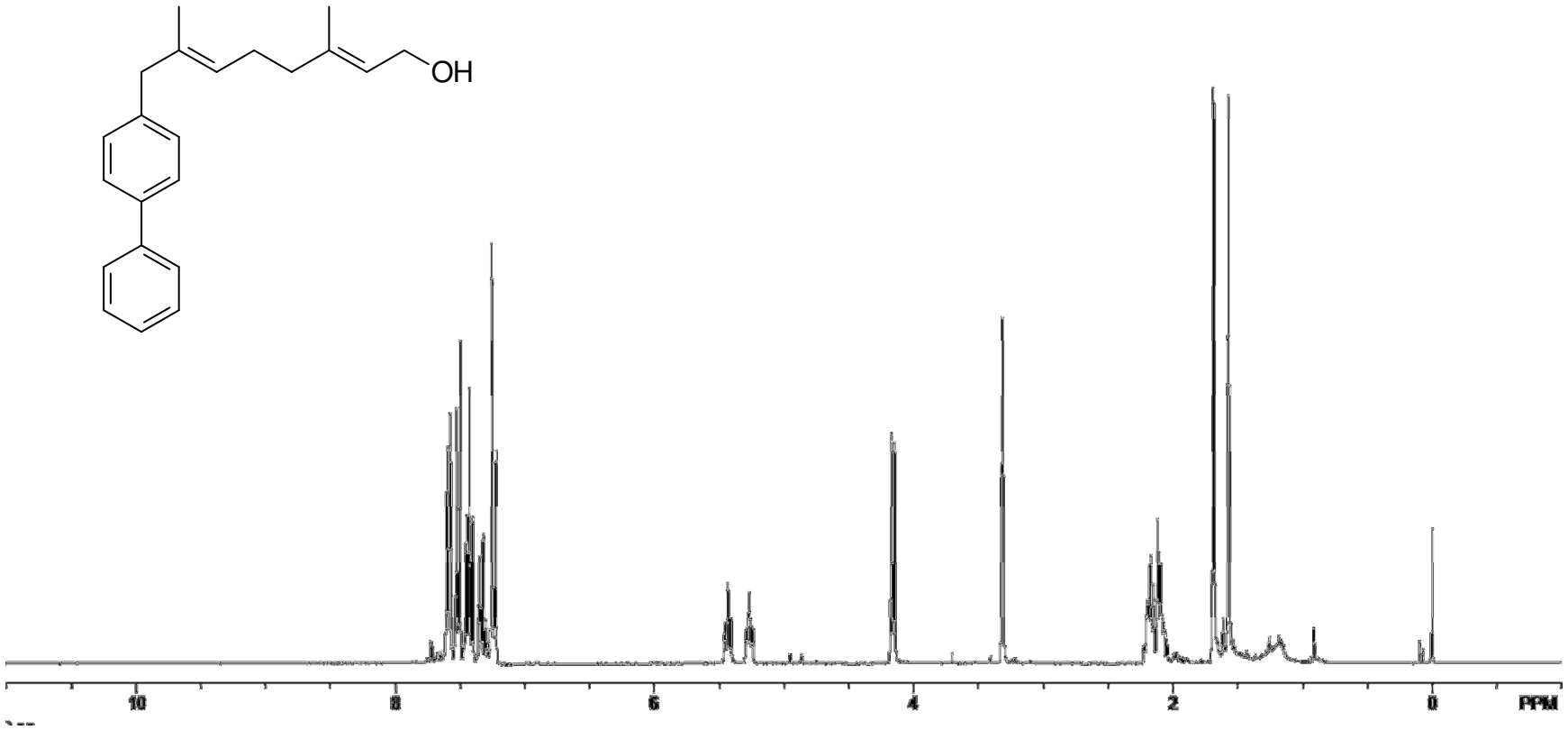


Figure A1. ^{1}H NMR of compound **38.**

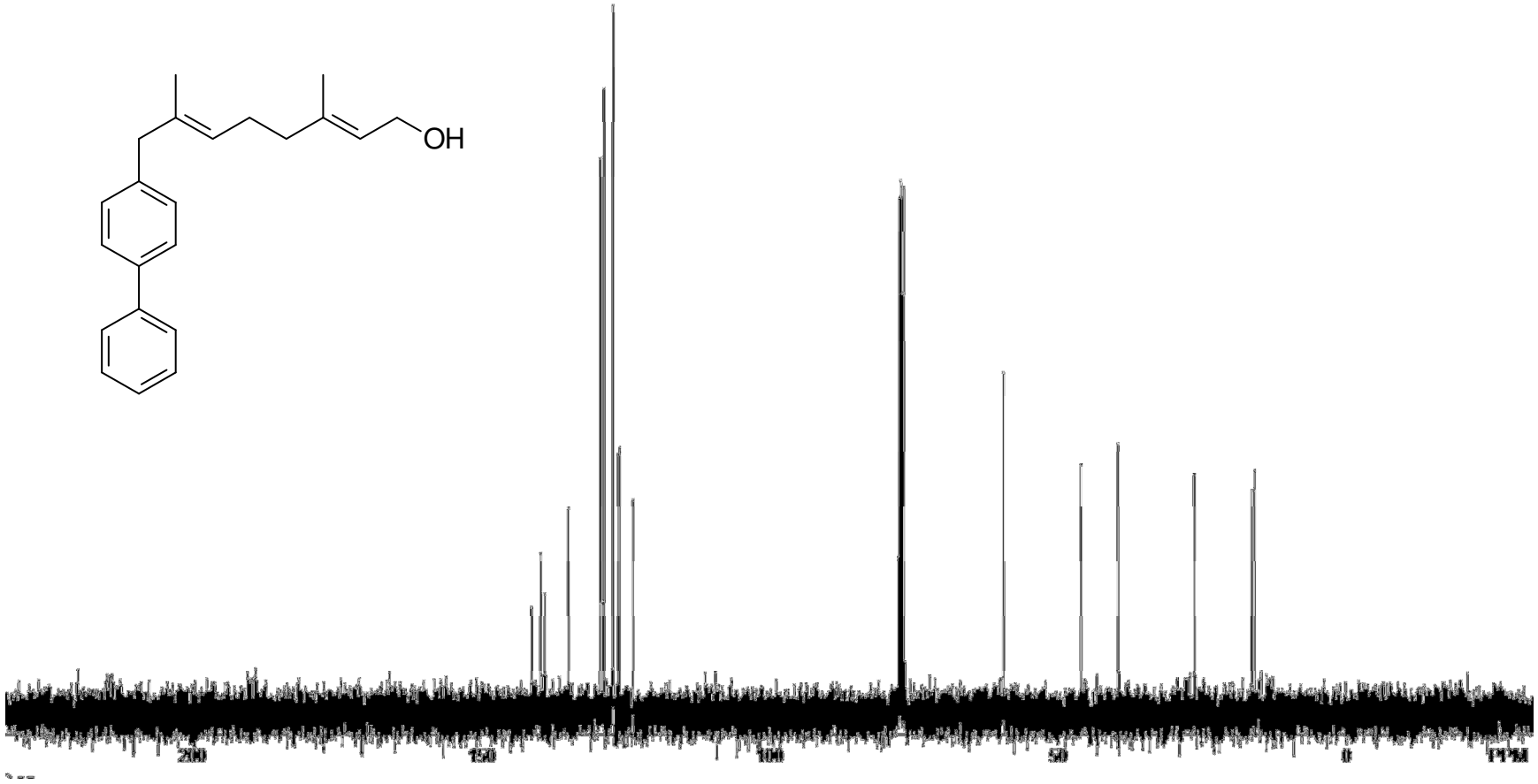


Figure A2. ^{13}C NMR of compound **38**.

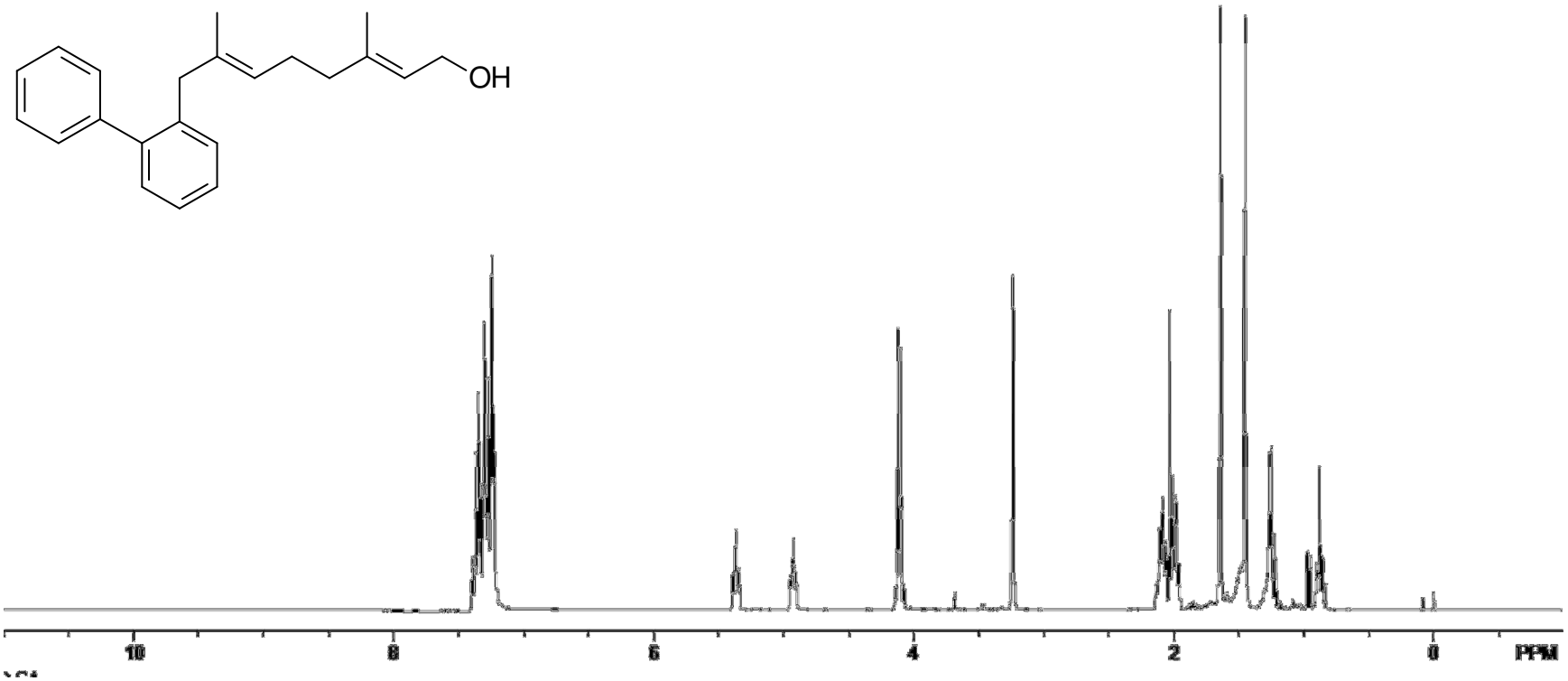


Figure A3. ^{1}H NMR spectrum of alcohol **36**.

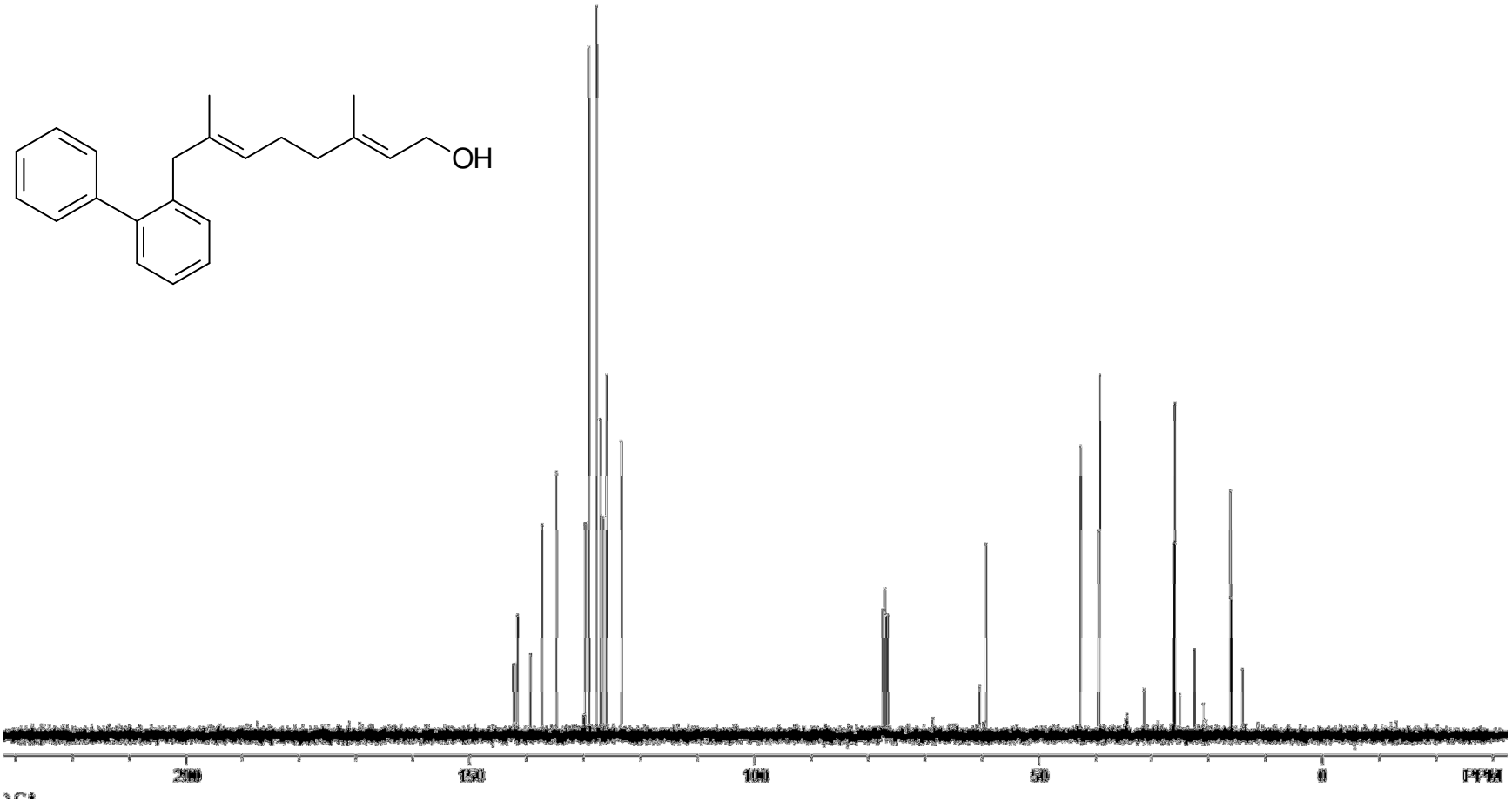


Figure A4. ^{13}C NMR spectrum of alcohol **36**.

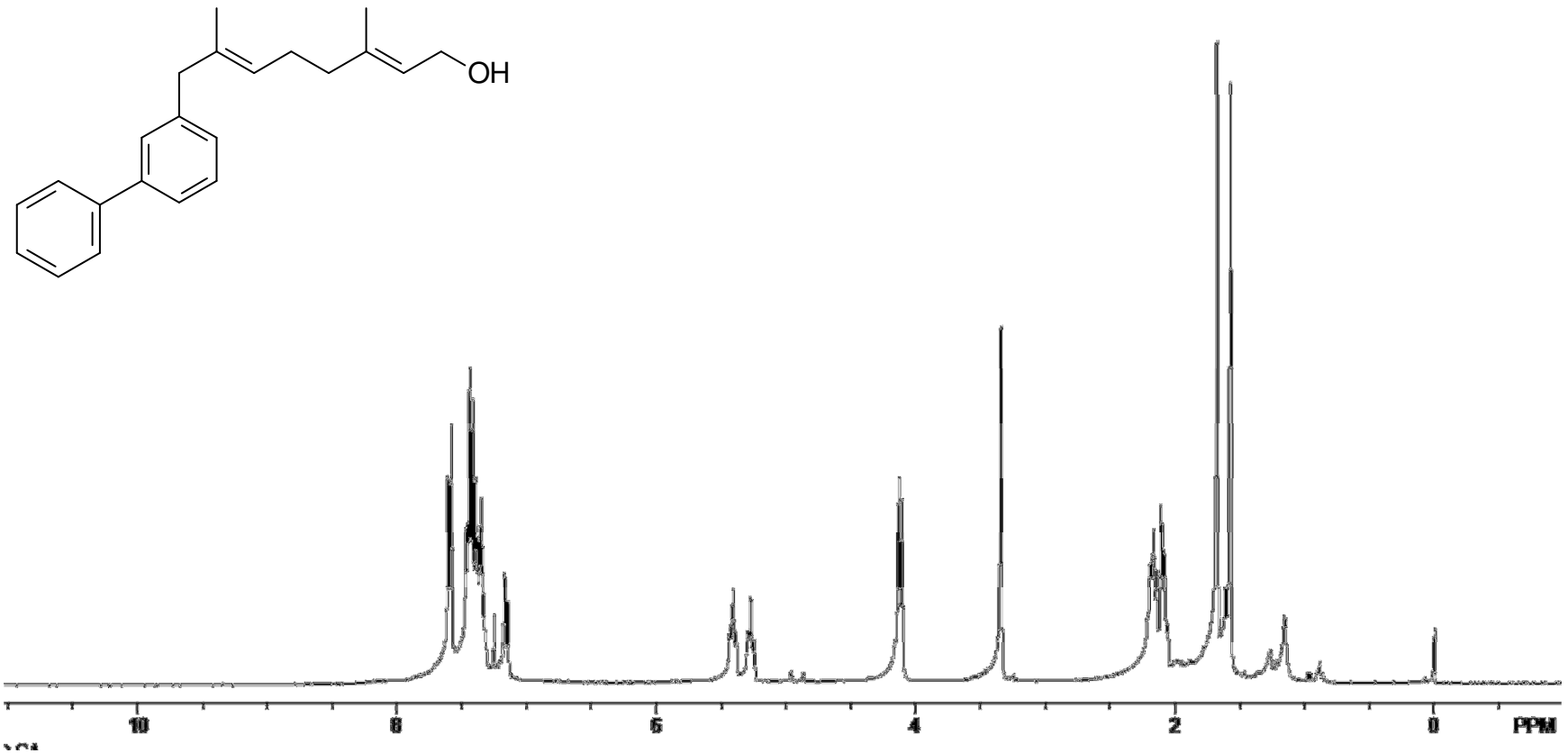


Figure A5. ^{1}H NMR spectrum of alcohol **37**.

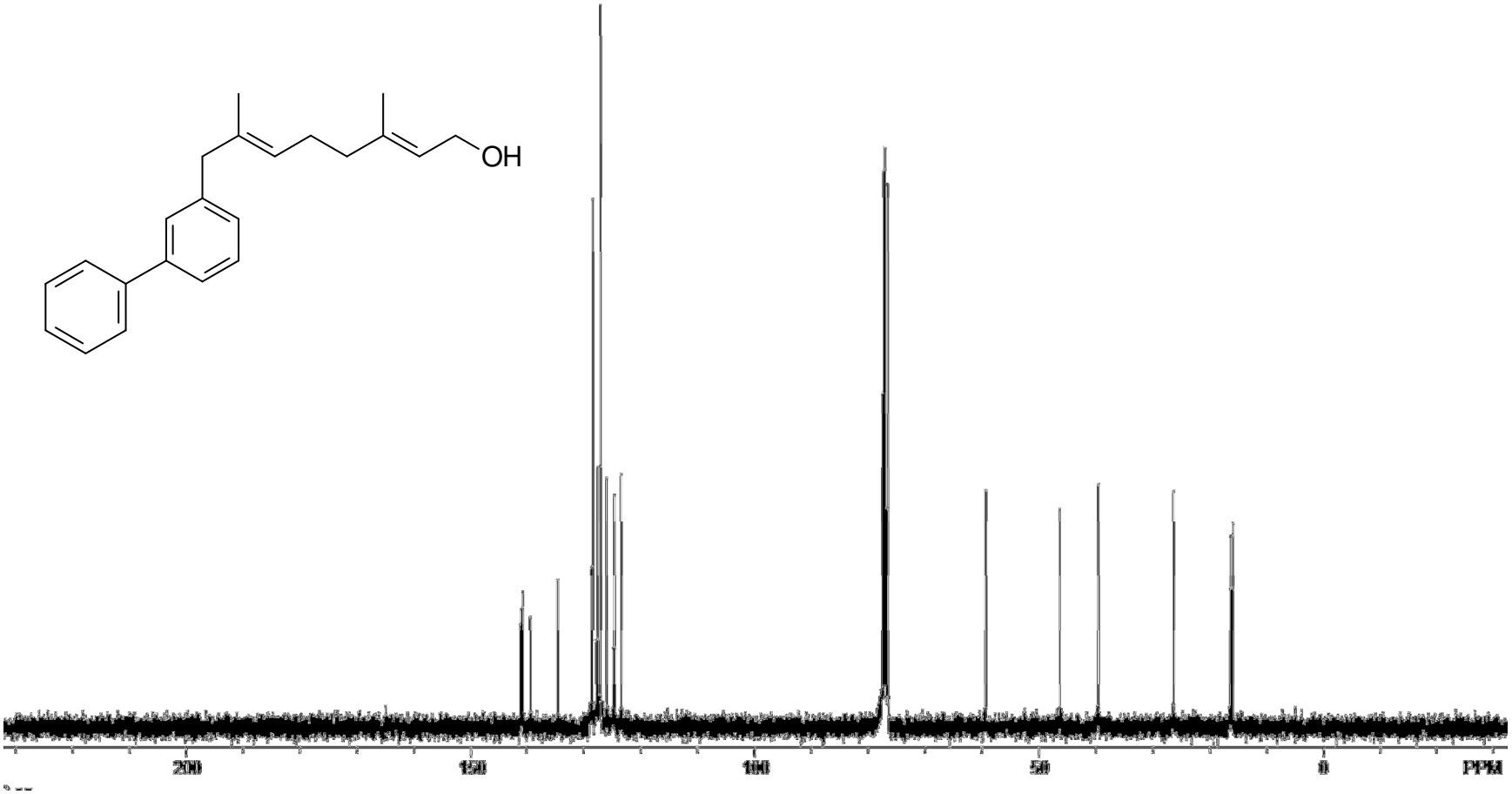


Figure A6. ^{13}C NMR spectrum of alcohol **37**.

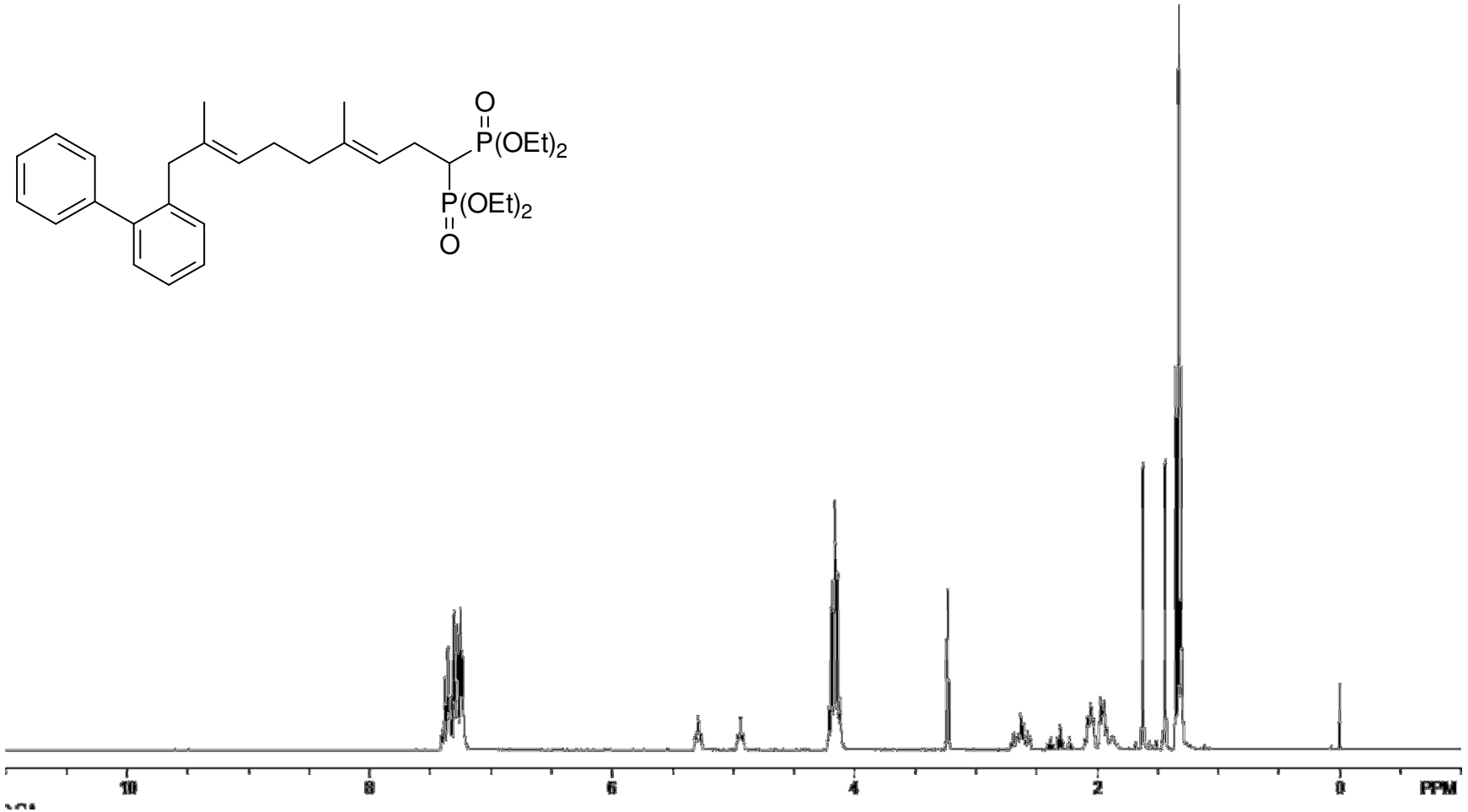


Figure A7. ^{1}H NMR spectrum of bisphosphonate **42**.

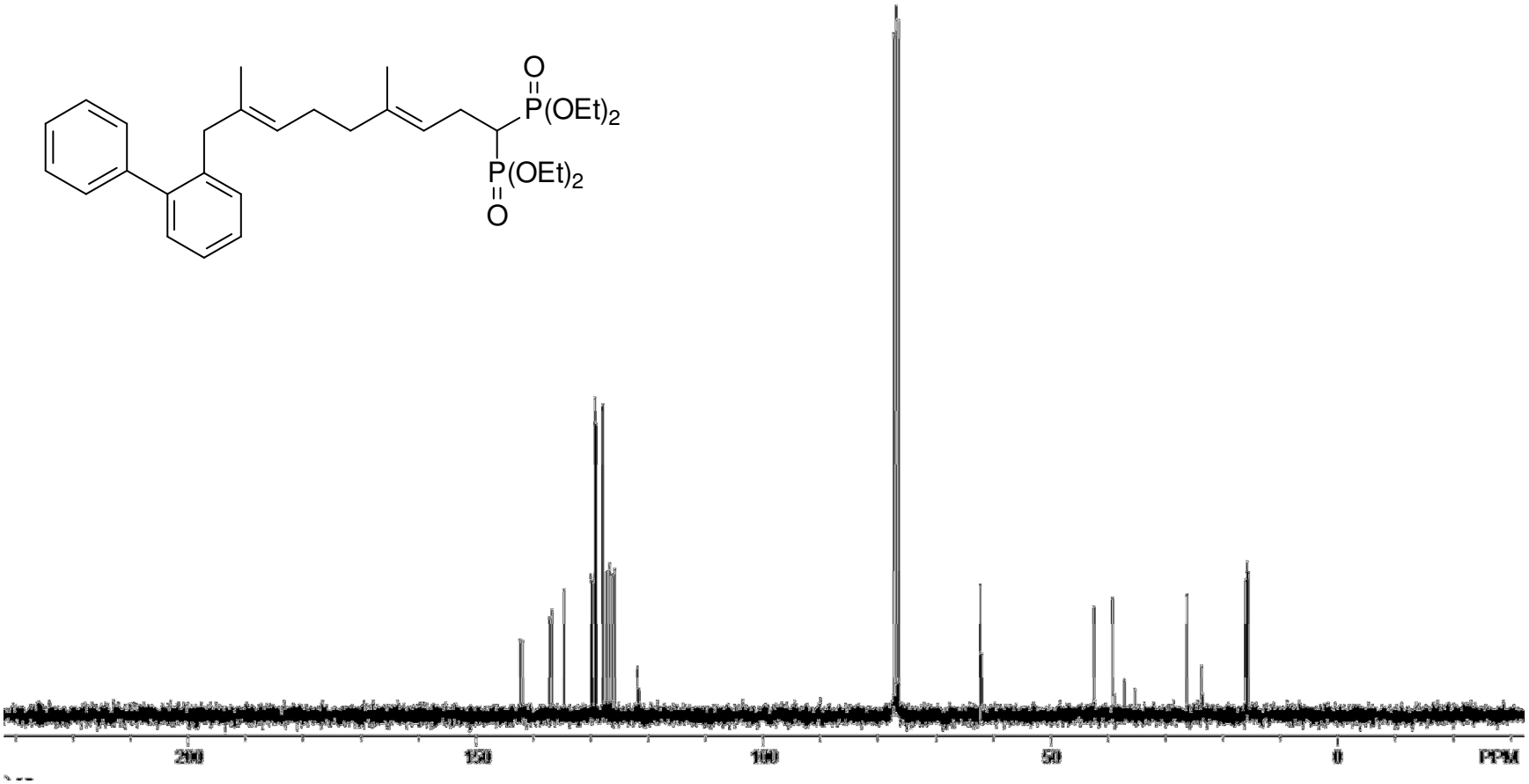


Figure A8. ^{13}C NMR spectrum of bisphosphonate **42**.

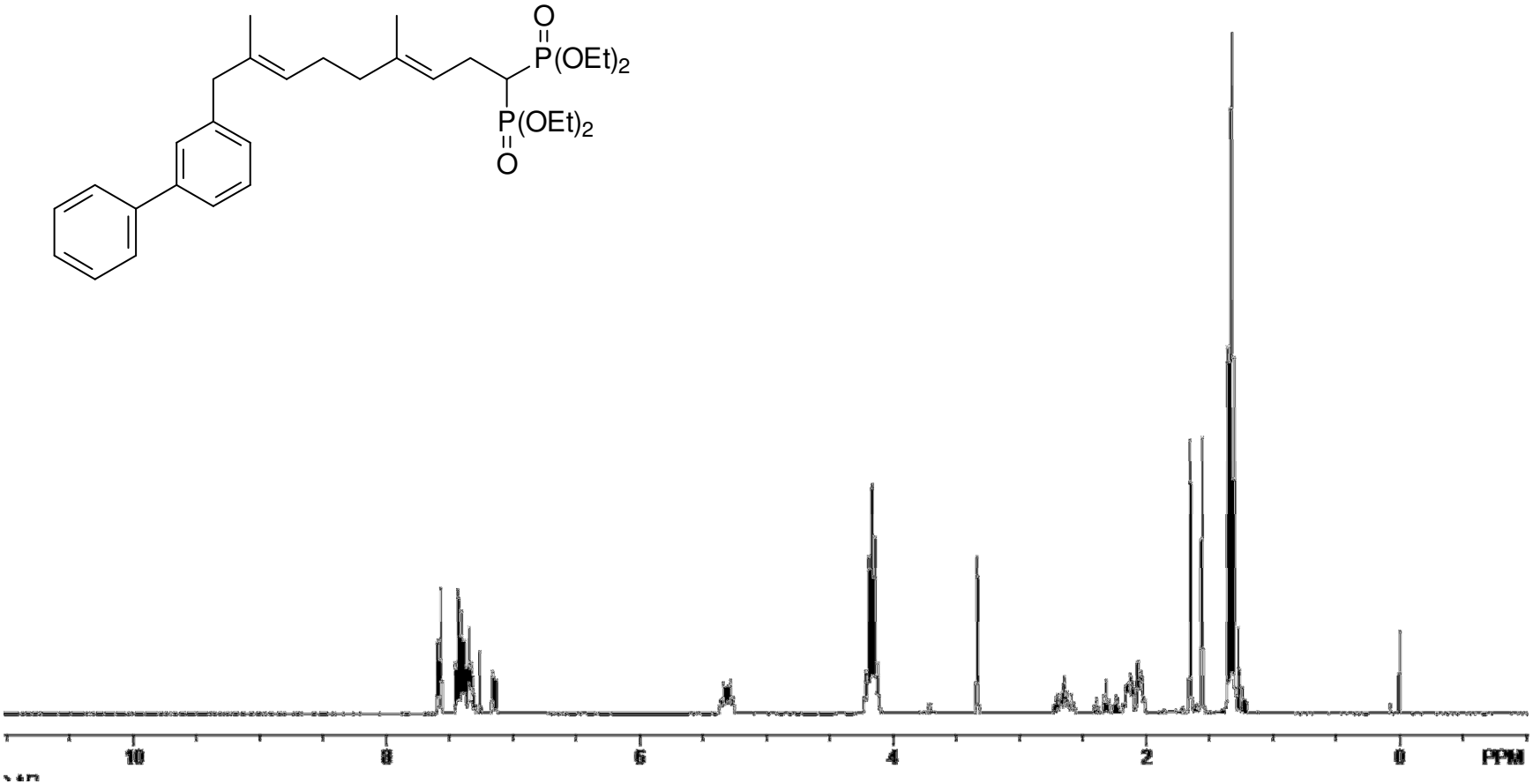


Figure A9. ^{1}H NMR spectrum of bisphosphonate **43**.

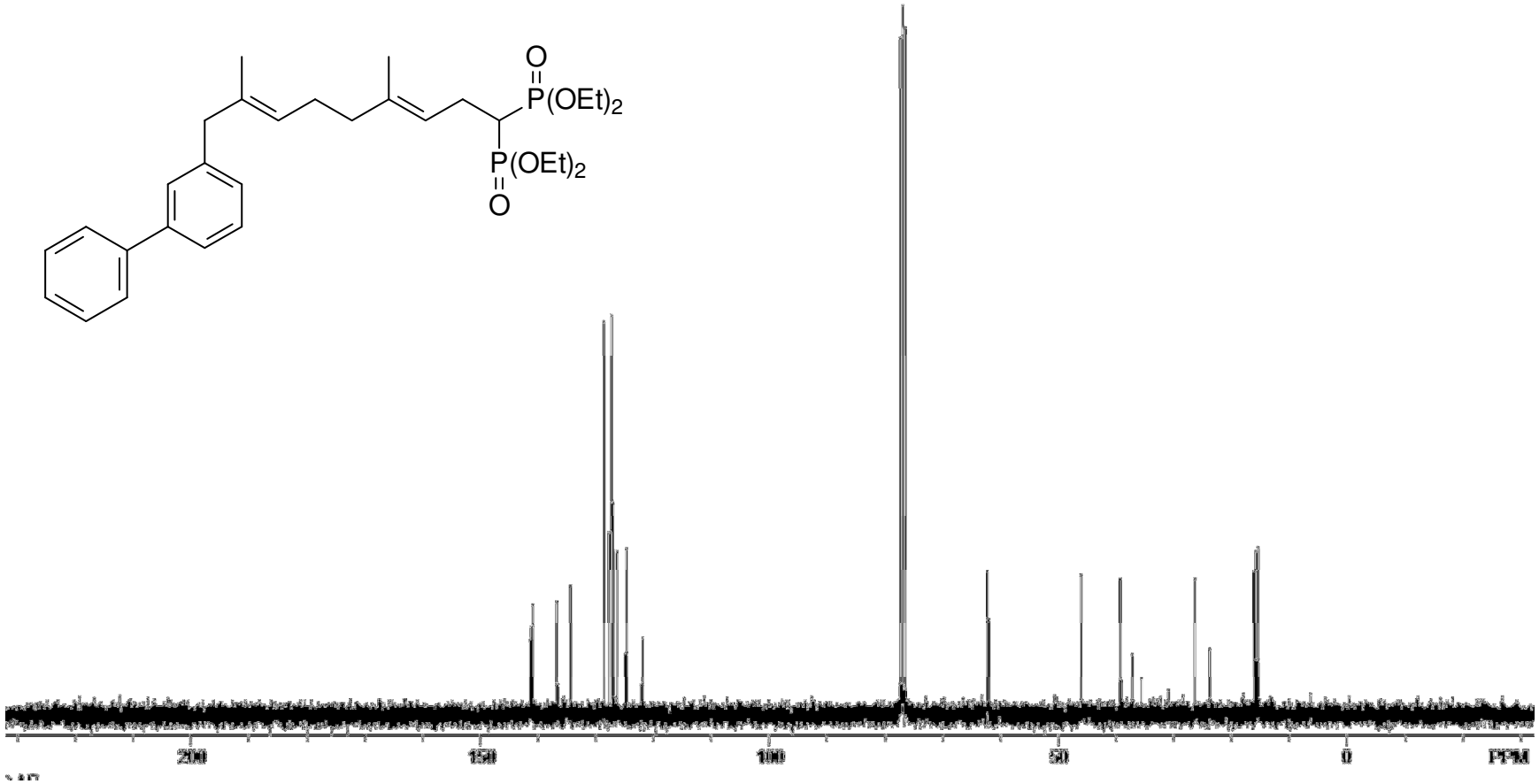


Figure A10. ^{13}C NMR spectrum of bisphosphonate **43**.

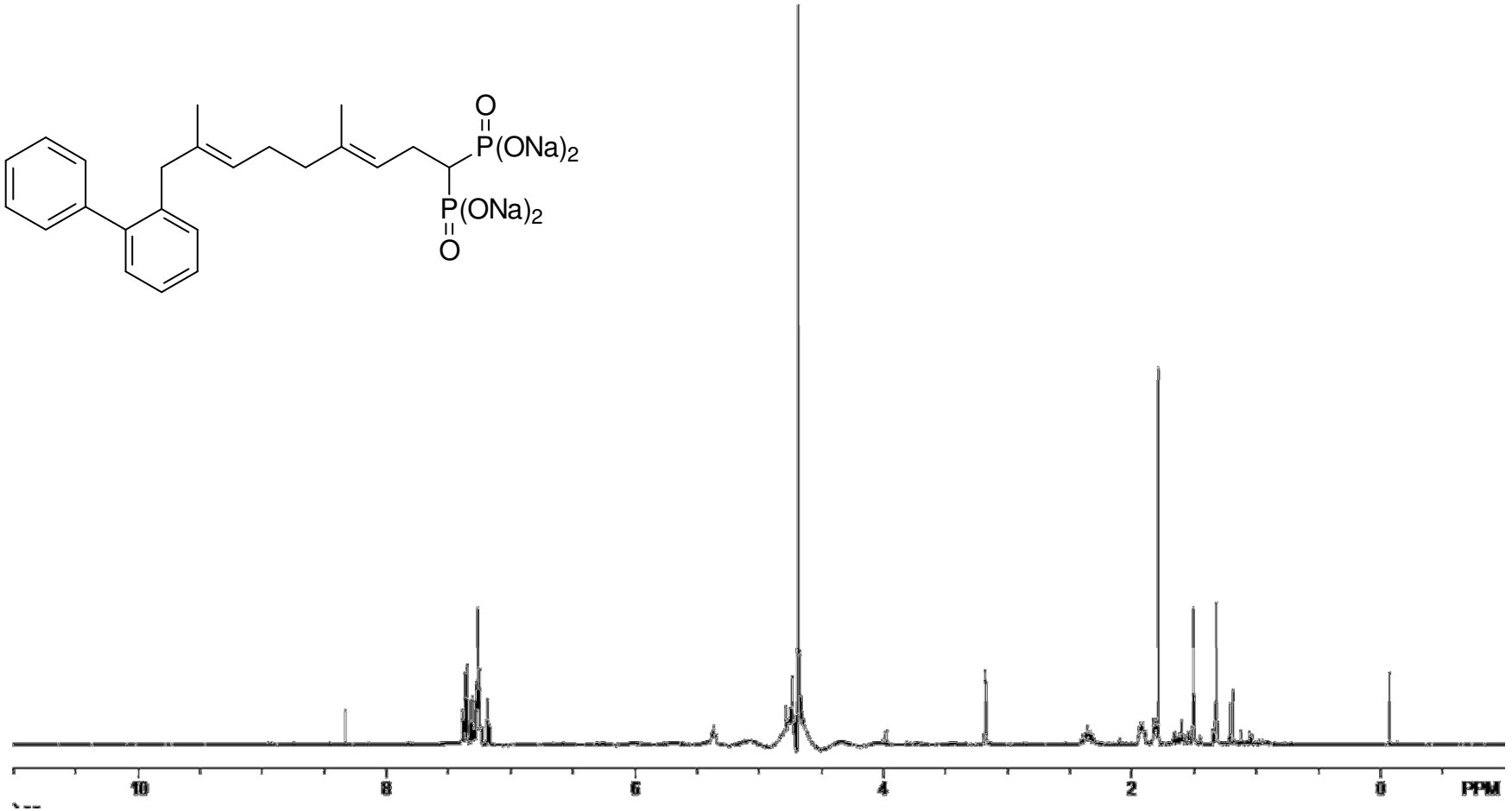


Figure A11. ^{1}H NMR spectrum of bisphosphonate **23**.

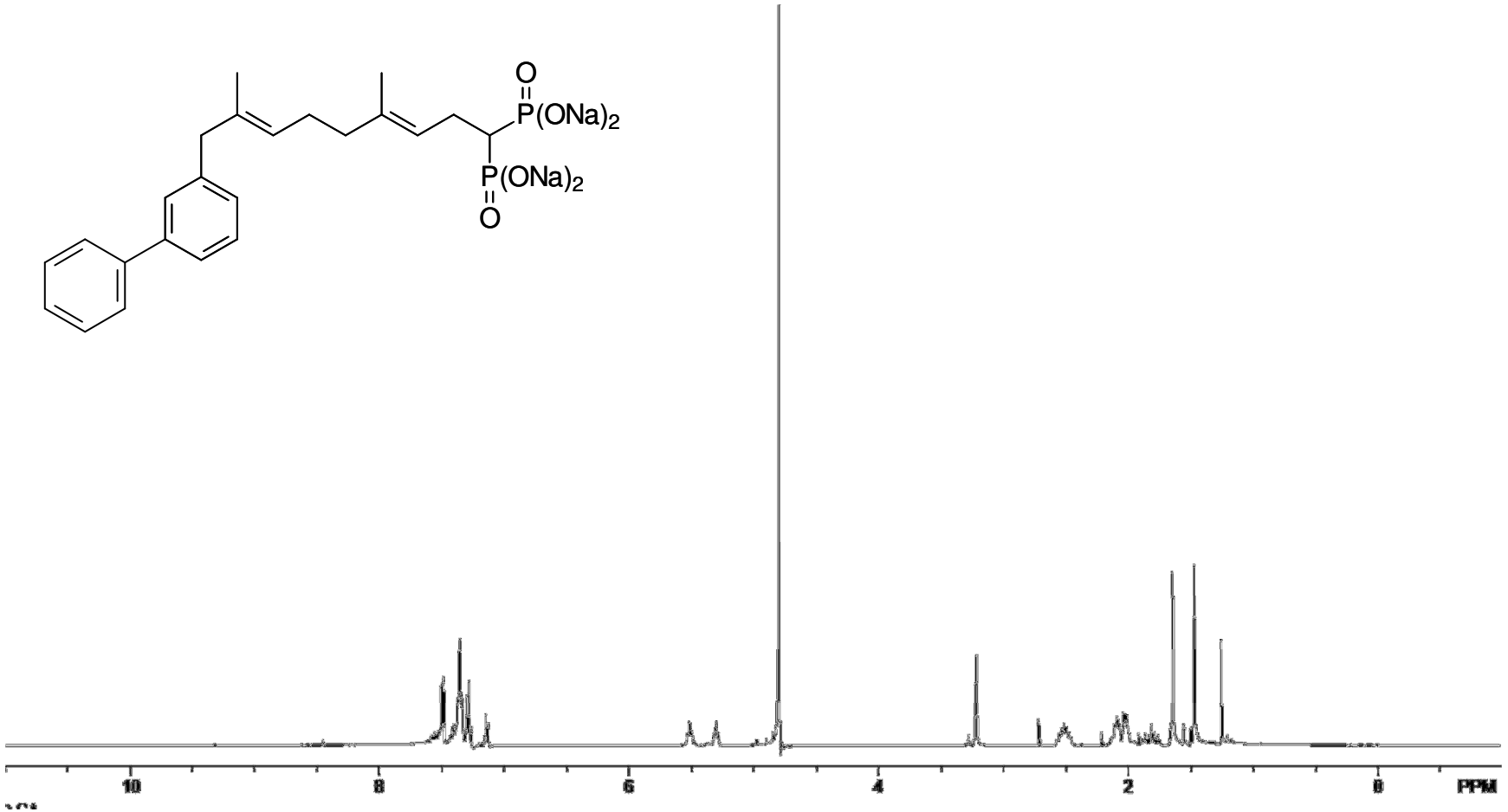


Figure A12. ^{1}H NMR spectrum of bisphosphonate **24**.

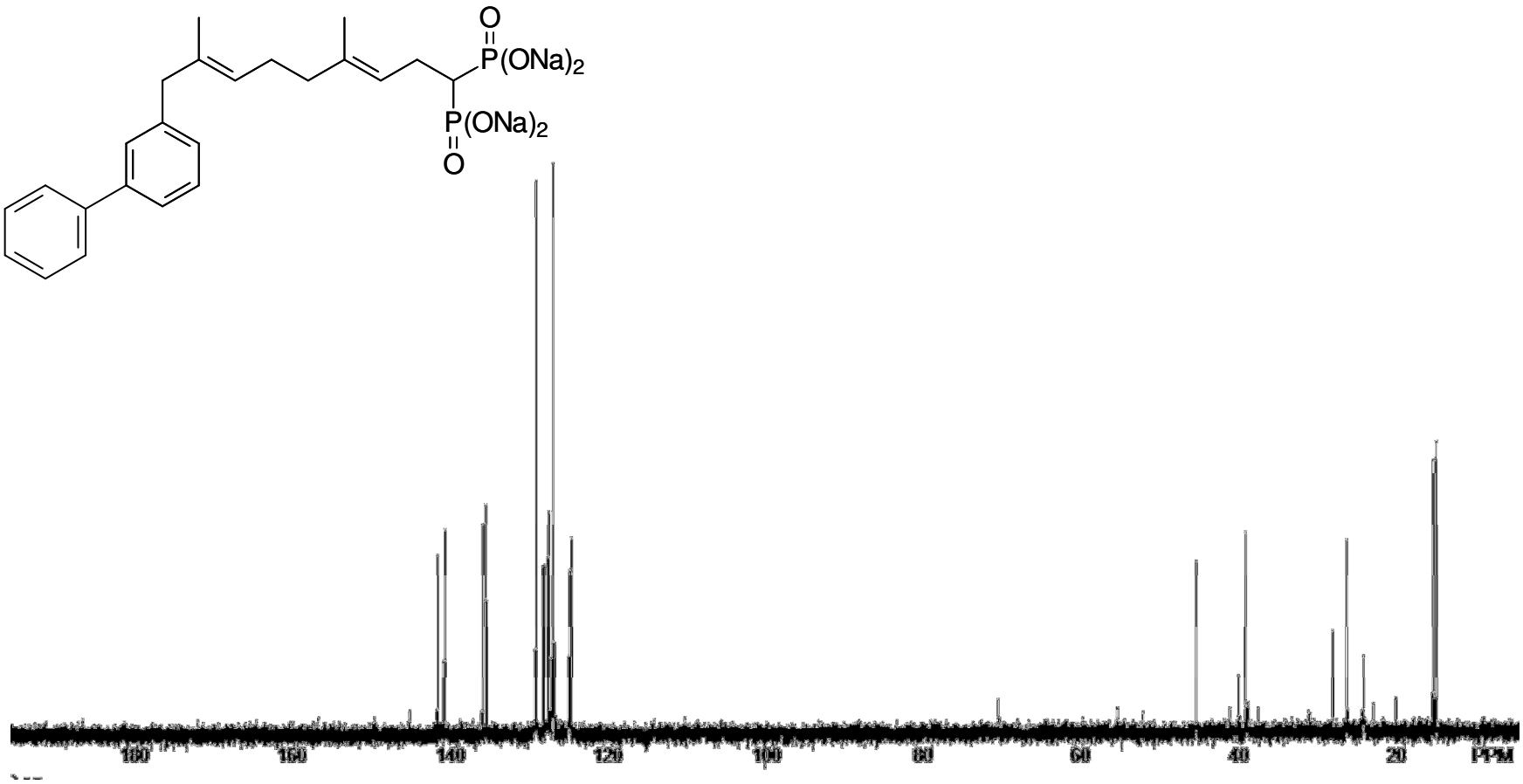


Figure A13. ^{13}C NMR spectrum of bisphosphonate **24**.

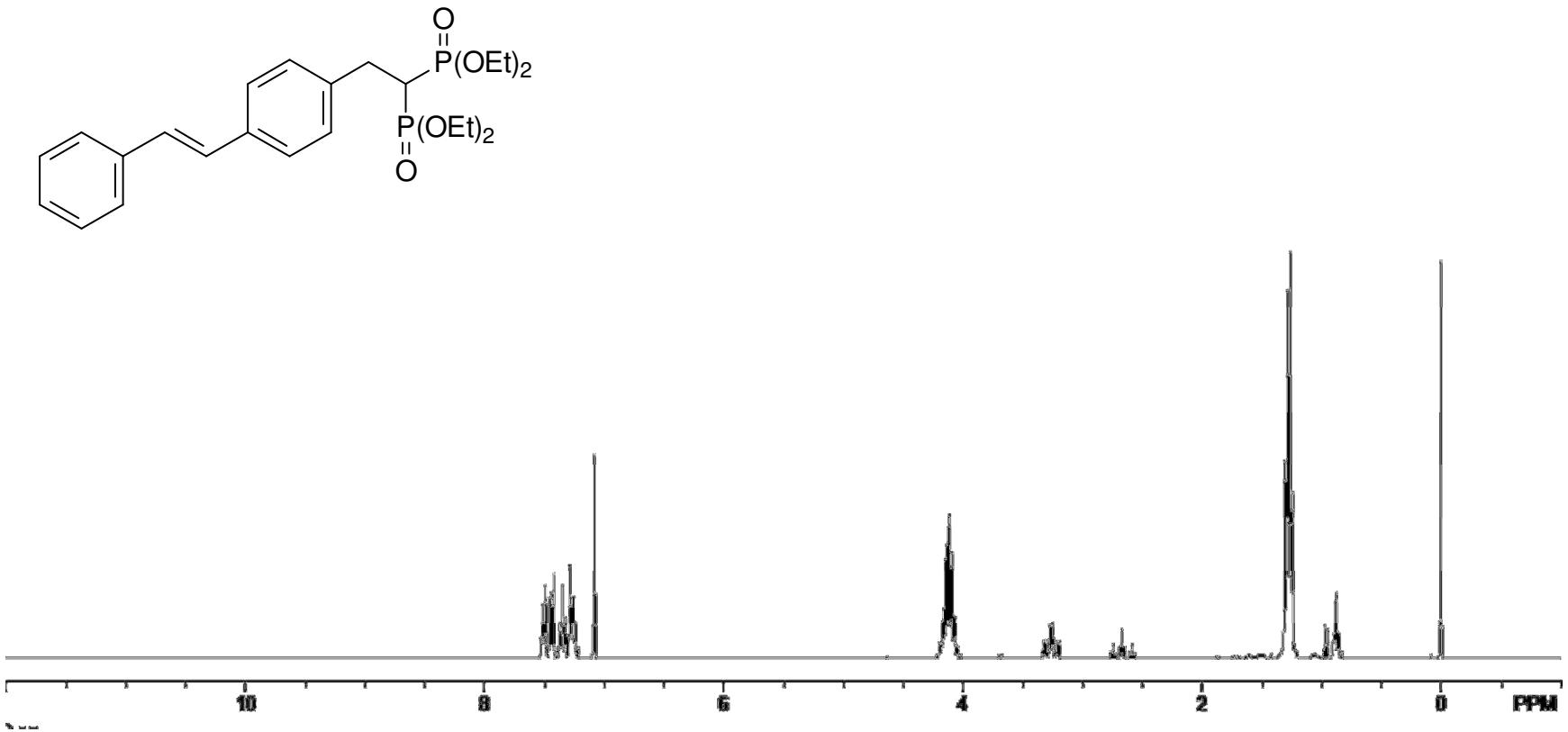


Figure A14. ^{1}H NMR spectrum of bisphosphonate **52**.

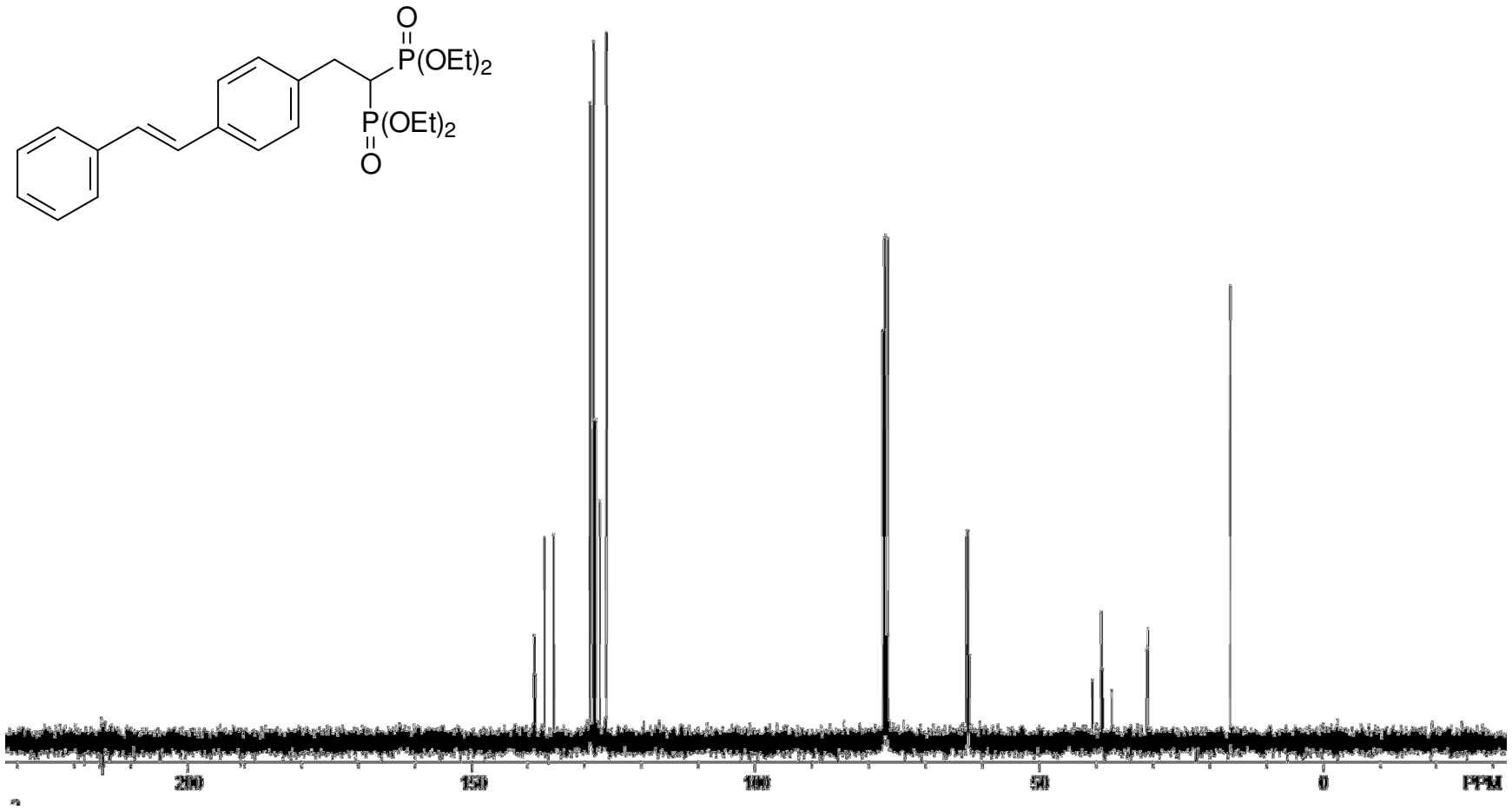


Figure A15. ^{13}C NMR spectrum of bisphosphonate **52**.

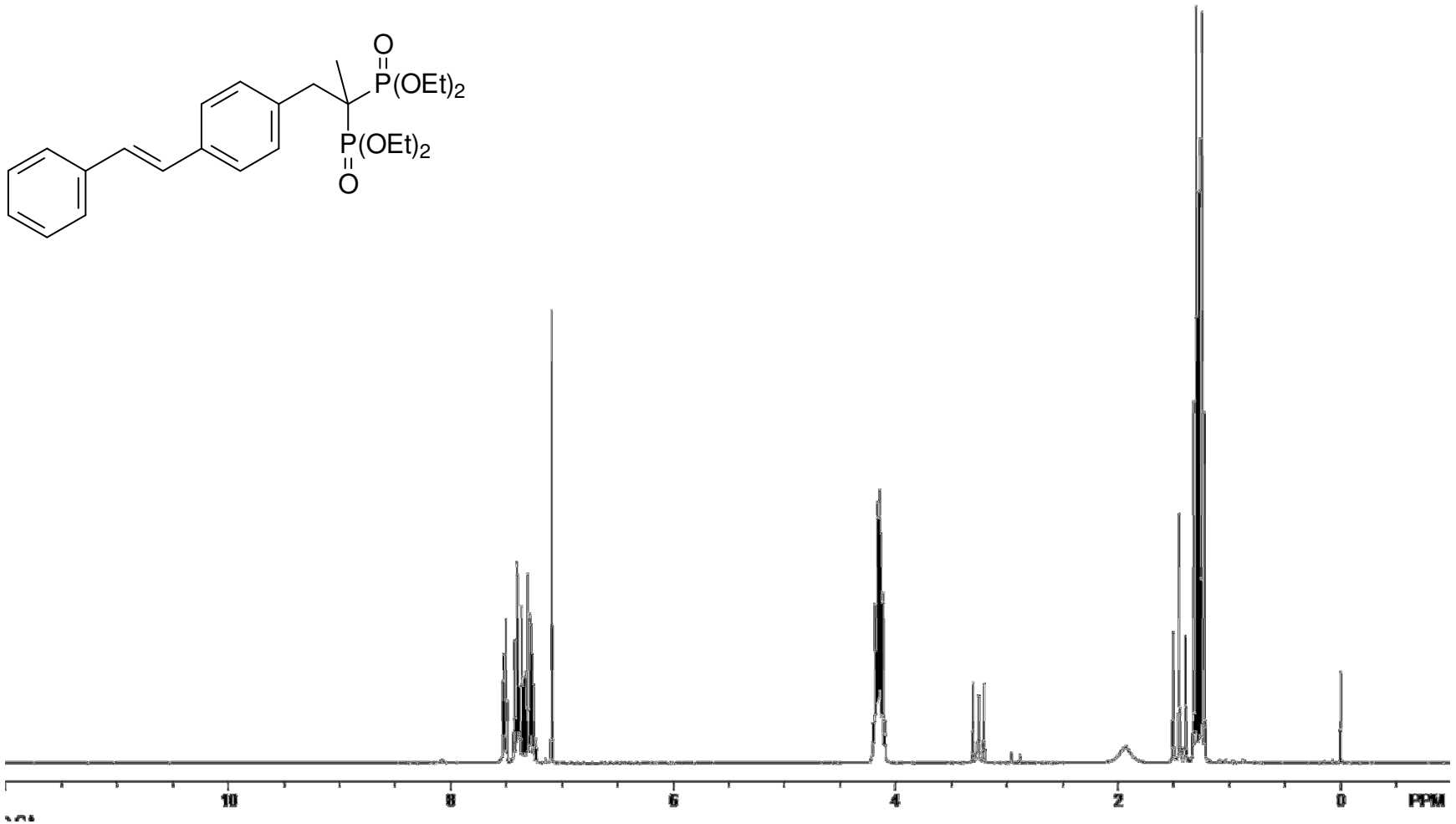


Figure A16. ^{1}H NMR spectrum of bisphosphonate **54**.

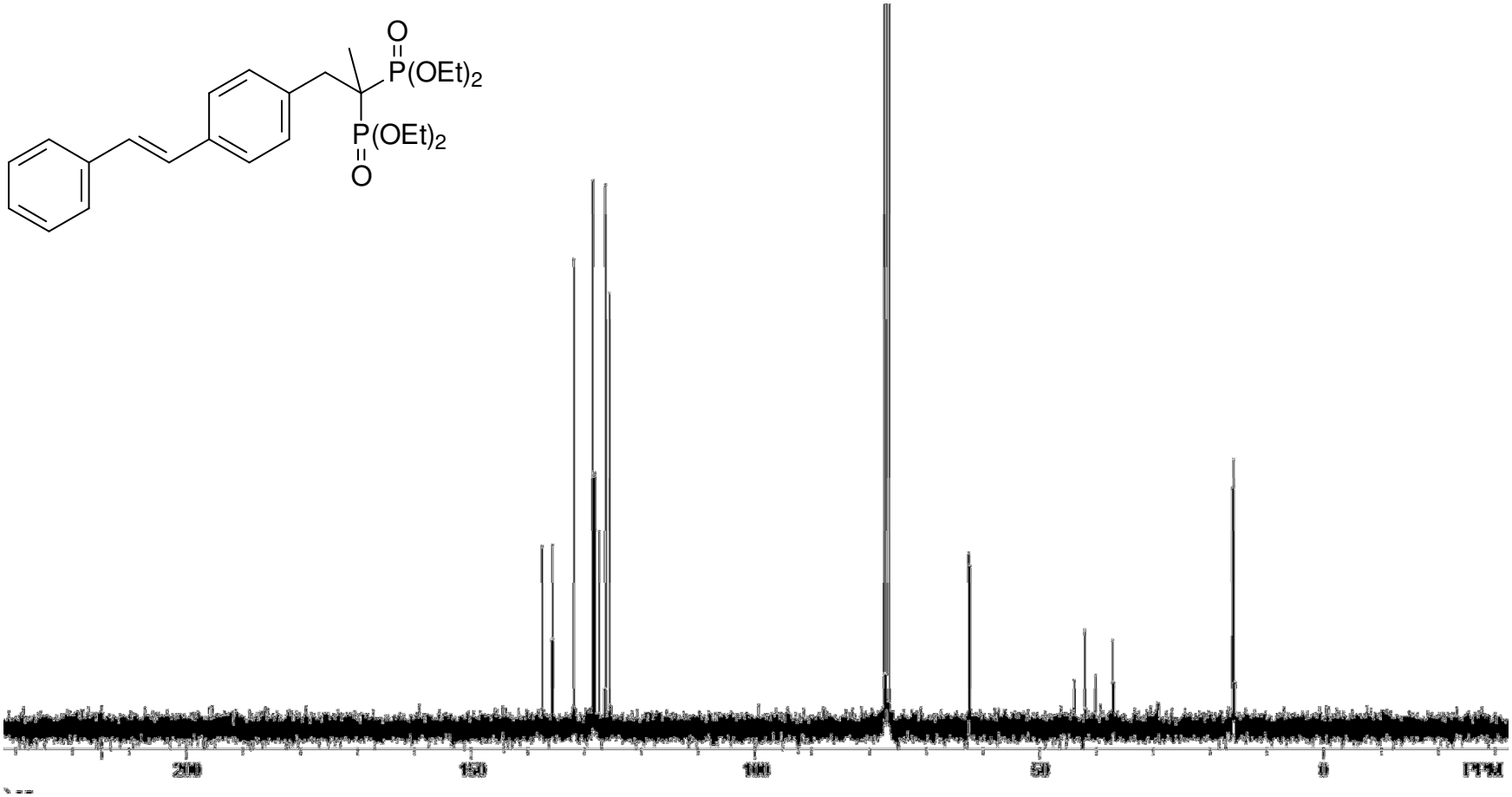


Figure A17. ^{13}C NMR spectrum of bisphosphonate **54**.

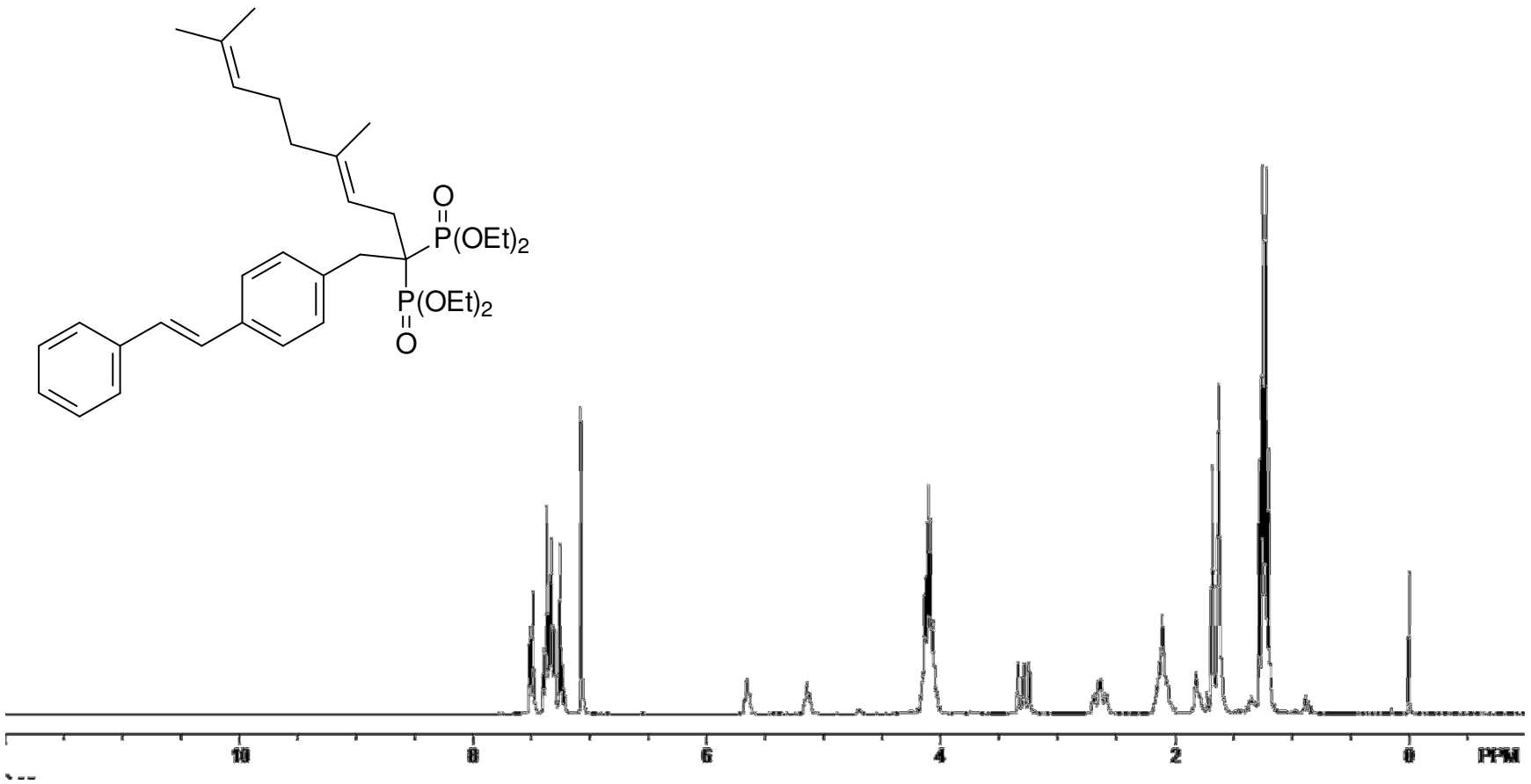


Figure A18. ^{1}H NMR spectrum of bisphosphonate **53**.

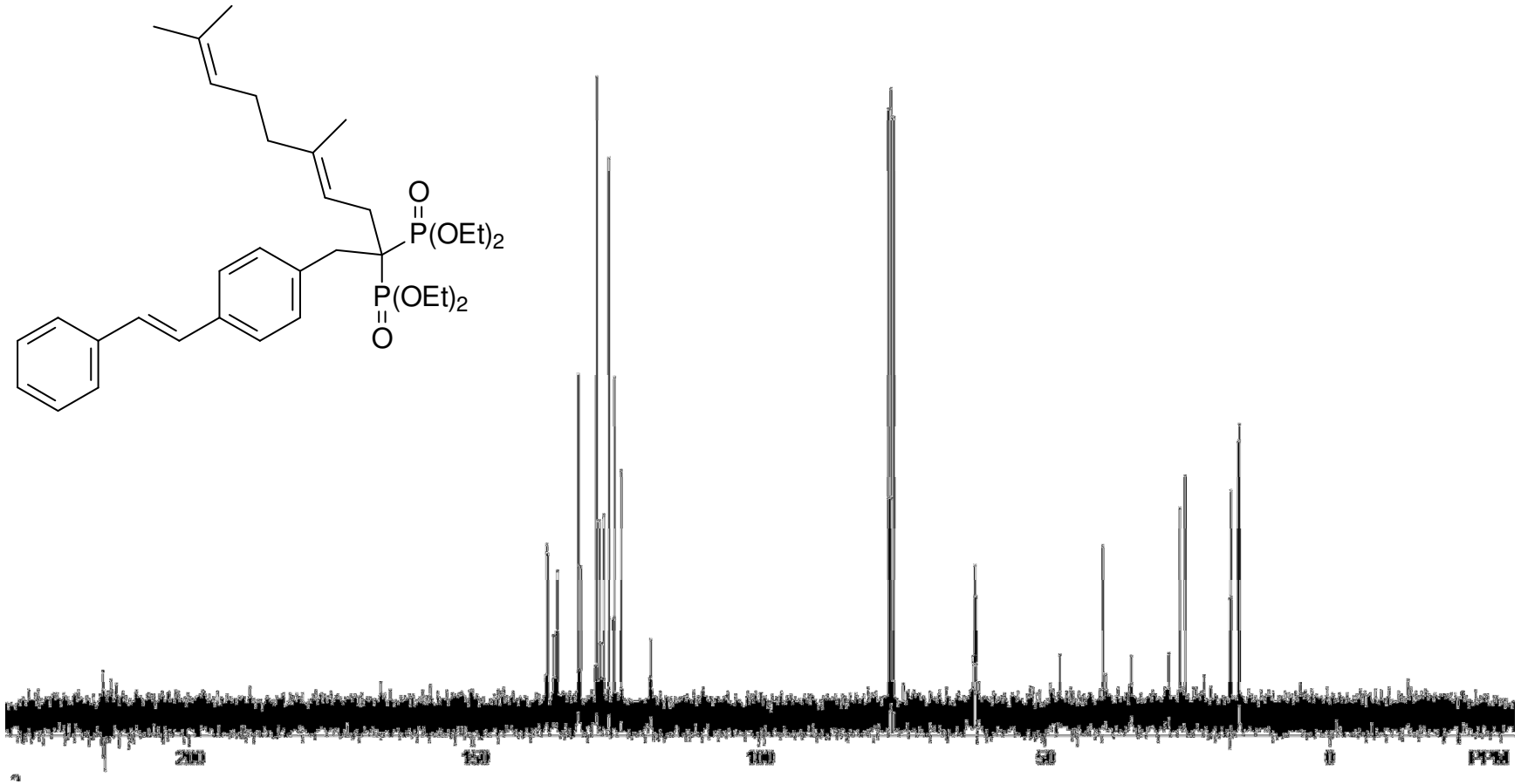


Figure A19. ^{13}C NMR spectrum of bisphosphonate **53**.

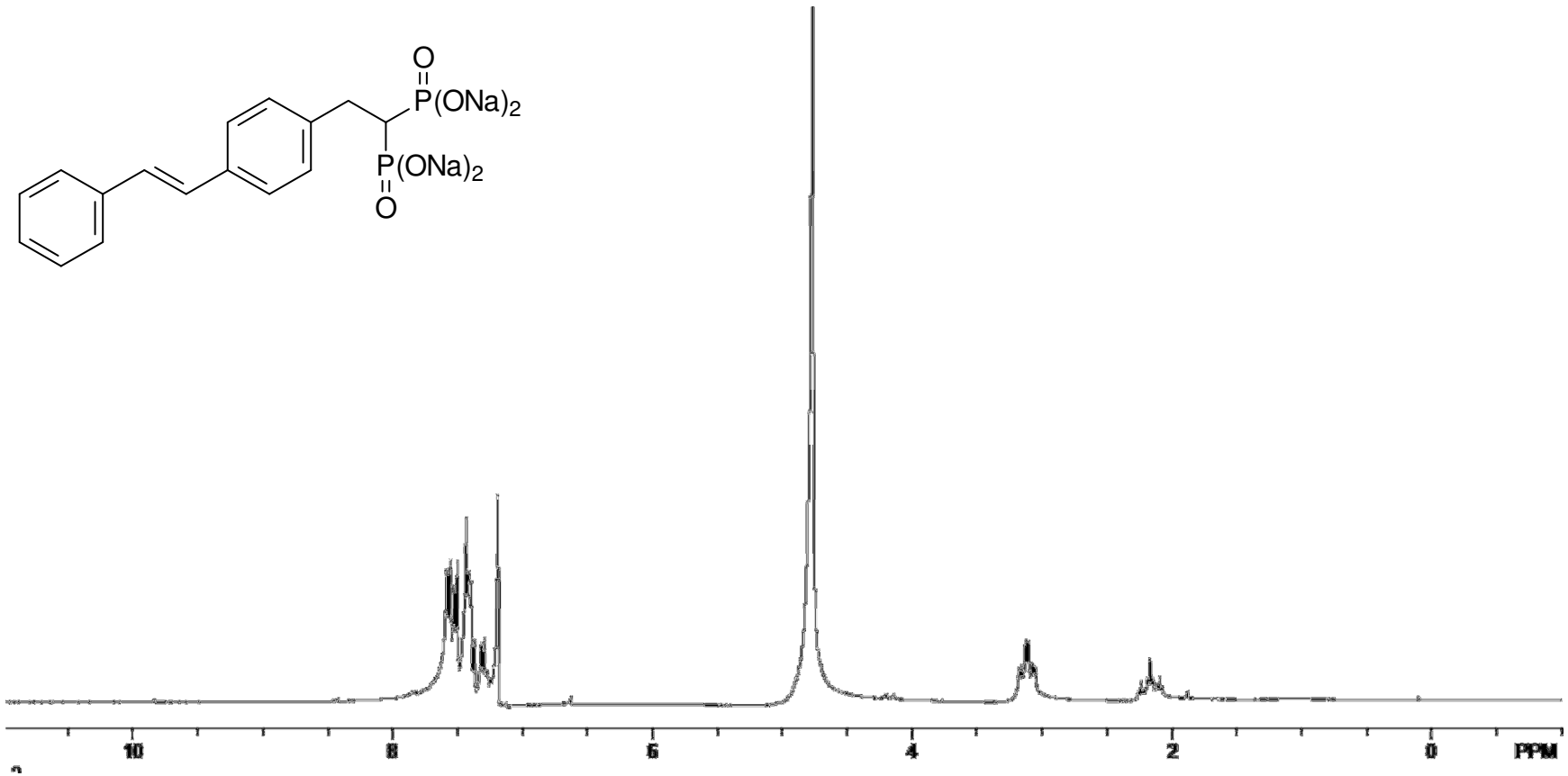


Figure A20. ^{1}H NMR spectrum of bisphosphonate **45**.

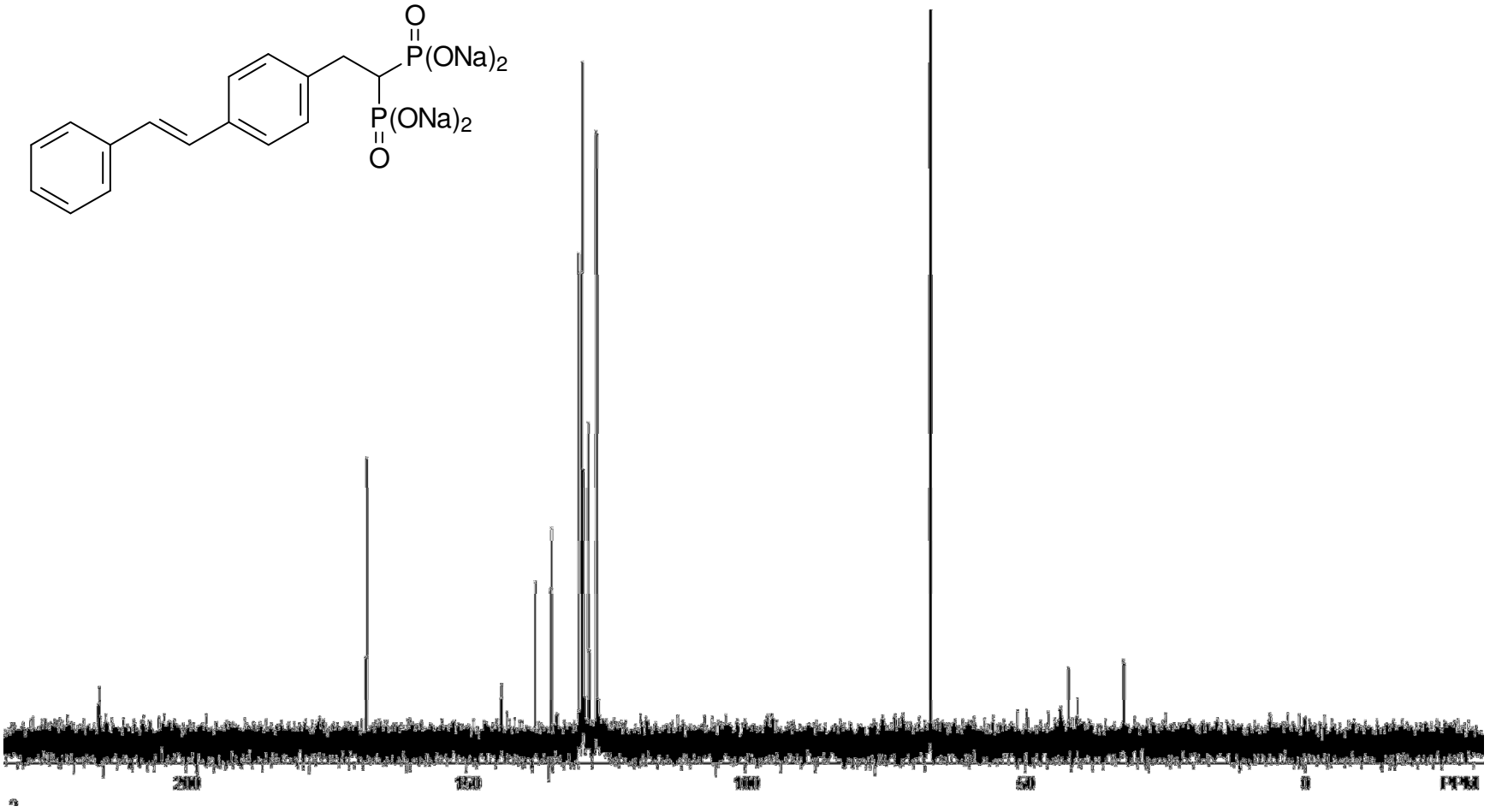


Figure A21. ^{13}C NMR spectrum of bisphosphonate **45**.

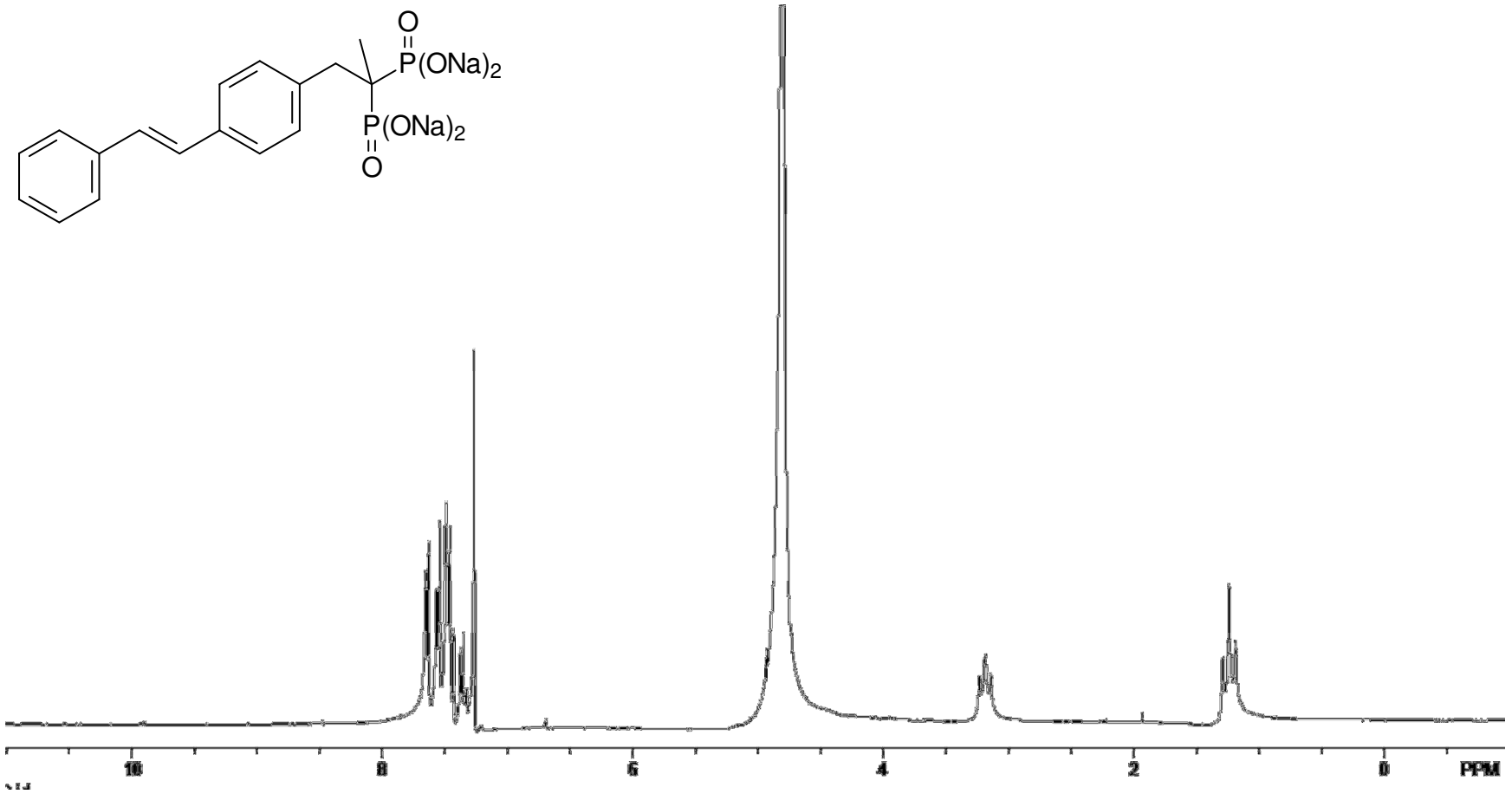


Figure A22. ^{1}H NMR spectrum of bisphosphonate **46**.

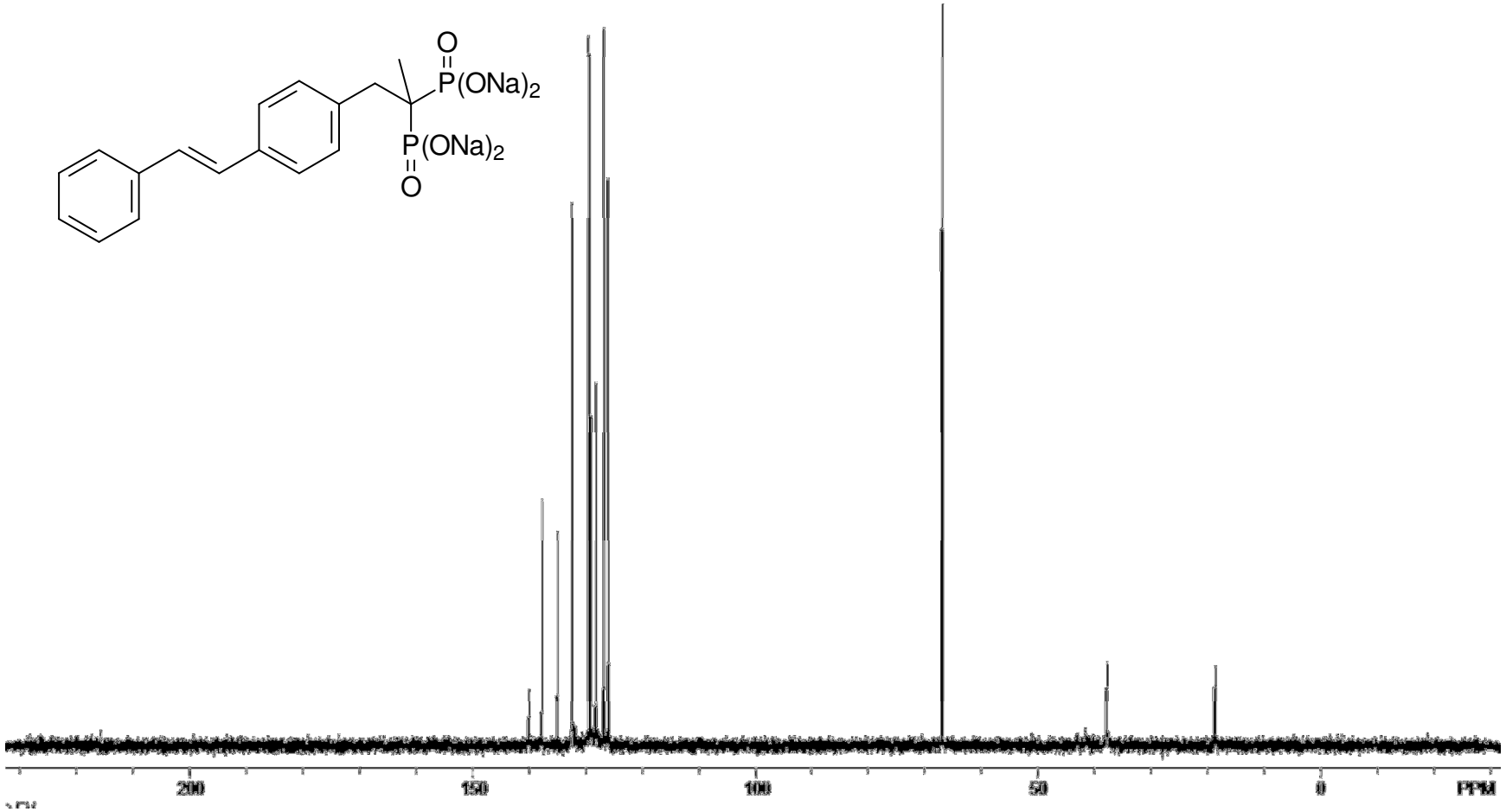


Figure A23. ^{13}C NMR spectrum of bisphosphonate **46**.

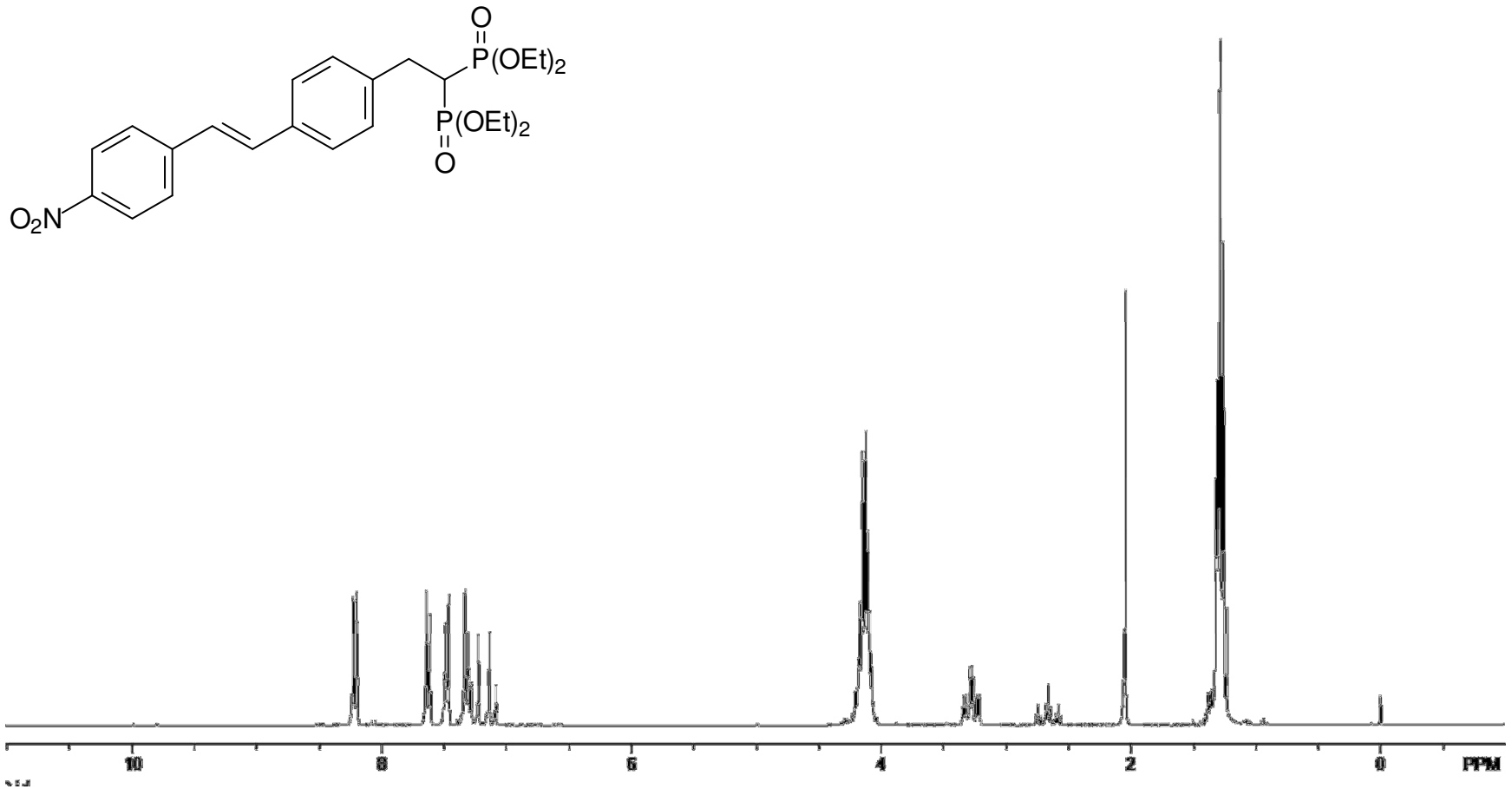


Figure A24. ^{1}H NMR spectrum of bisphosphonate **65**.

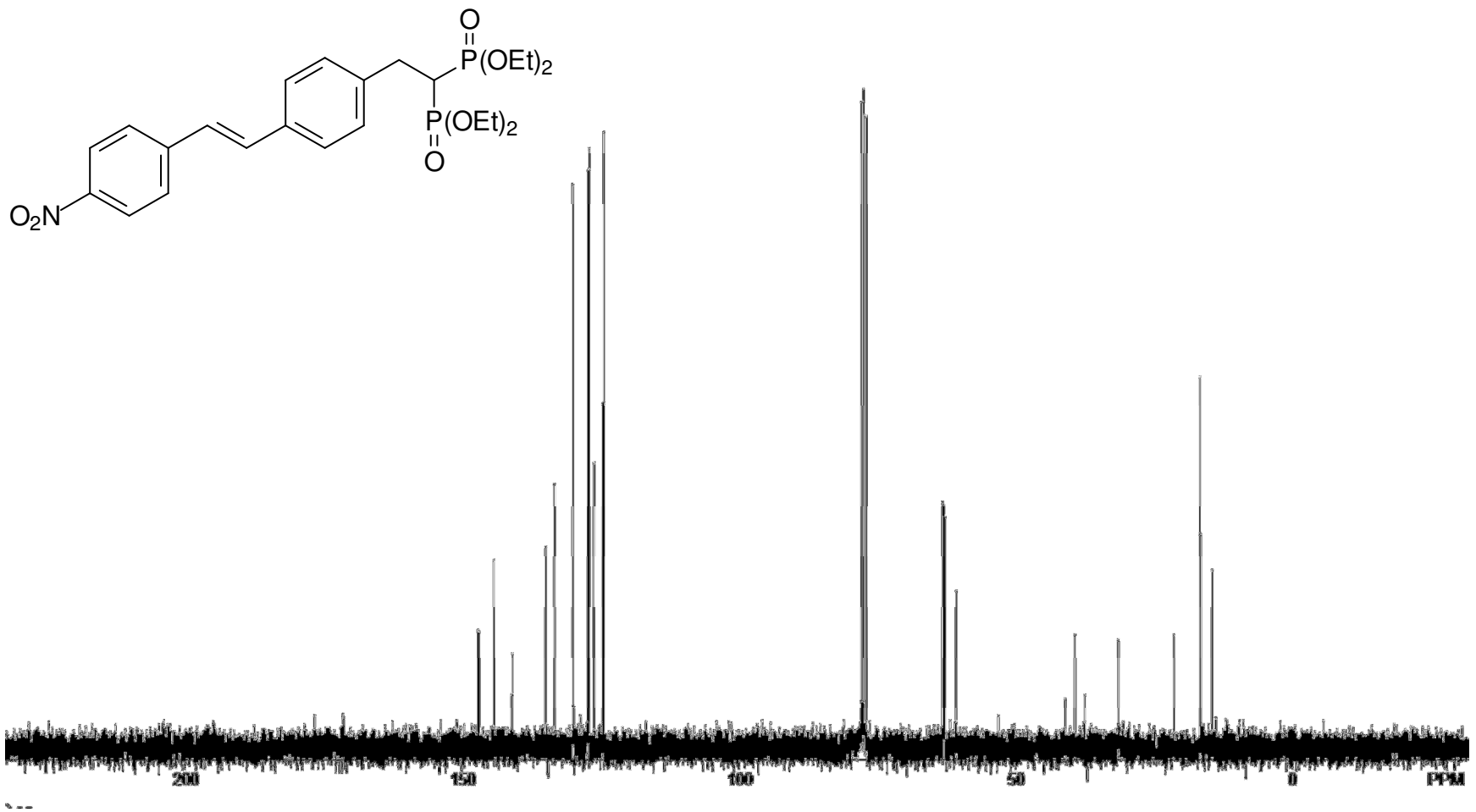


Figure A25. ^{13}C NMR spectrum of bisphosphonate **65**.

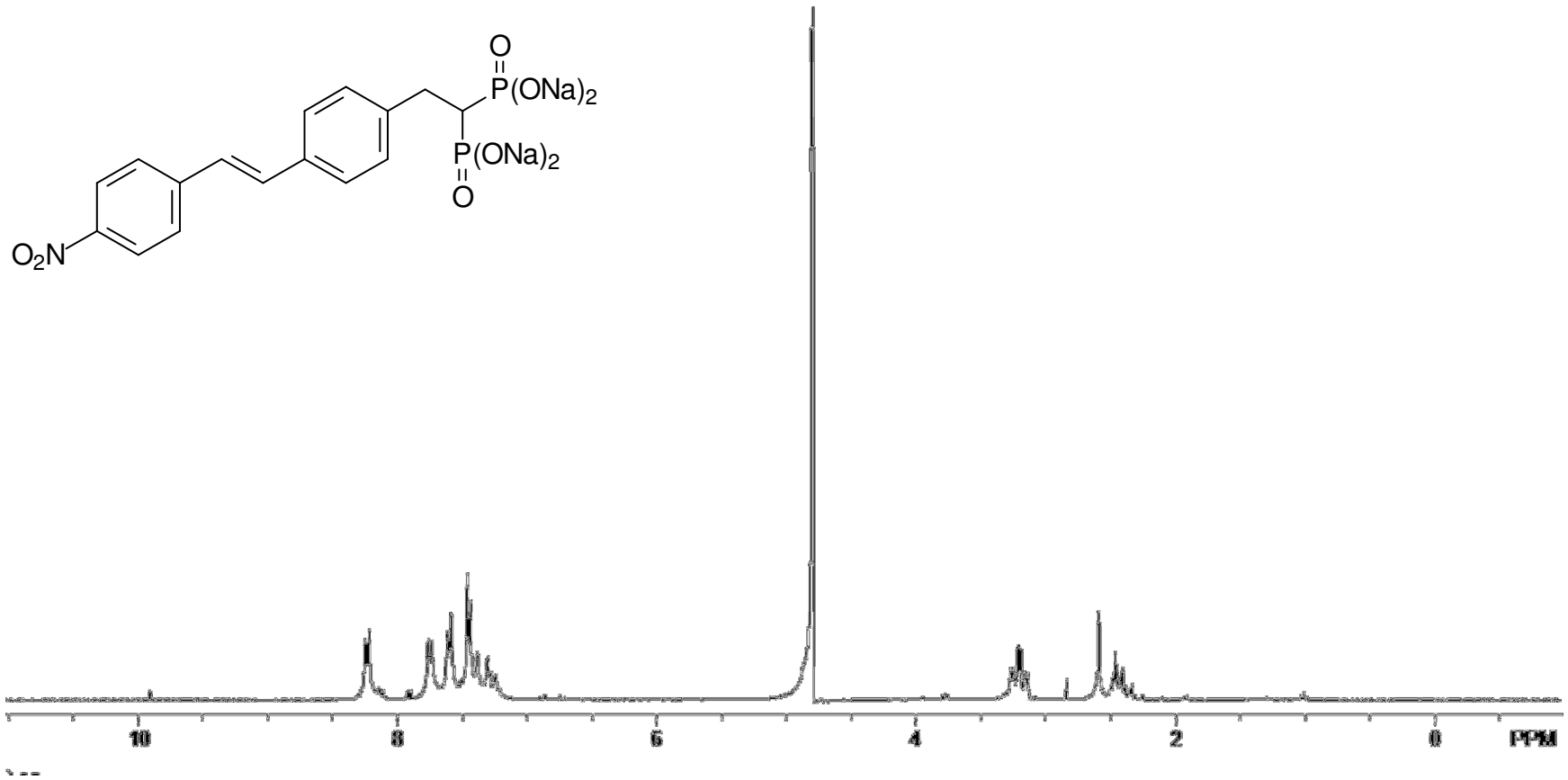


Figure A26. ^{1}H NMR spectrum of bisphosphonate **59.**

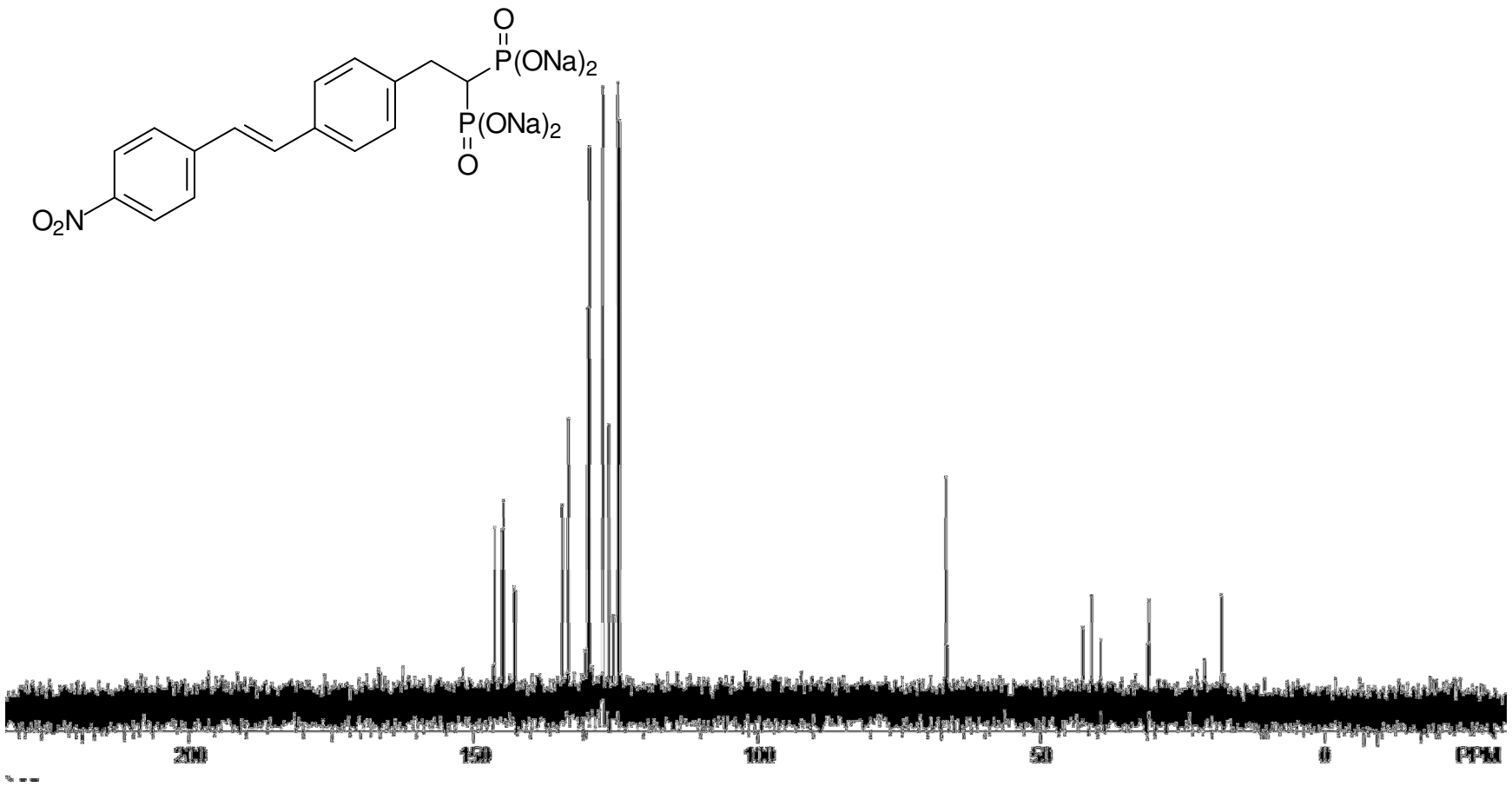


Figure A27. ^{13}C NMR spectrum of bisphosphonate **59.**

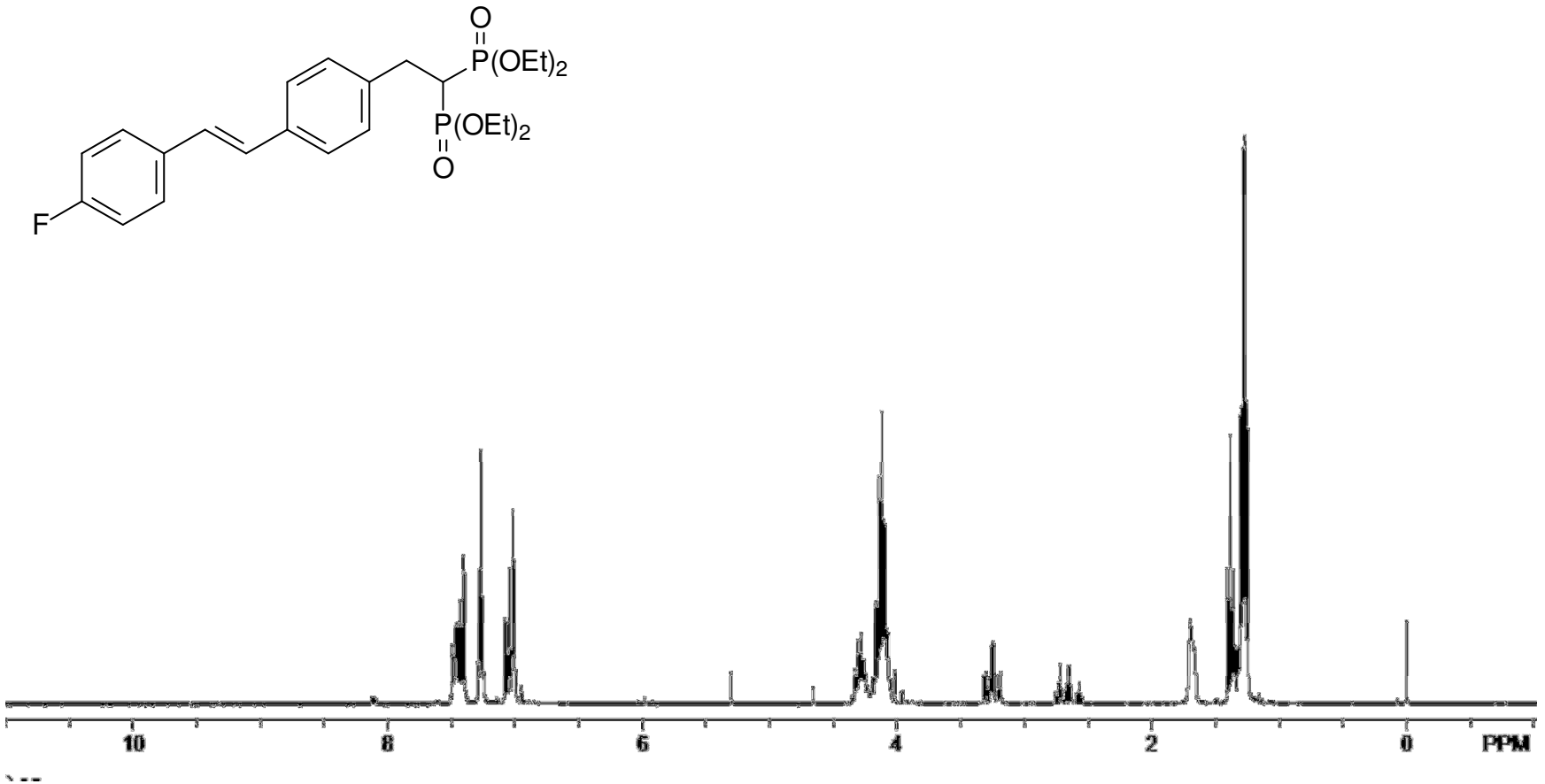


Figure A28. ^{1}H NMR spectrum of bisphosphonate **73.**

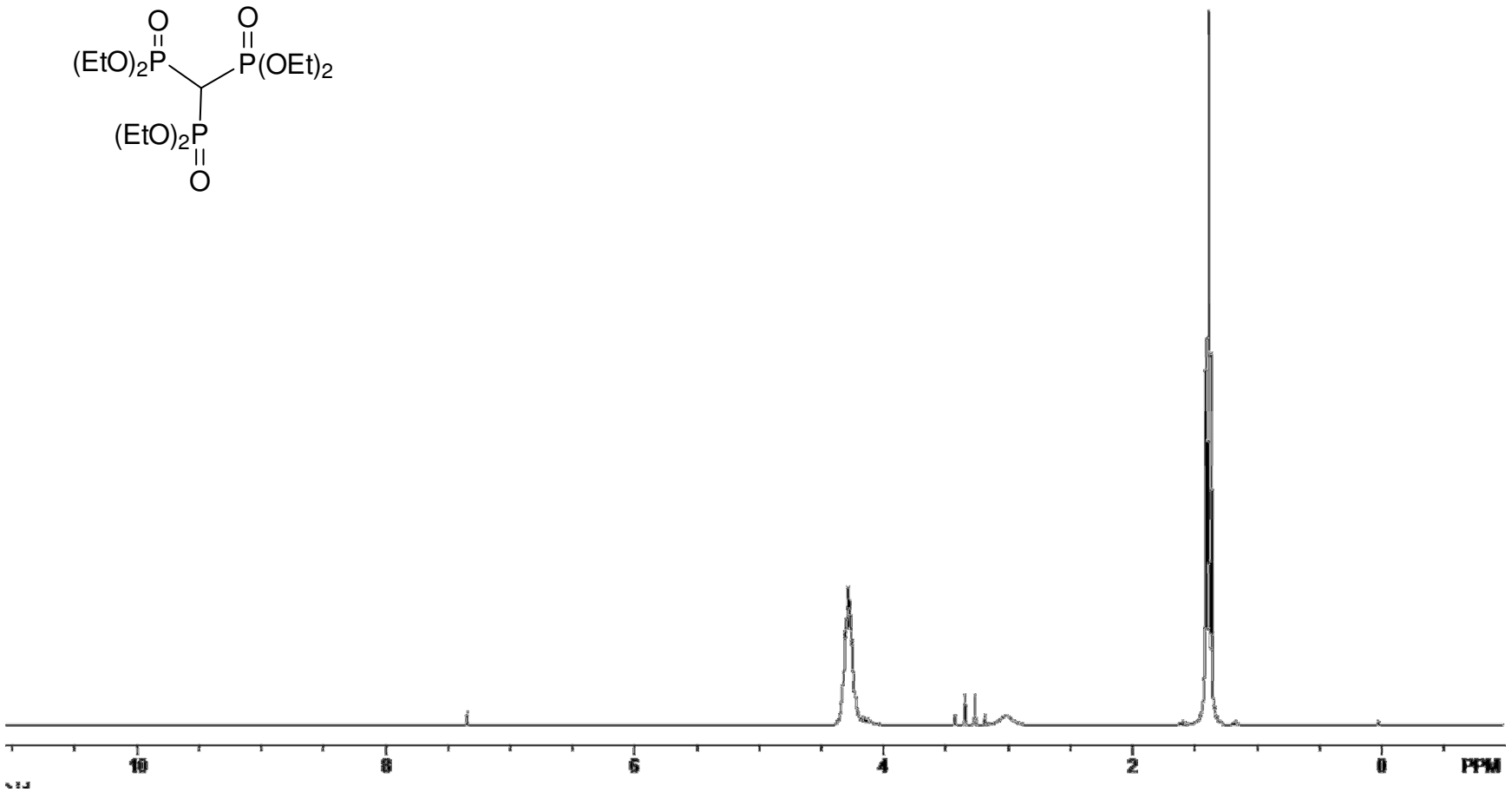


Figure A29. ^{1}H NMR spectrum of trisphosphonate **75.**

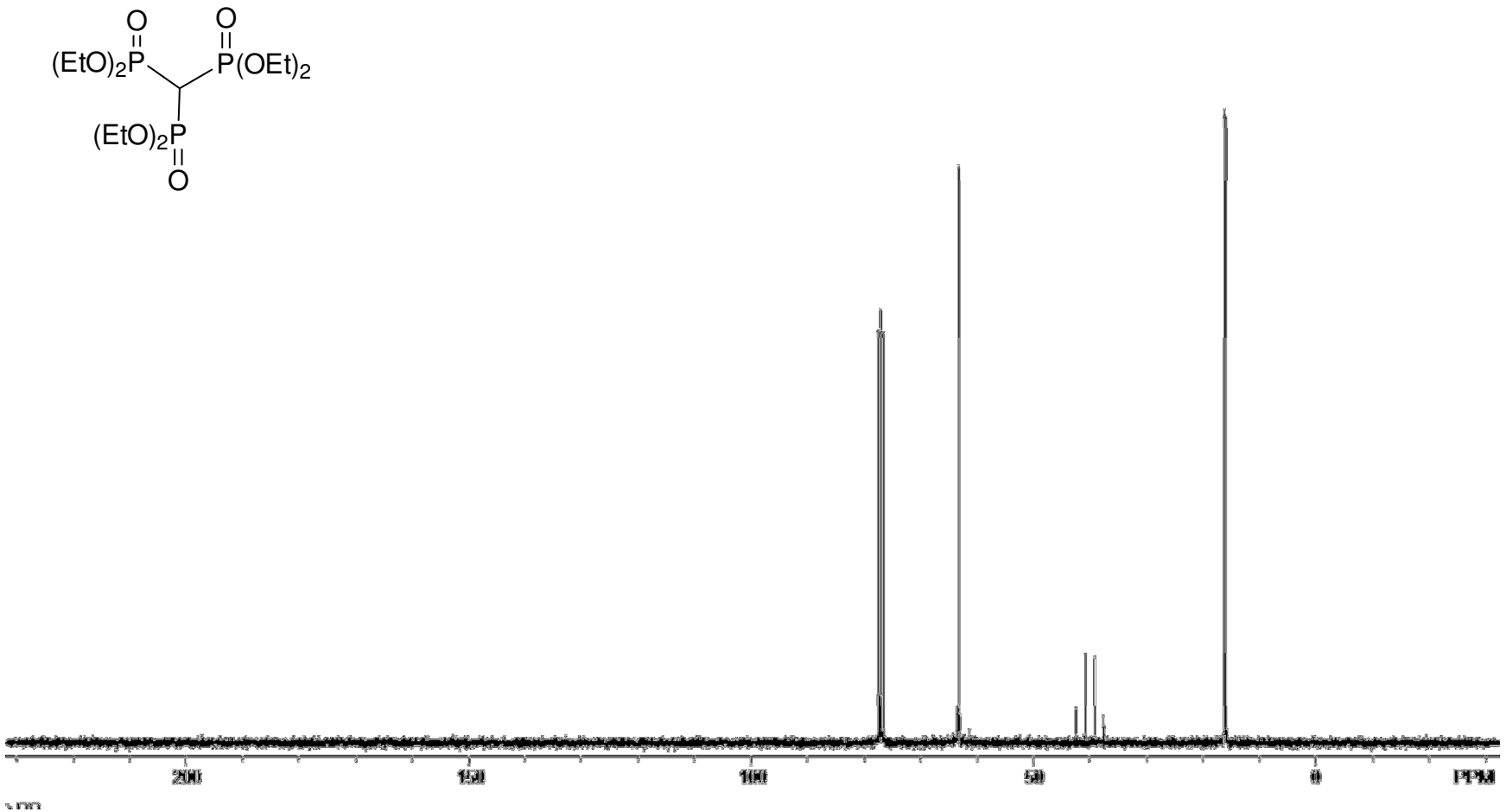


Figure A30. ^{13}C NMR spectrum of trisphosphonate **75.**

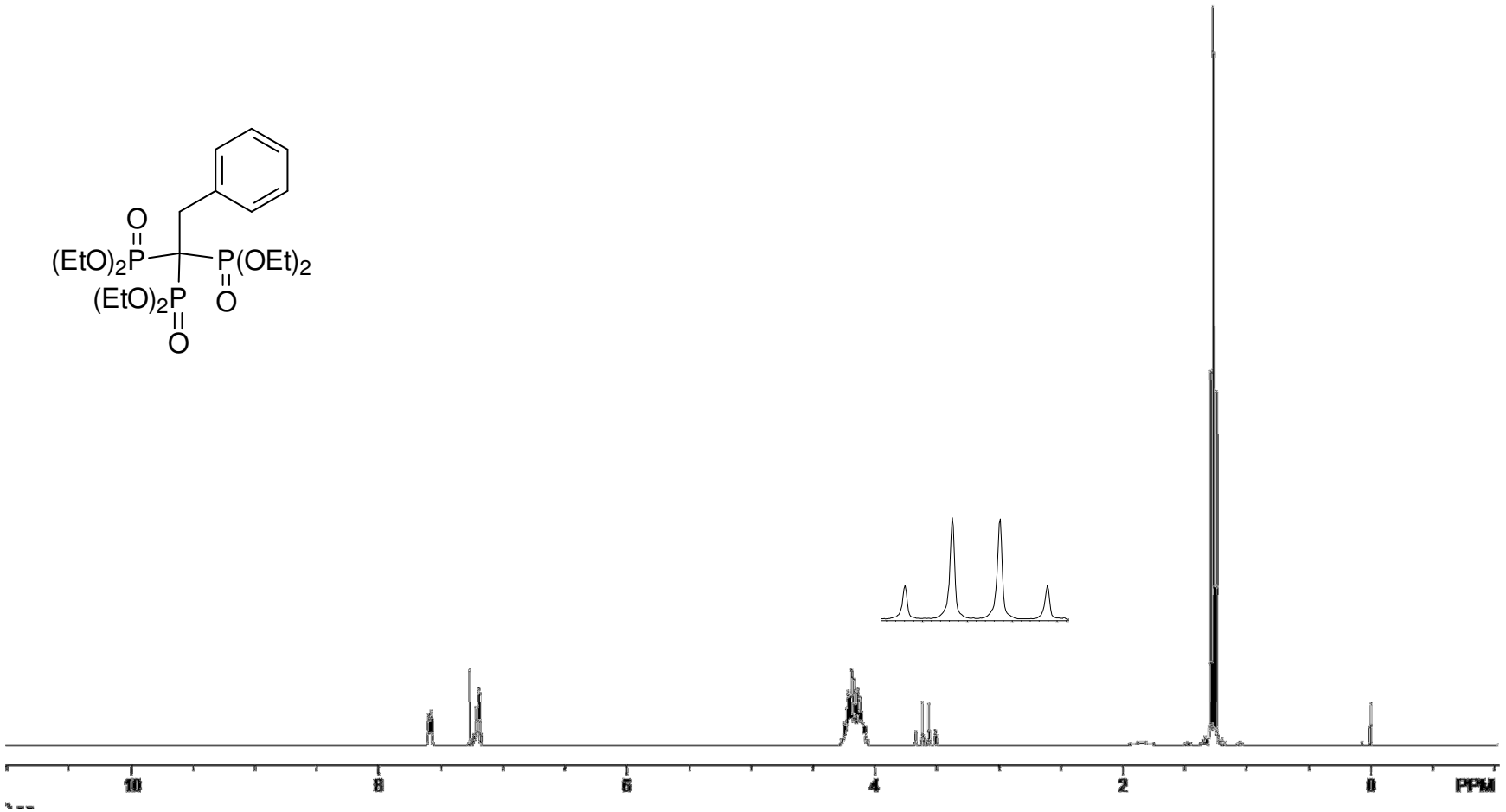


Figure A31. ^{1}H NMR spectrum of trisphosphonate **83**.

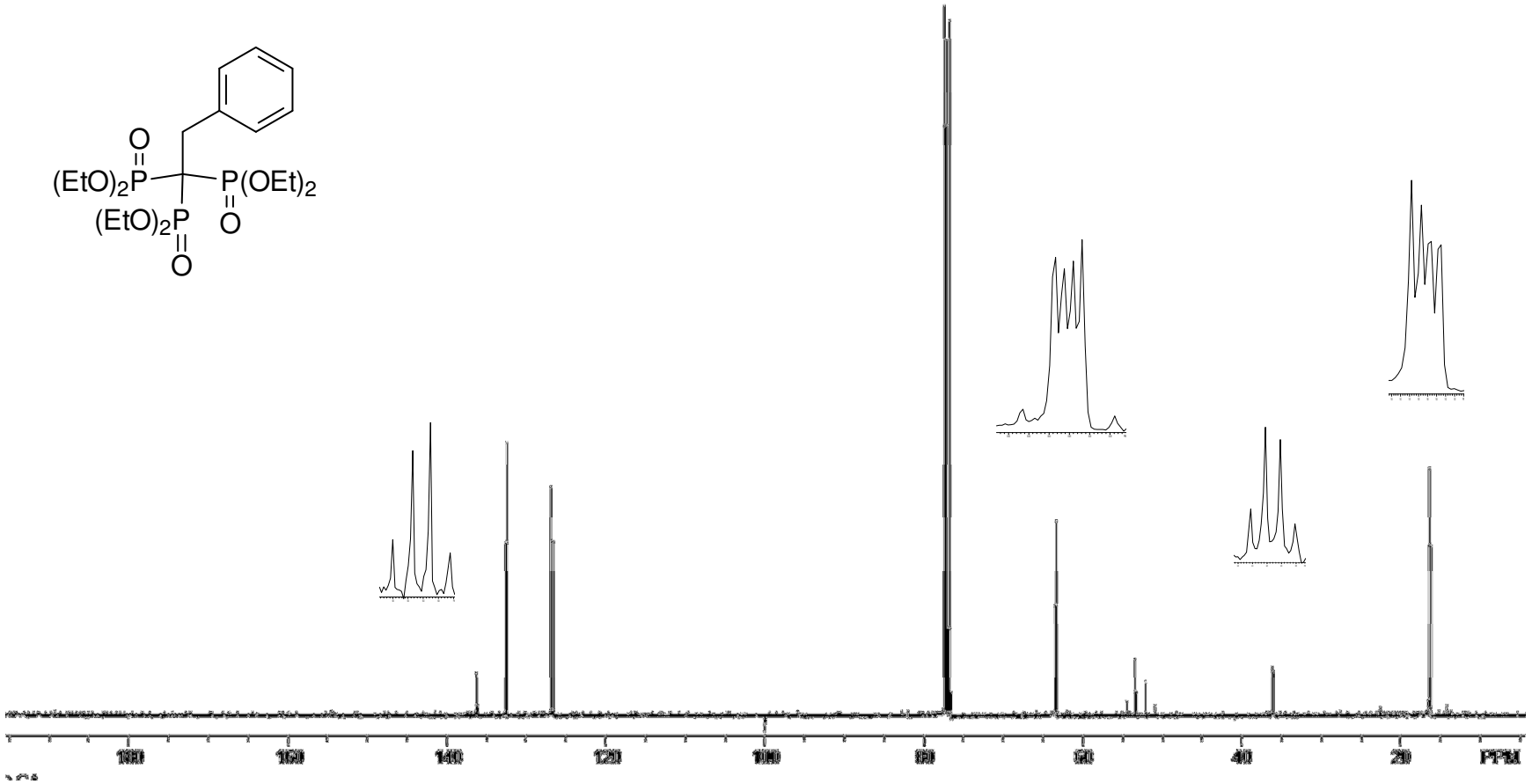


Figure A32. ^{13}C NMR spectrum of trisphosphonate **83**.

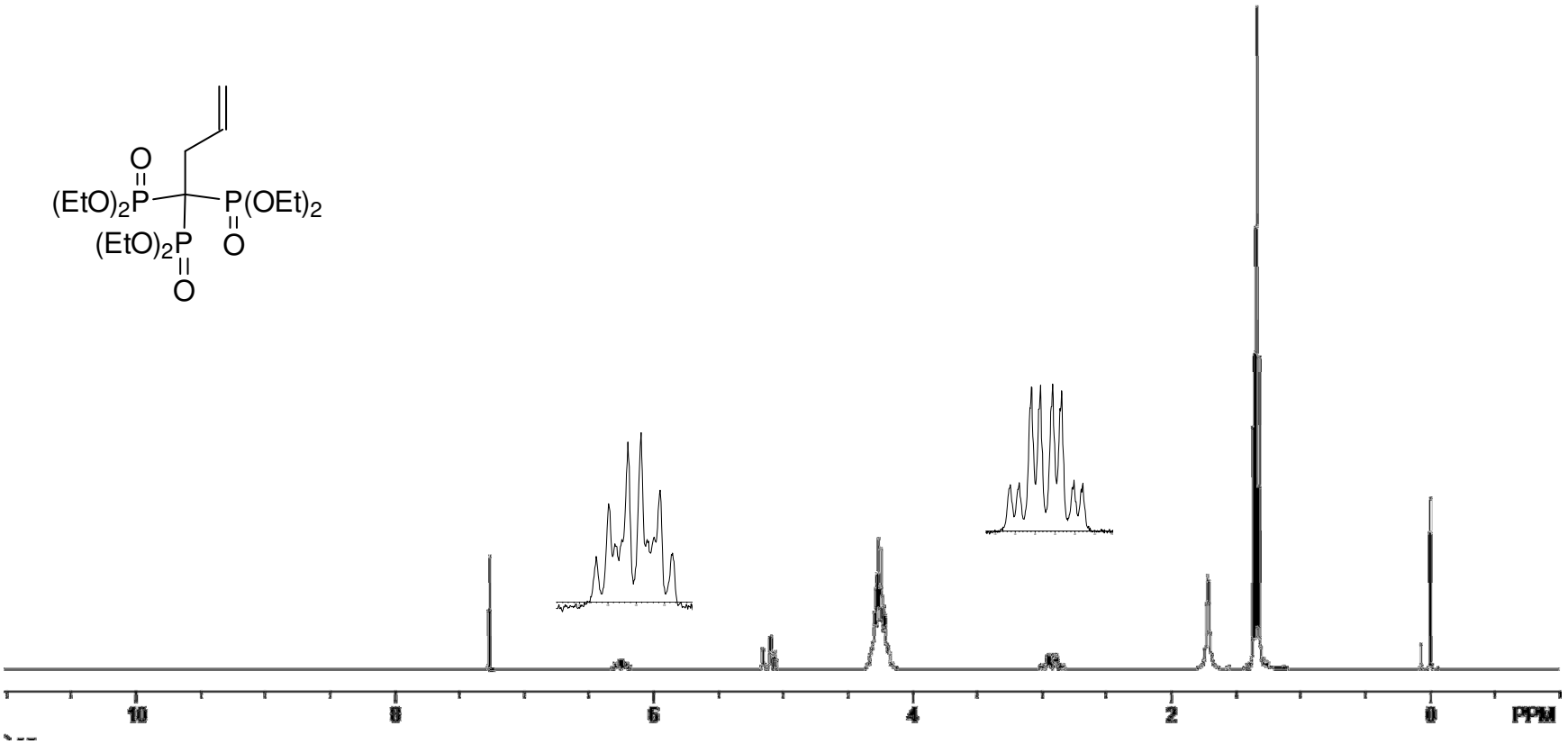


Figure A33. ^{1}H NMR spectrum of trisphosphonate **97**.

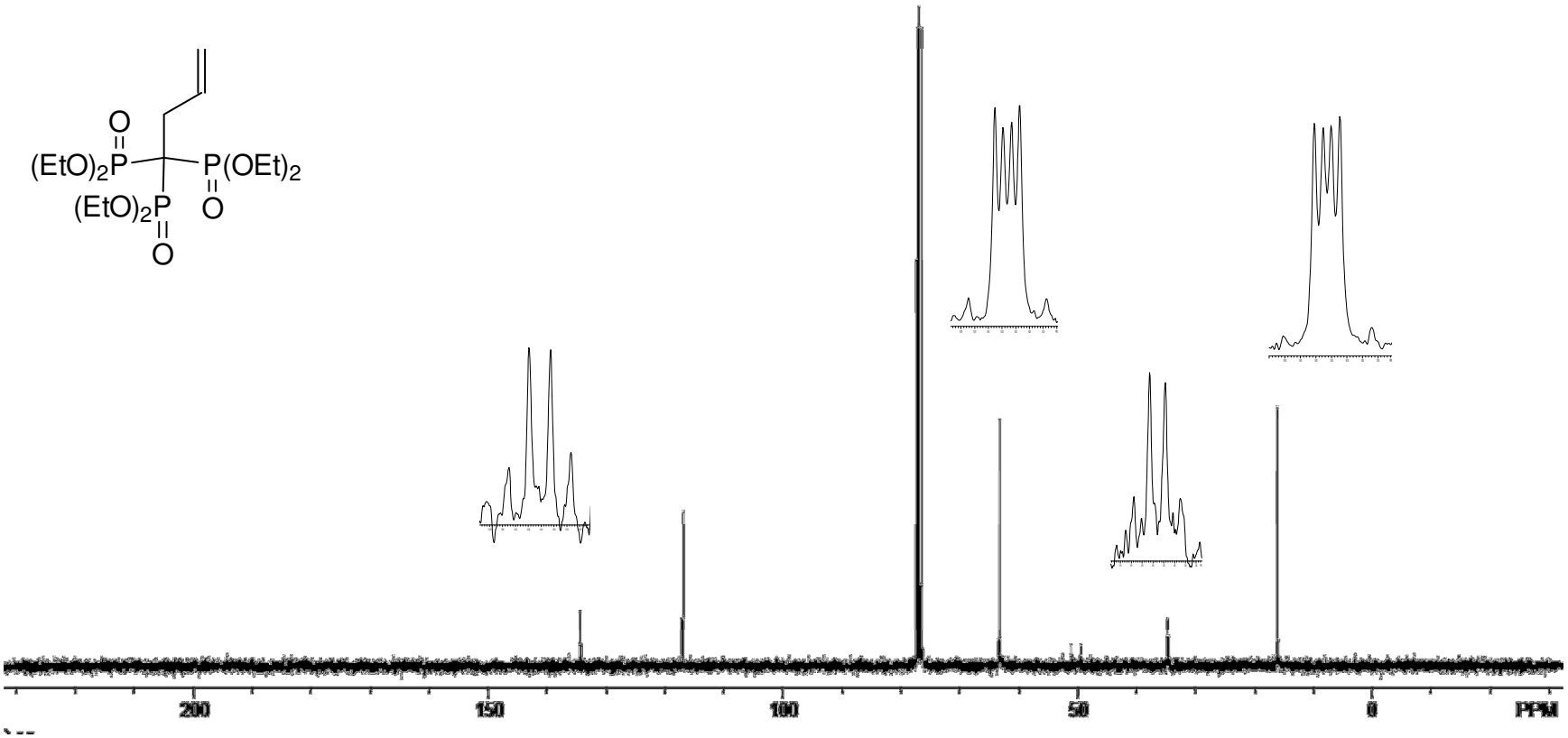


Figure A34. ^{13}C NMR spectrum of trisphosphonate **97**.

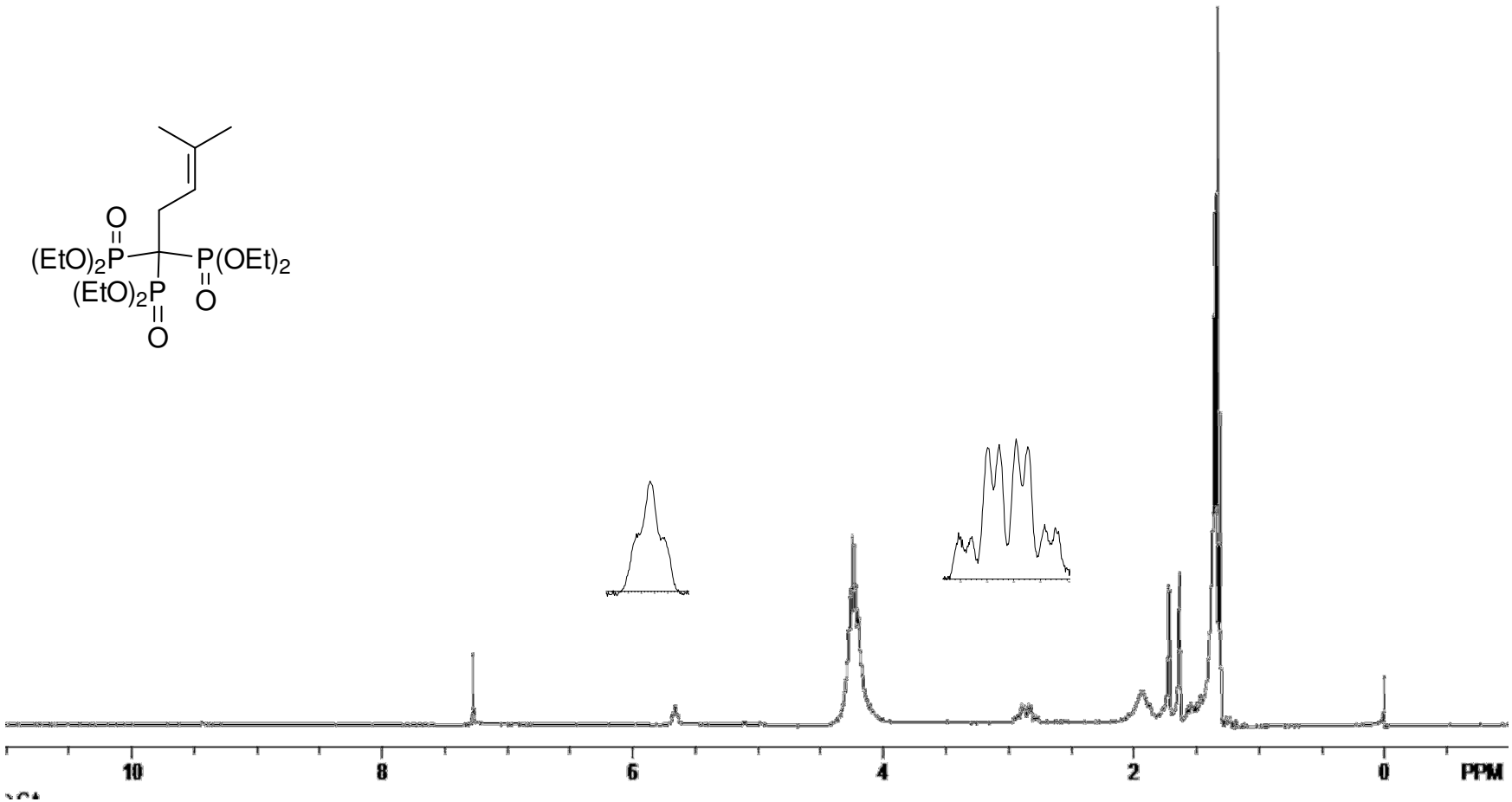


Figure A35. ^{1}H NMR spectrum of trisphosphonate **98.**

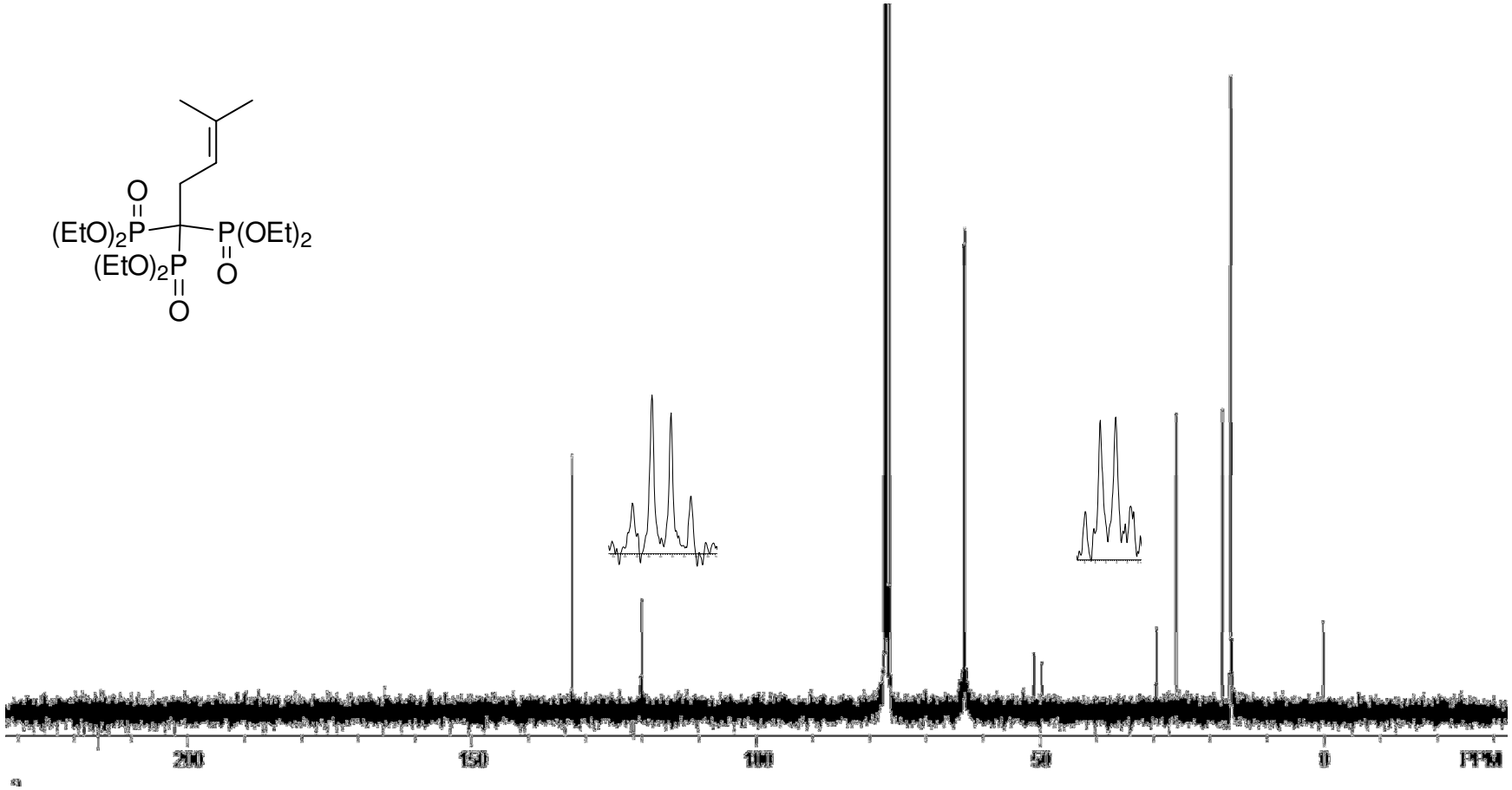


Figure A36. ^{13}C NMR spectrum of trisphosphonate **98.**

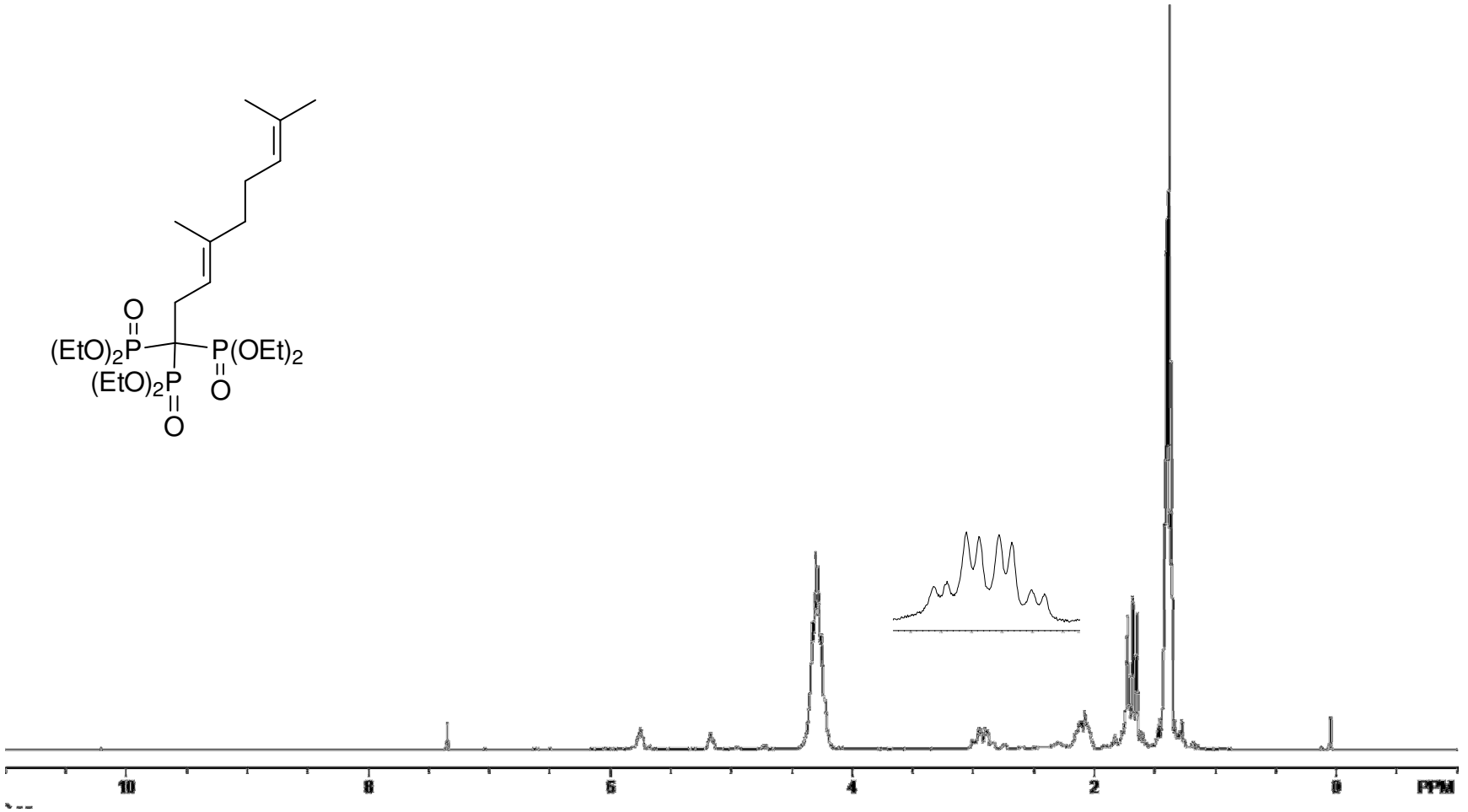


Figure A37. ^{1}H NMR spectrum of trisphosphonate **99.**

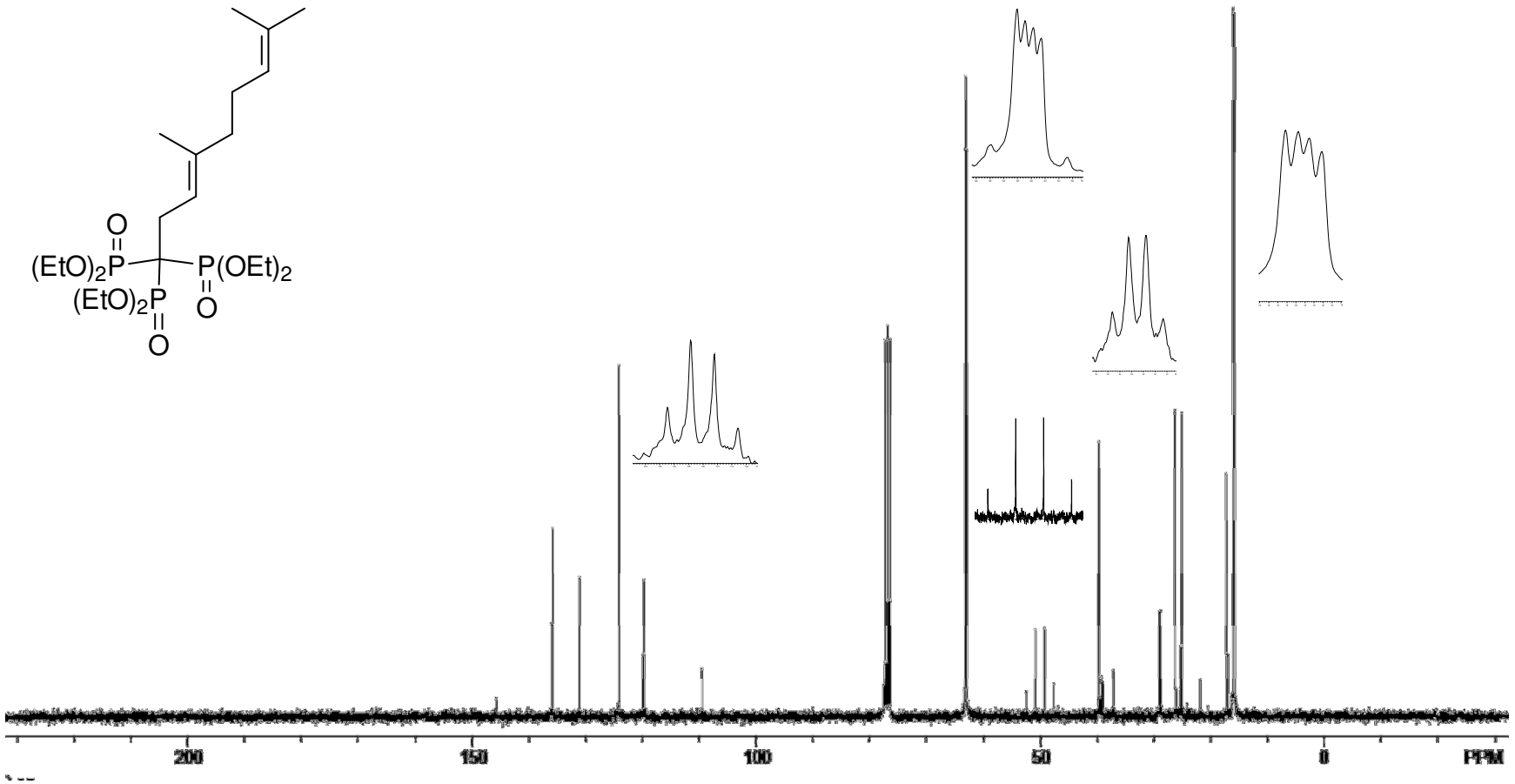


Figure A38. ^{13}C NMR spectrum of trisphosphonate **99**.

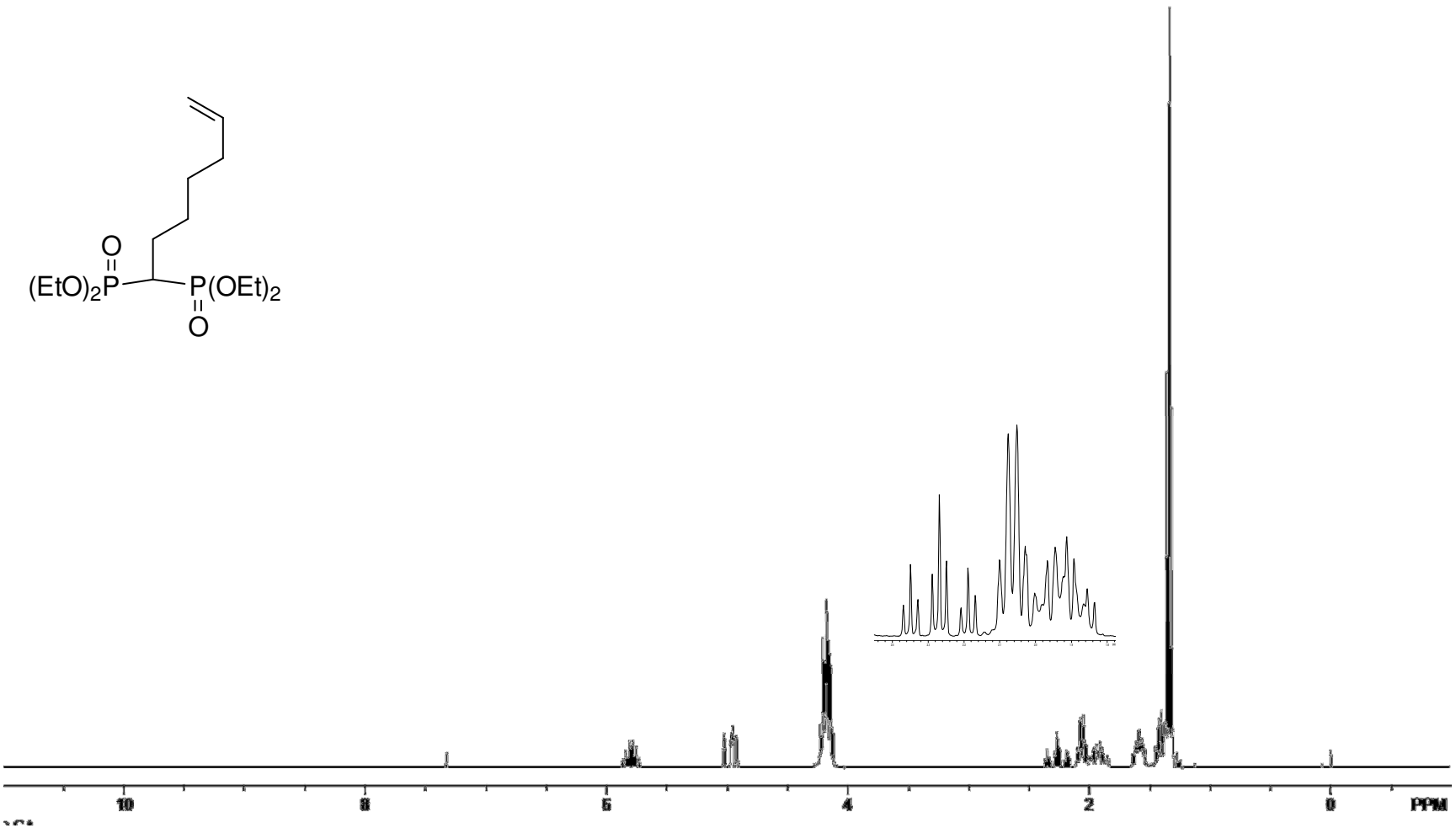


Figure A39. ^{1}H NMR spectrum of bisphosphonate **120**.

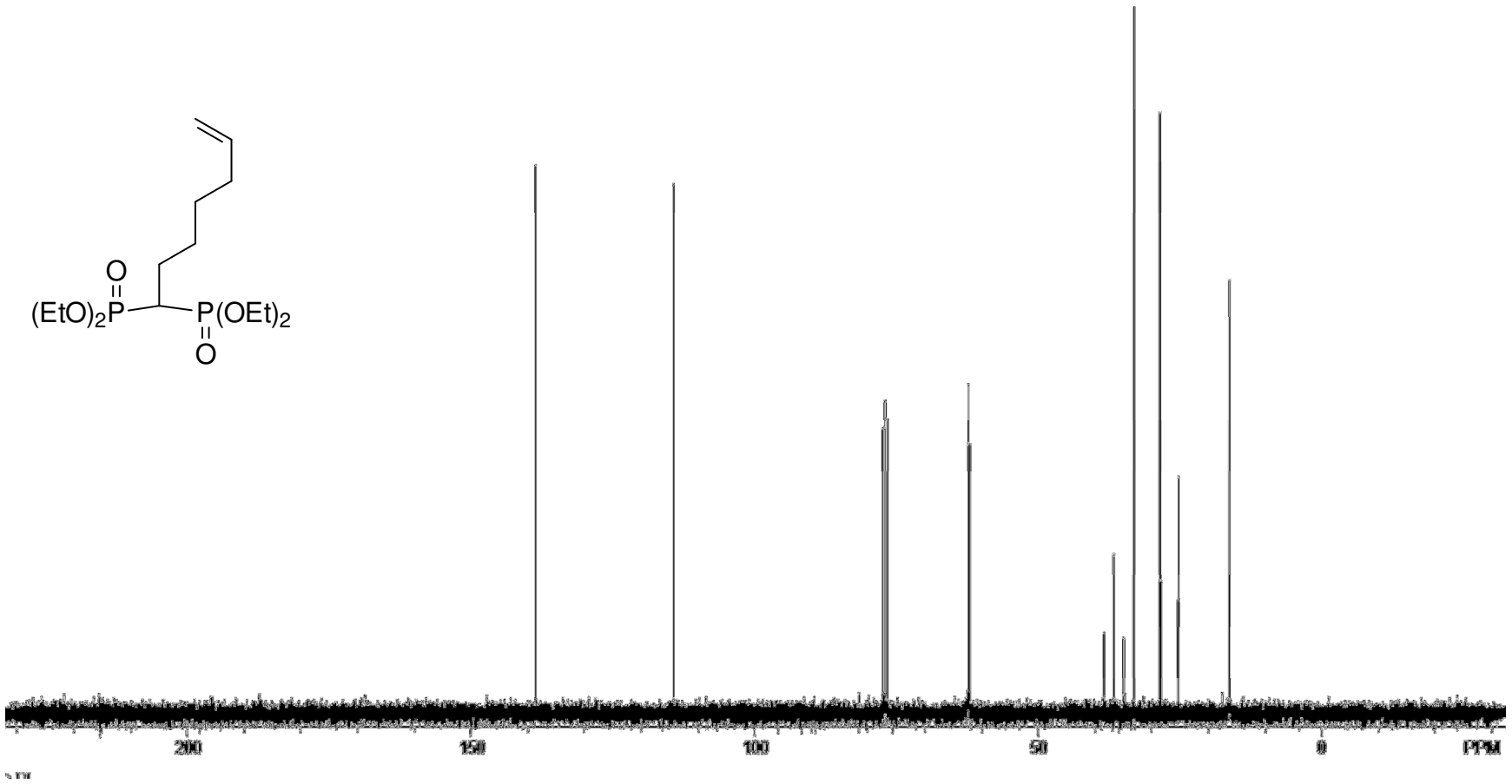


Figure A40. ^{13}C NMR spectrum of bisphosphonate **120**.

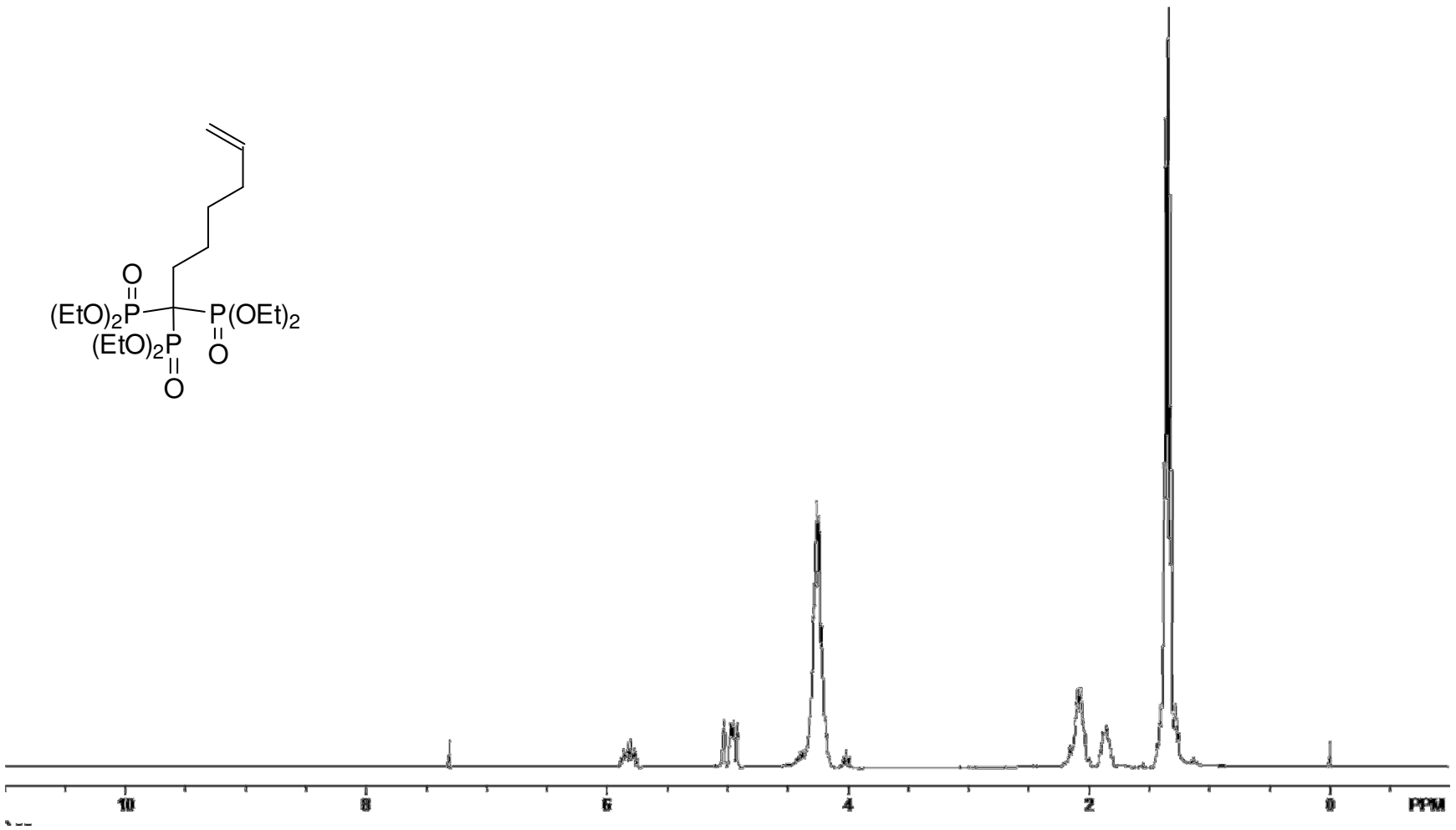


Figure A41. ^{1}H NMR spectrum of trisphosphonate **121**.

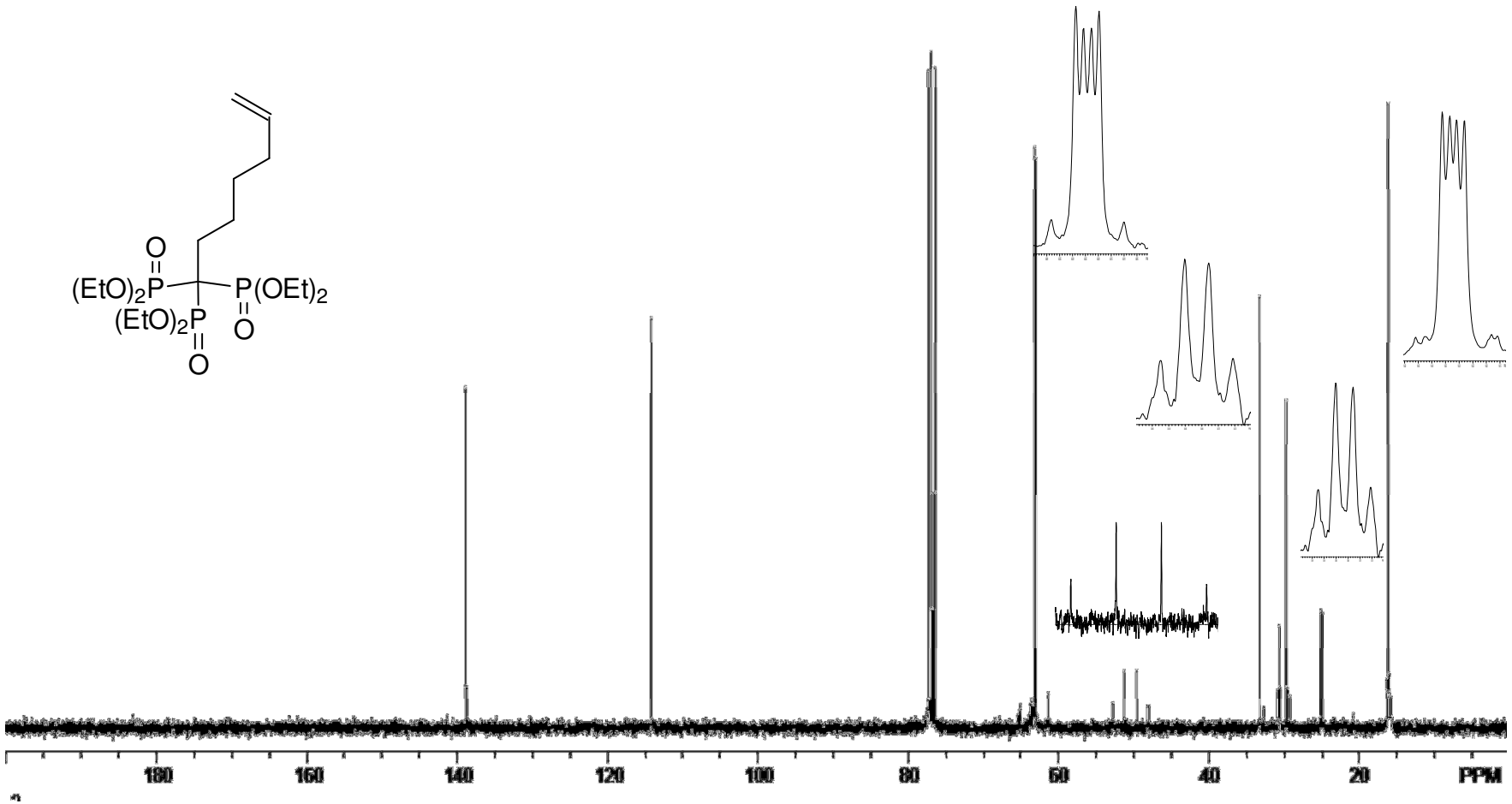


Figure A42. ^{13}C NMR spectrum of trisphosphonate **121.**

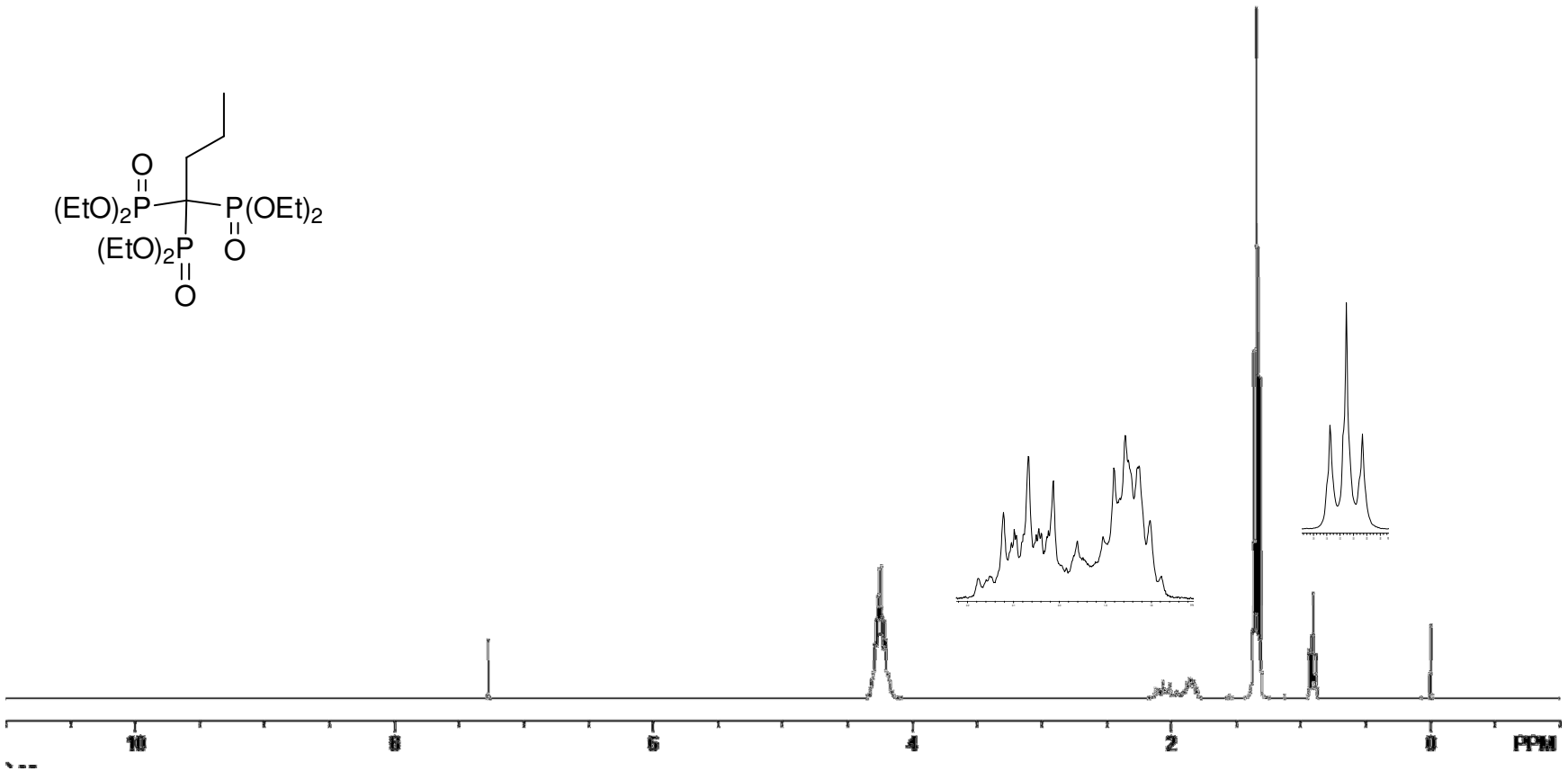


Figure A43. ^{1}H NMR spectrum of trisphosphonate **100**.

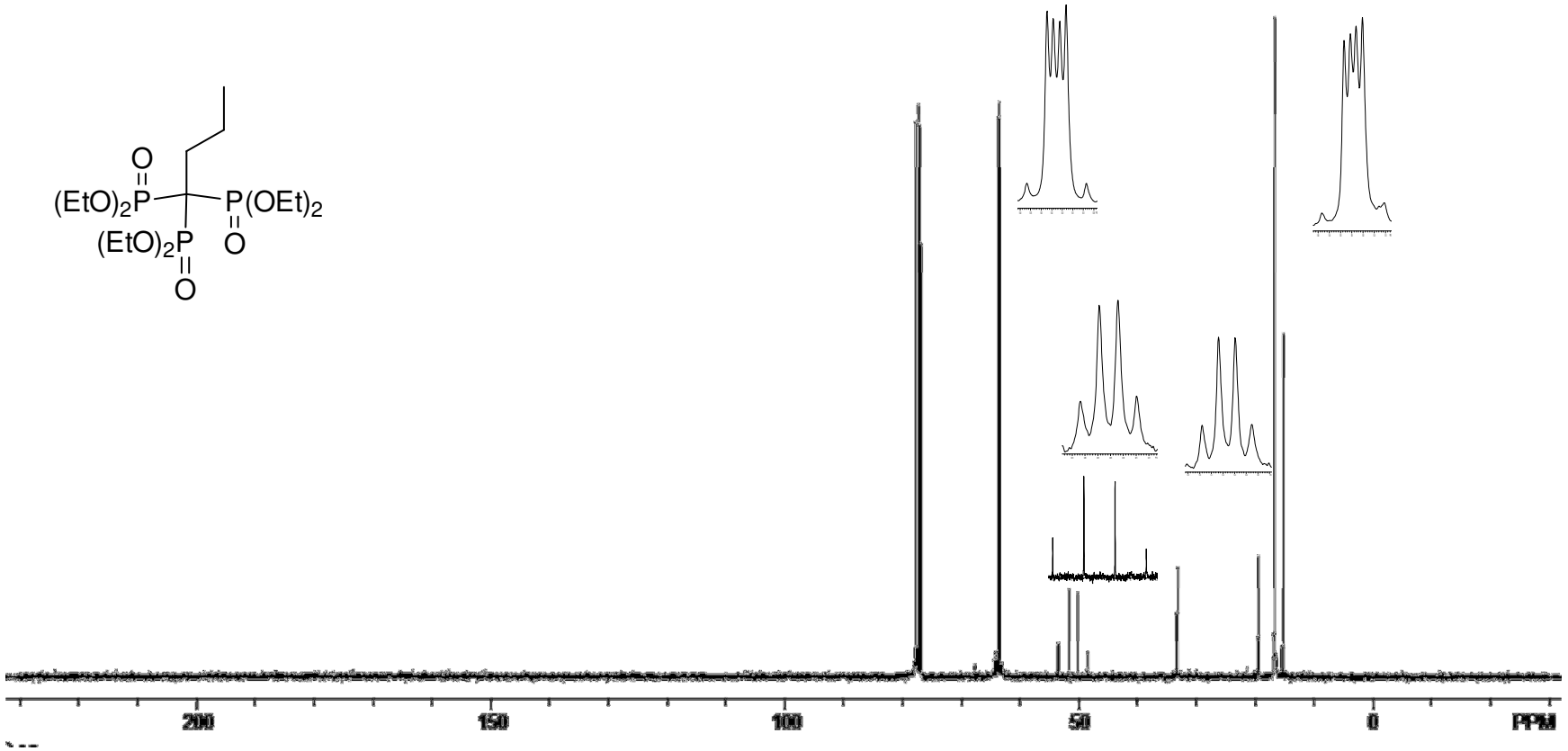


Figure A44. ^{13}C NMR spectrum of trisphosphonate **100**.

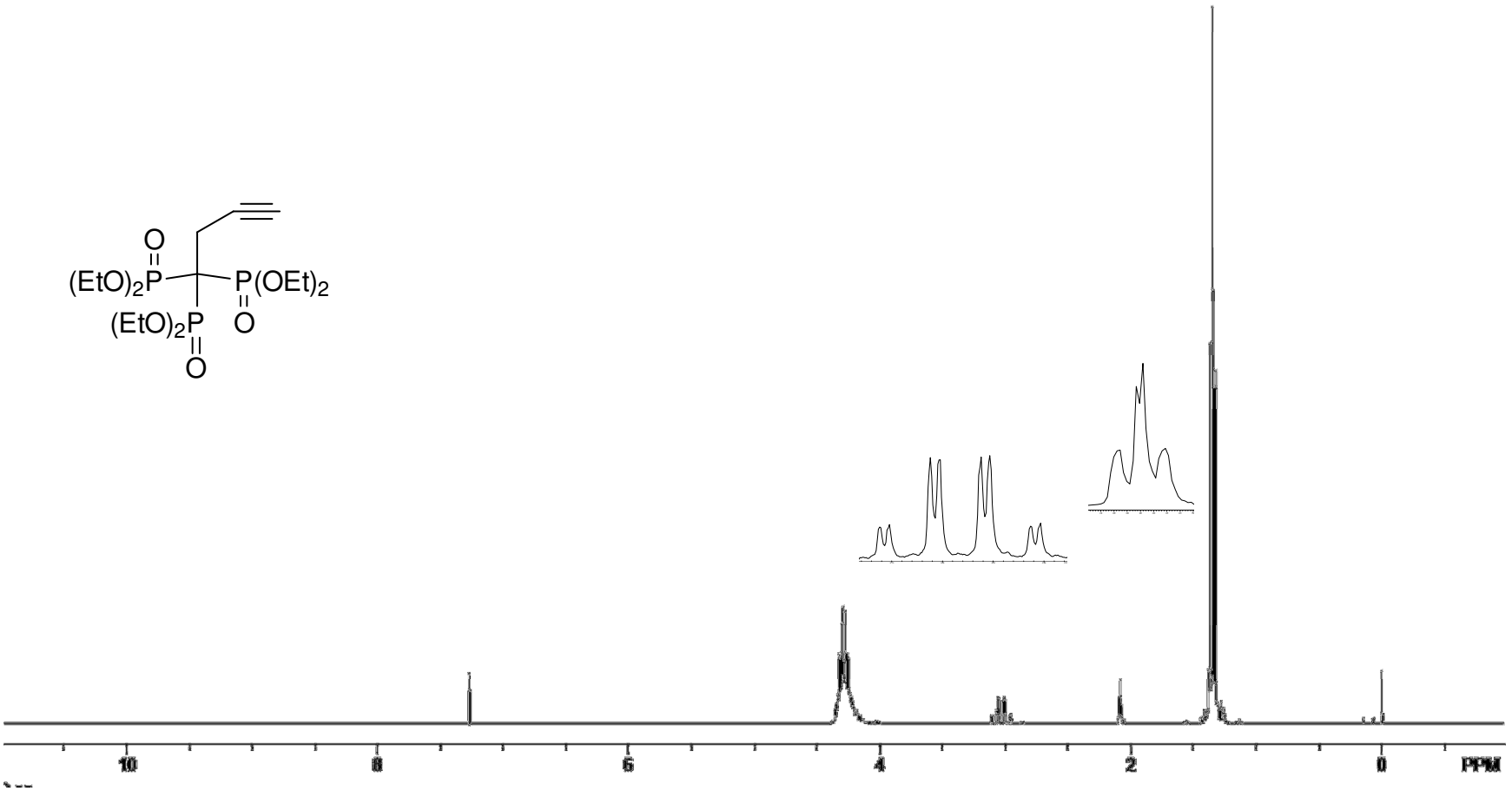


Figure A45. ^{1}H NMR spectrum of trisphosphonate **101**.

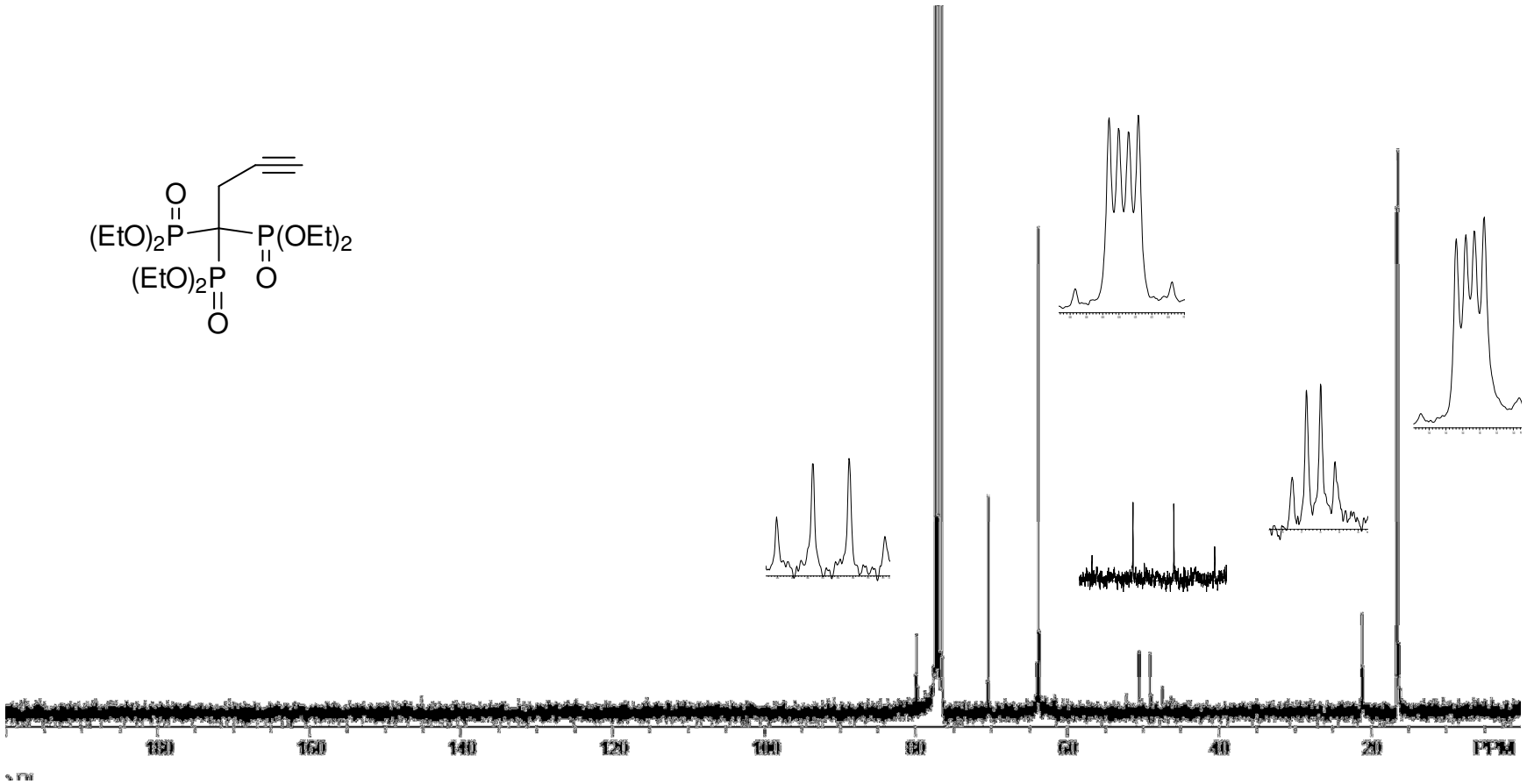


Figure A46. ^{13}C NMR spectrum of trisphosphonate **101.**

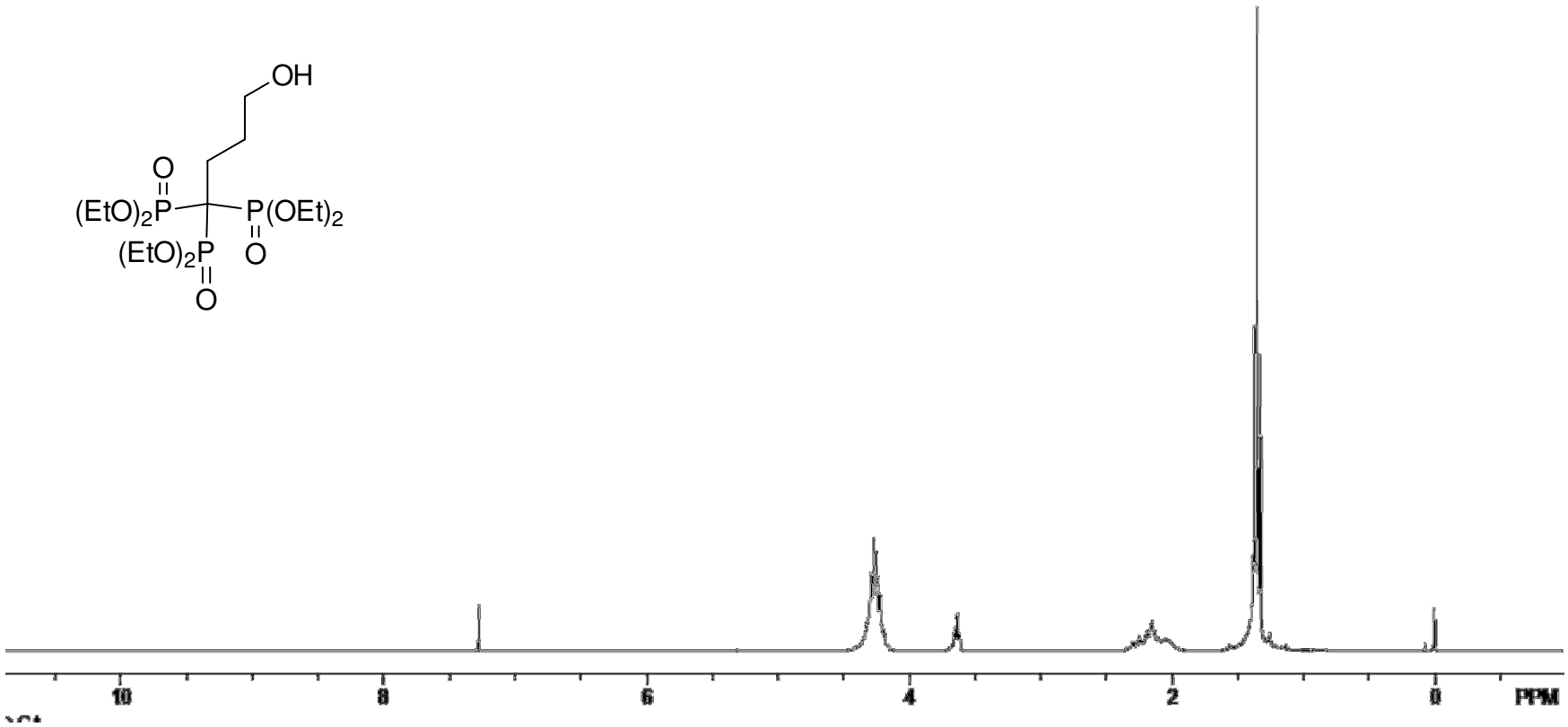


Figure A47. ^{1}H NMR spectrum of trisphosphonate **105.**

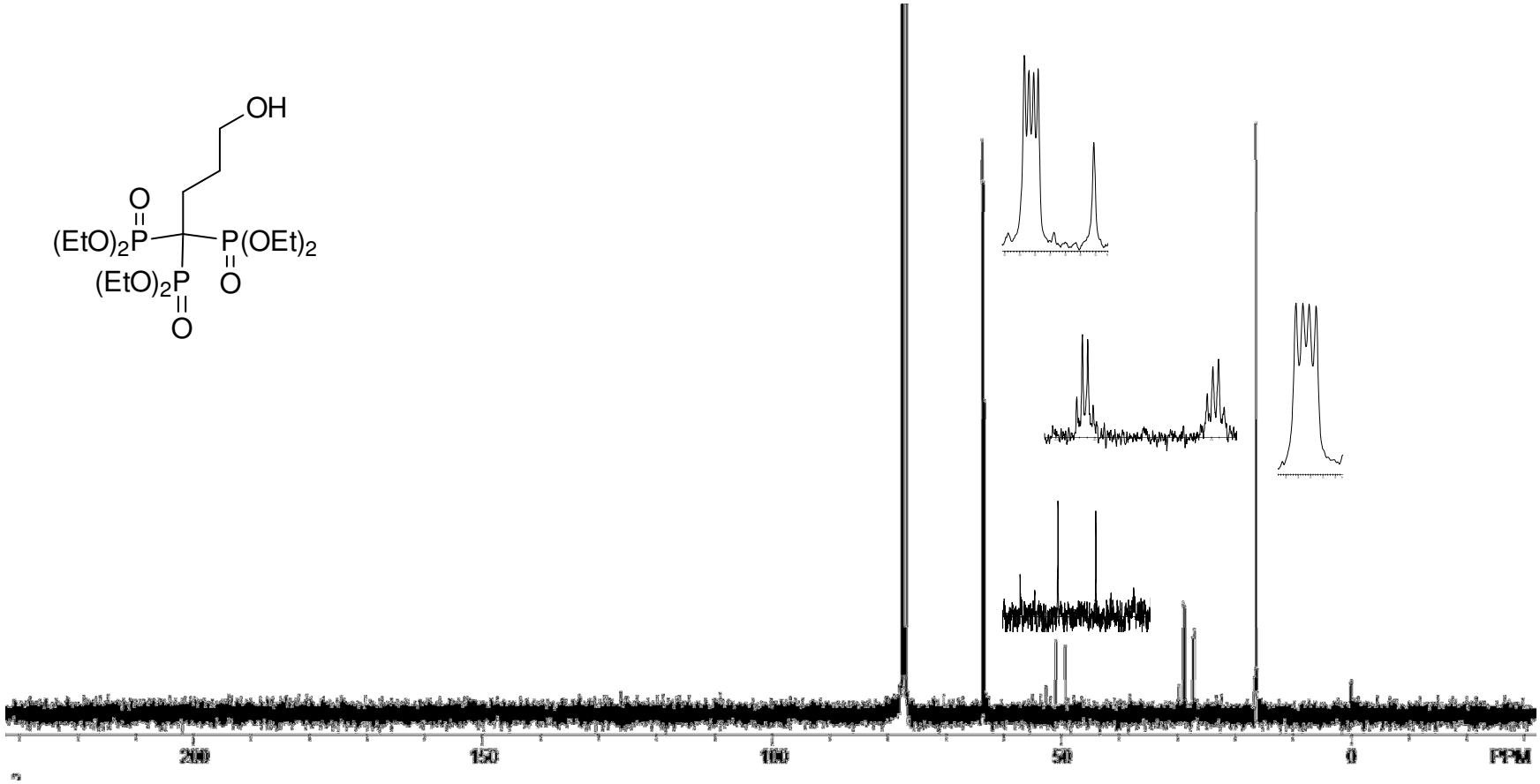


Figure A48. ^{13}C NMR spectrum of trisphosphonate **105.**

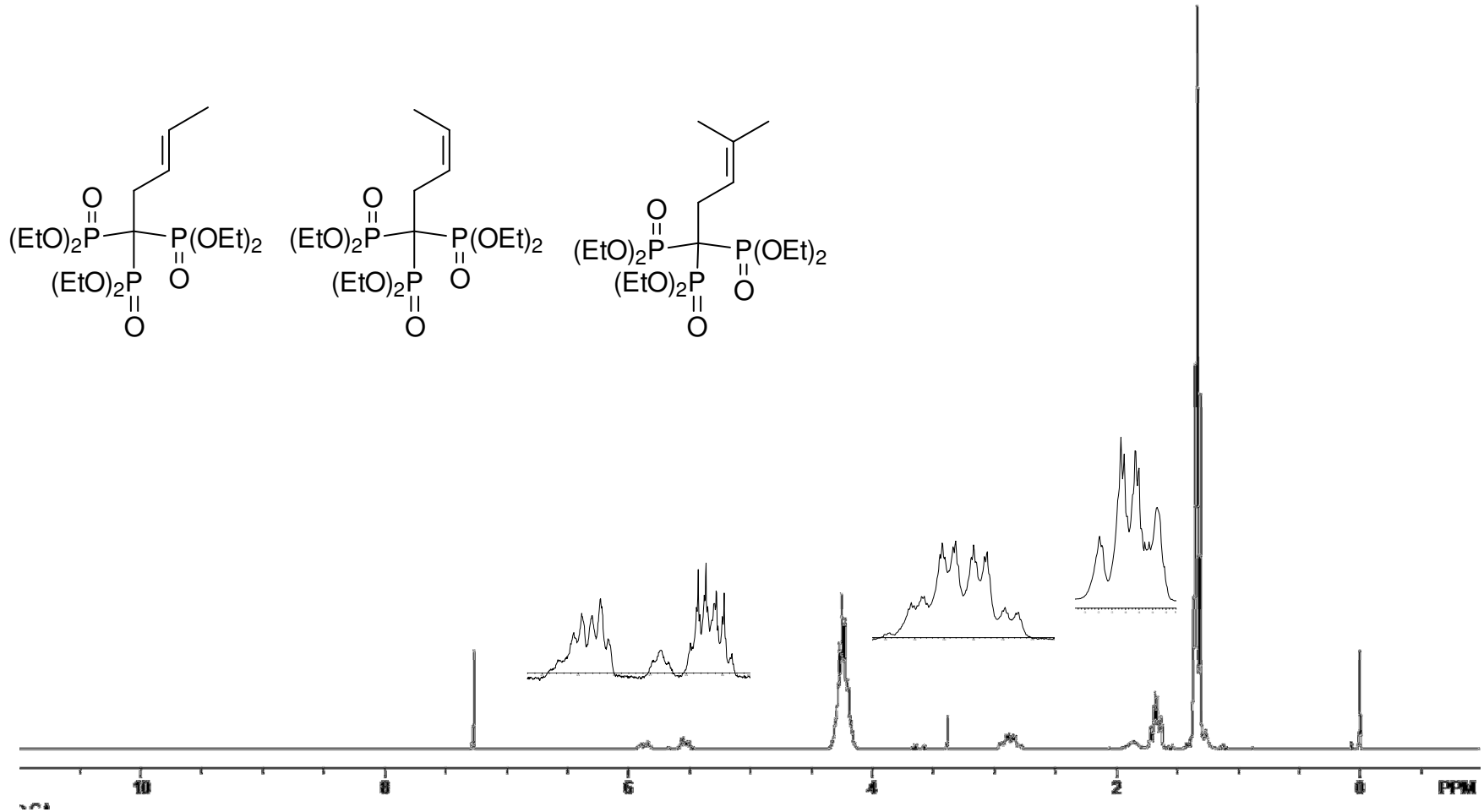


Figure A49. ^{1}H NMR of compounds **115**, **116** and **98**.

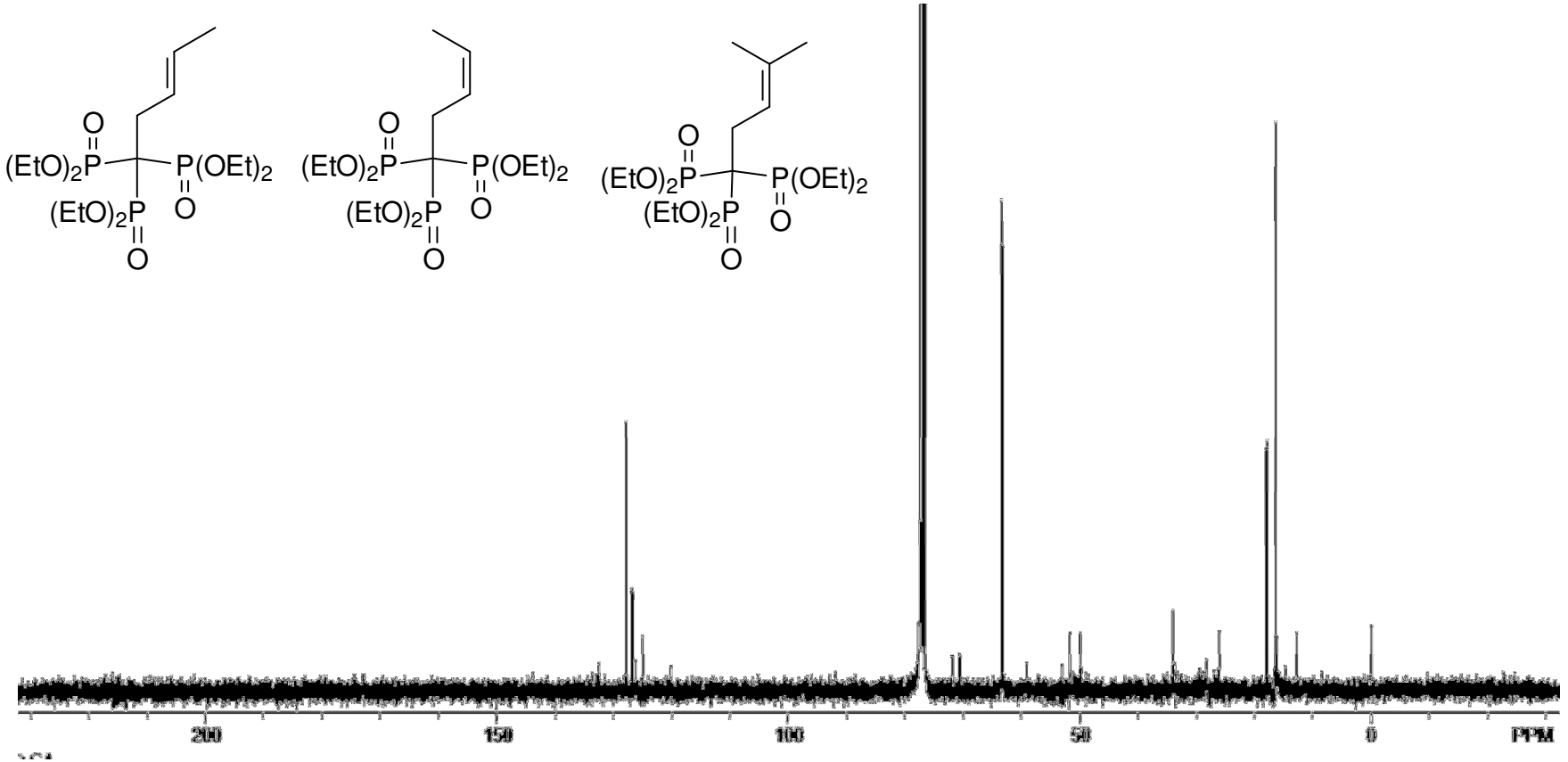


Figure A50. ^{13}C NMR of compounds**115**, **116** and **98**.

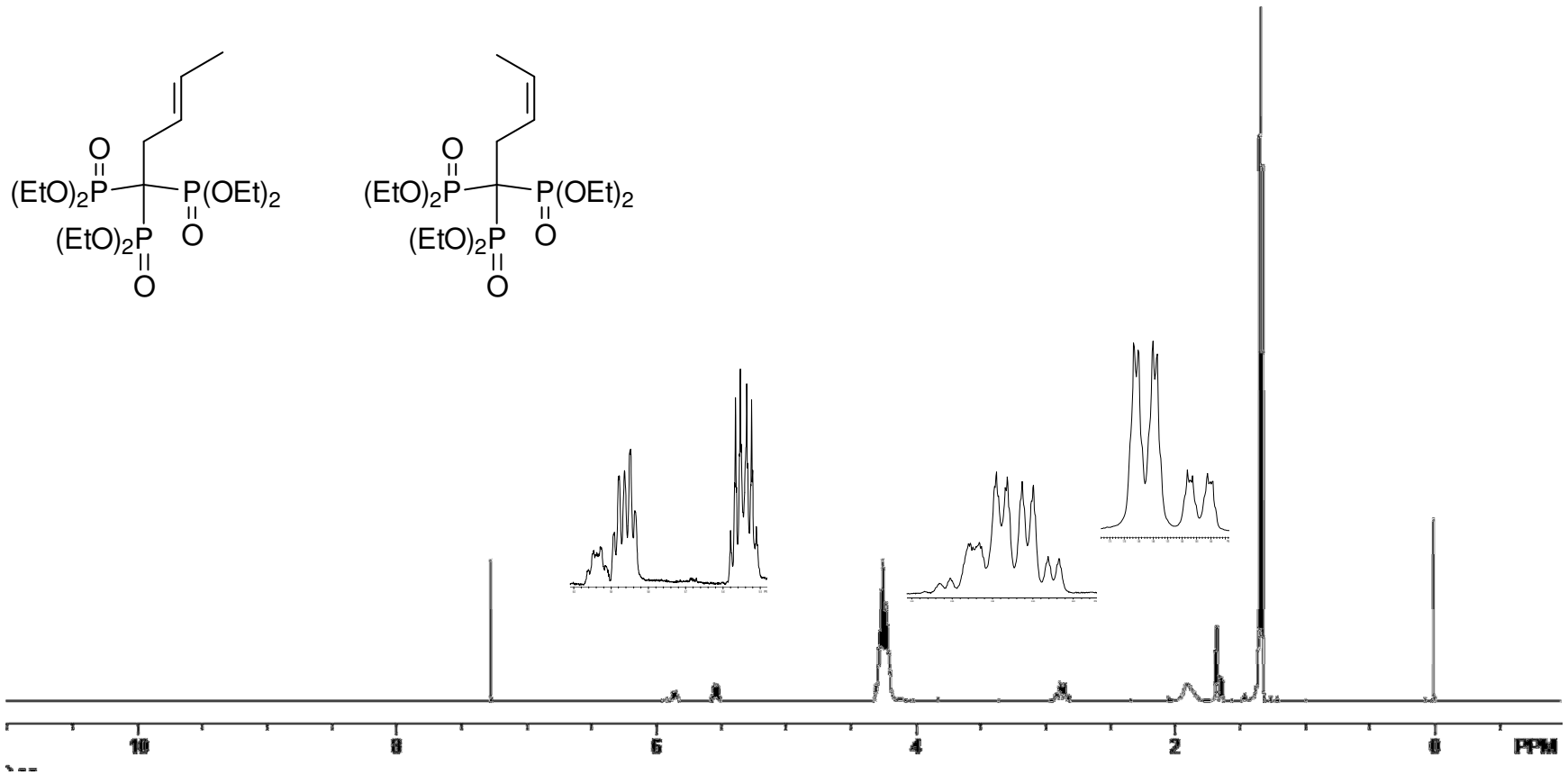


Figure A51. ^{1}H NMR of compounds **115** and **116**.

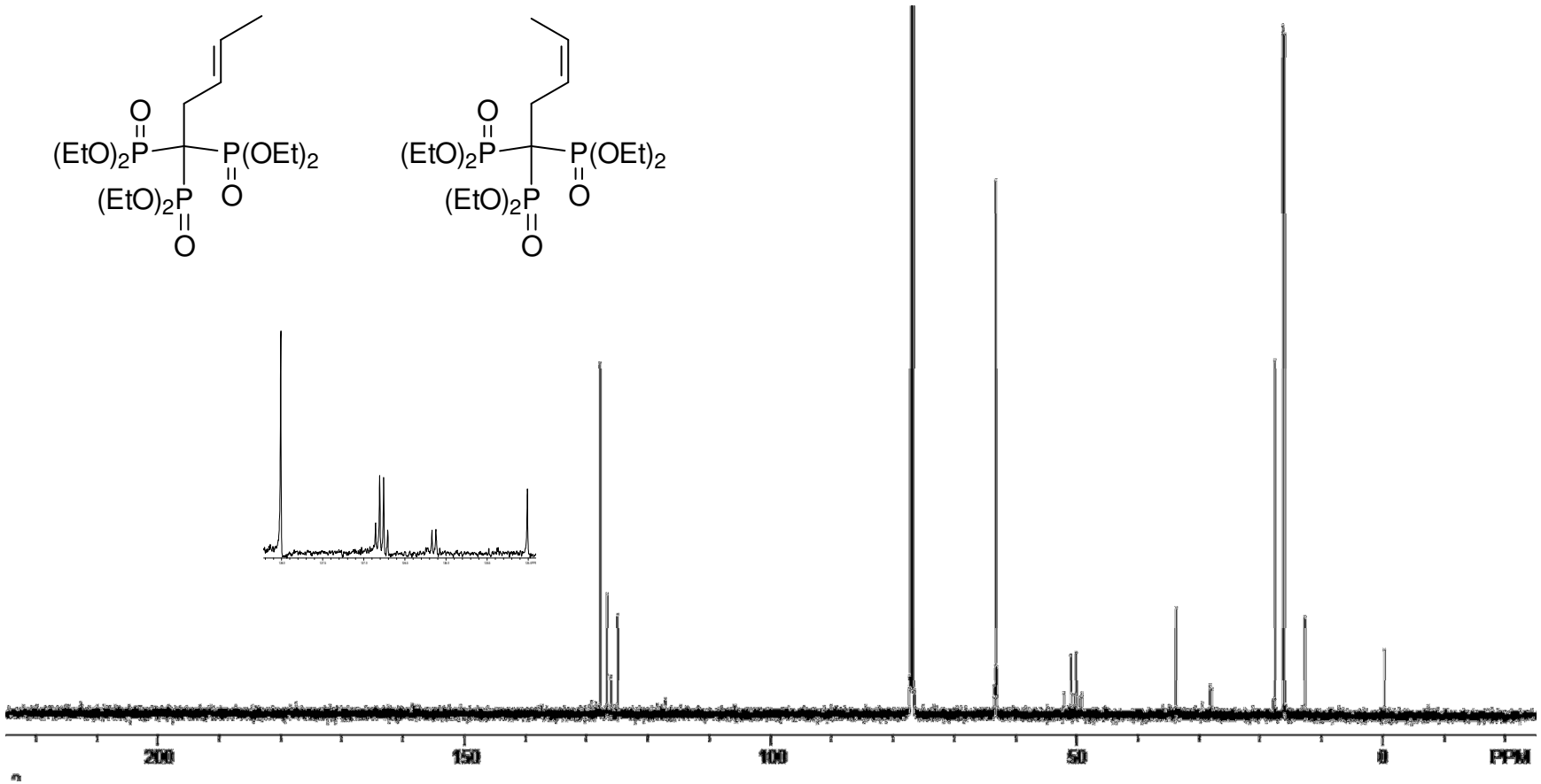


Figure A52. ^{13}C NMR of compounds **115** and **116**.

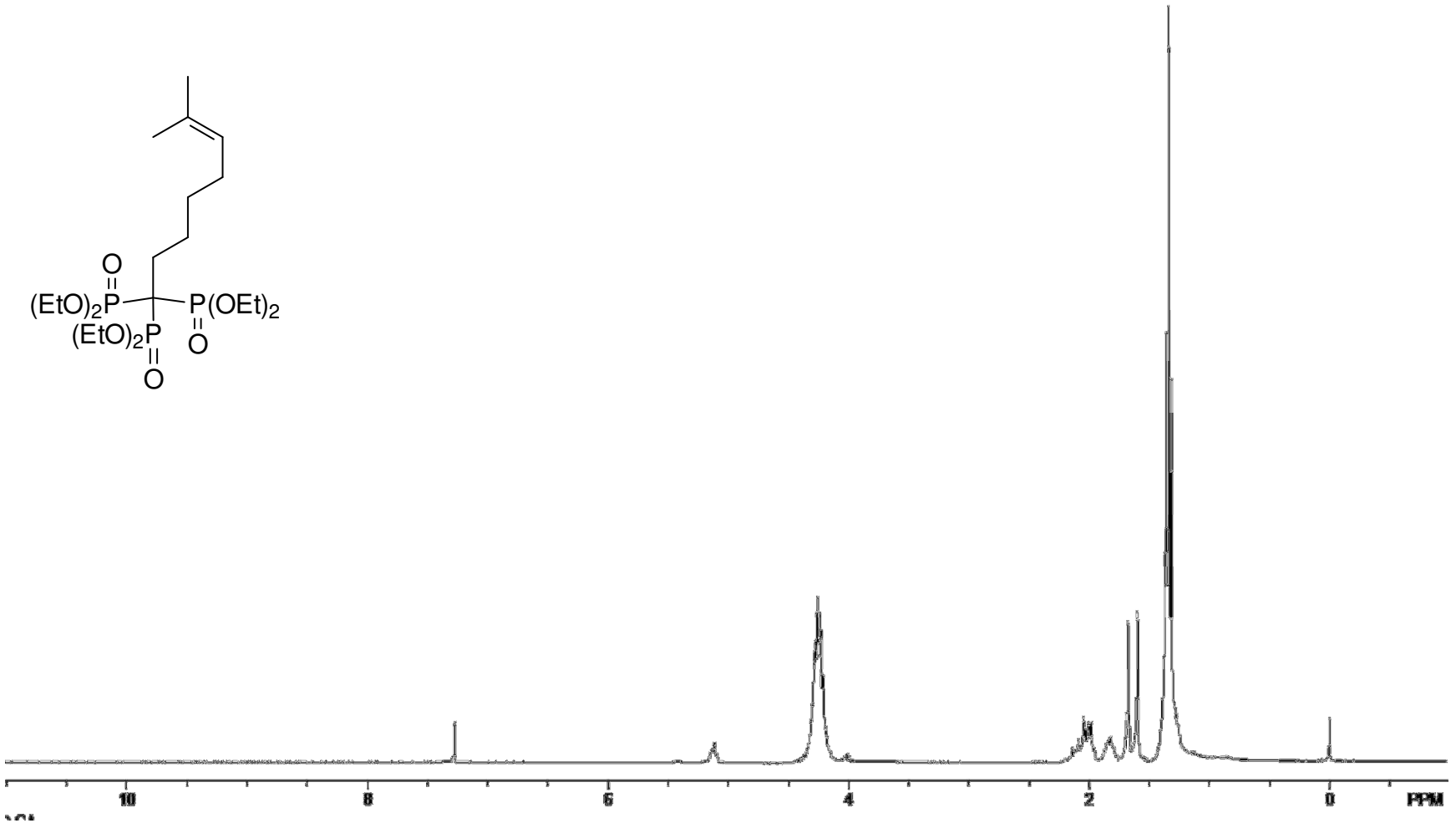


Figure A53. ^{1}H NMR spectrum of trisphosphonate **122**.

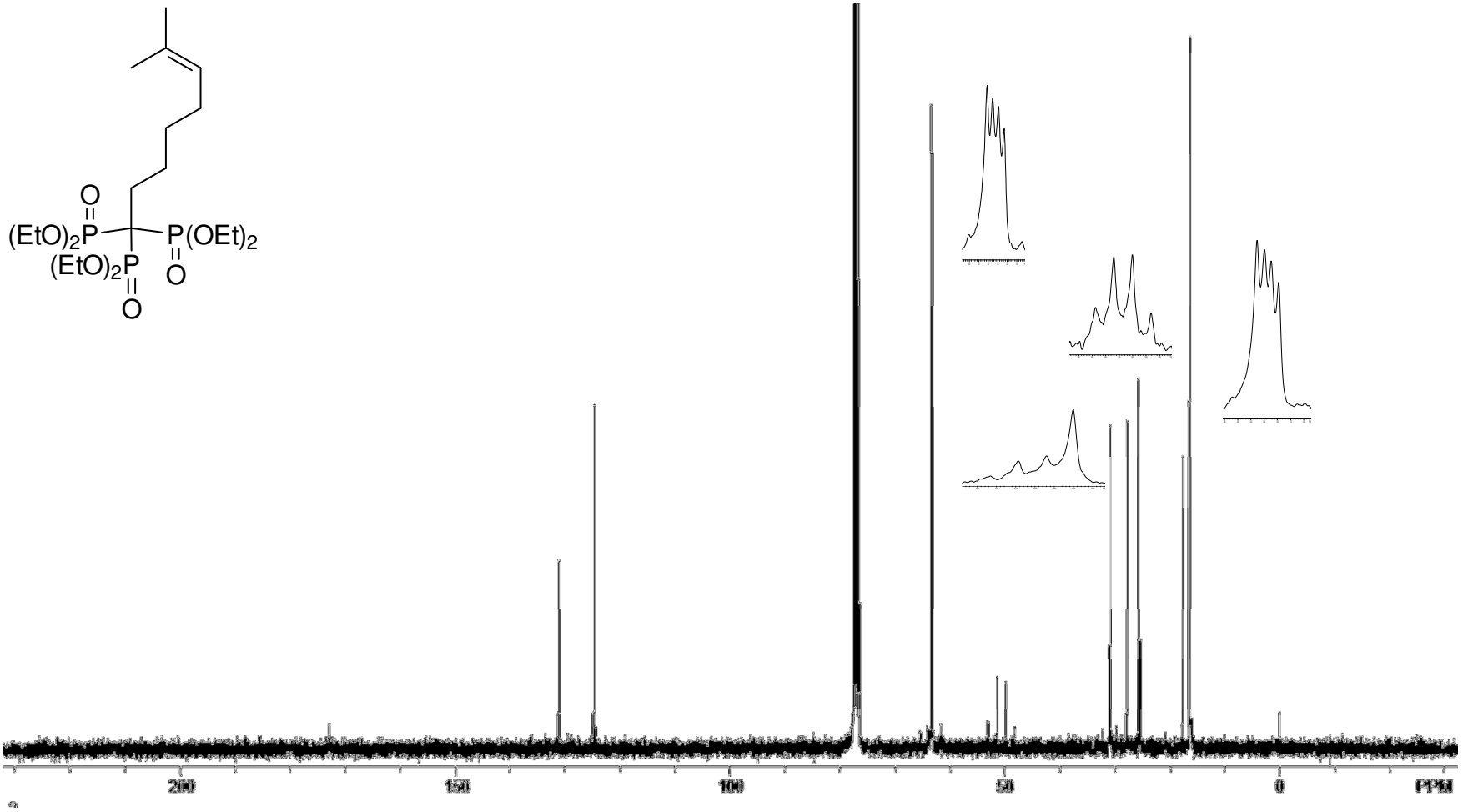


Figure A54. ^{13}C NMR spectrum of trisphosphonate **122**.

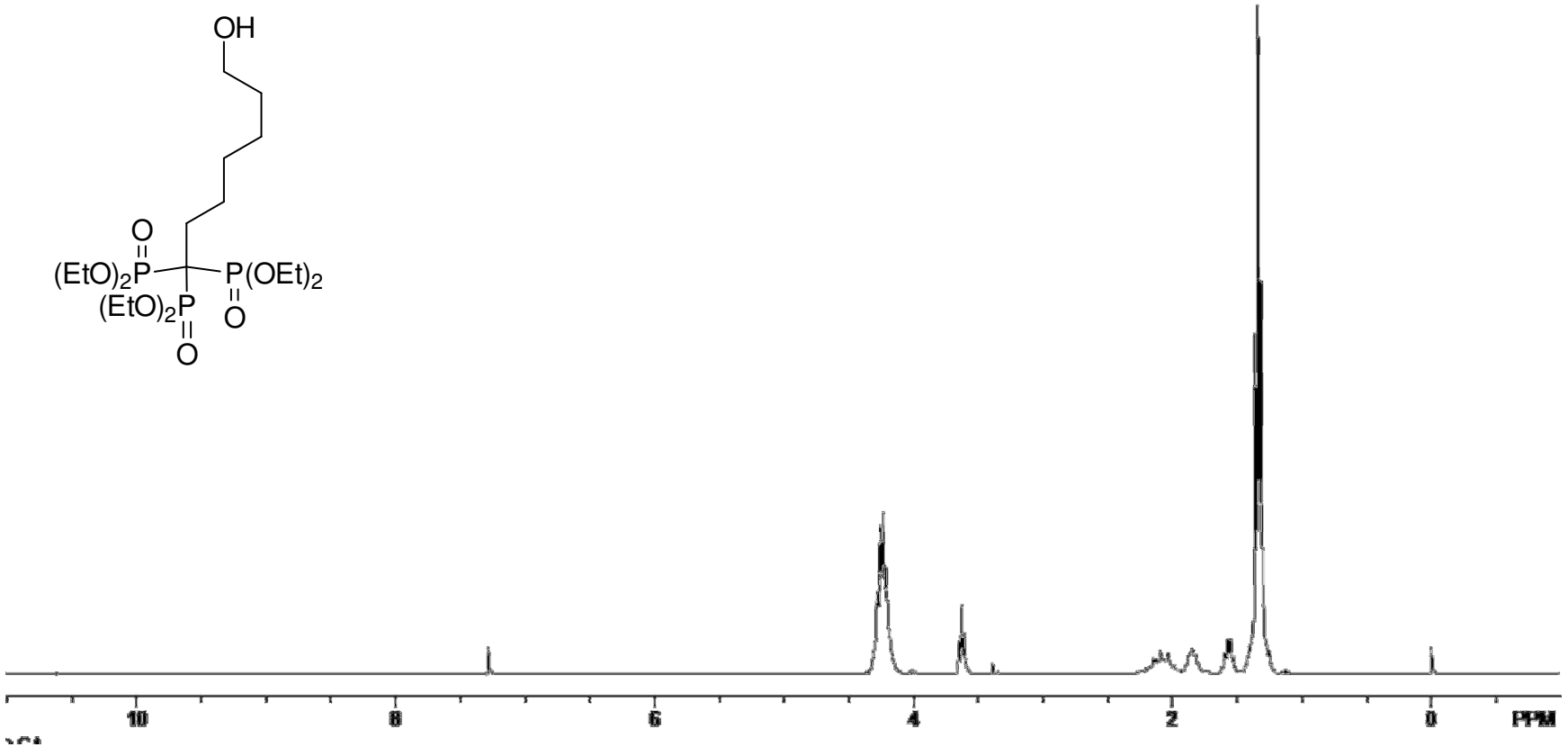


Figure A55. ^{1}H NMR spectrum of trisphosphonate **123**.

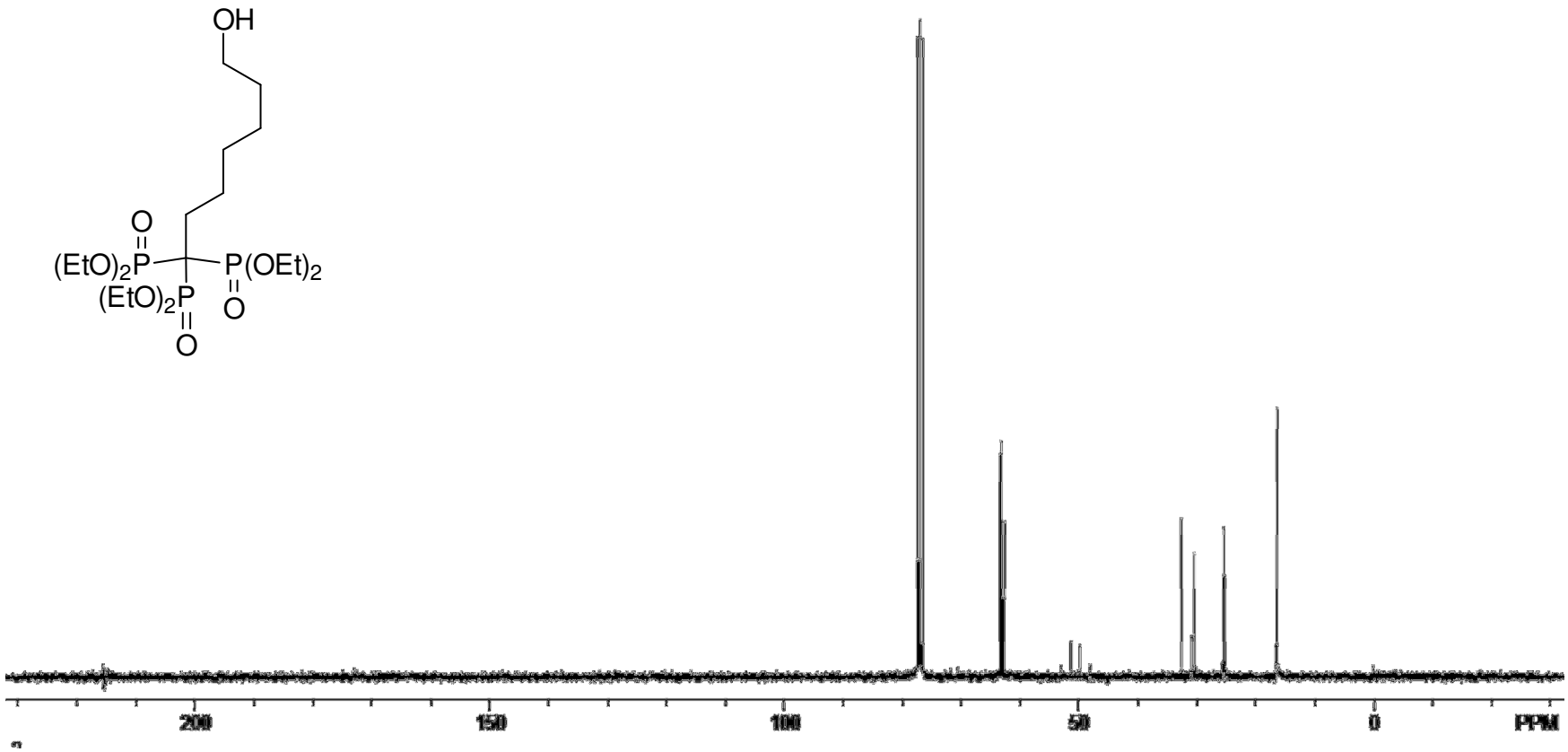


Figure A56. ^{13}C NMR spectrum of trisphosphonate **123**.

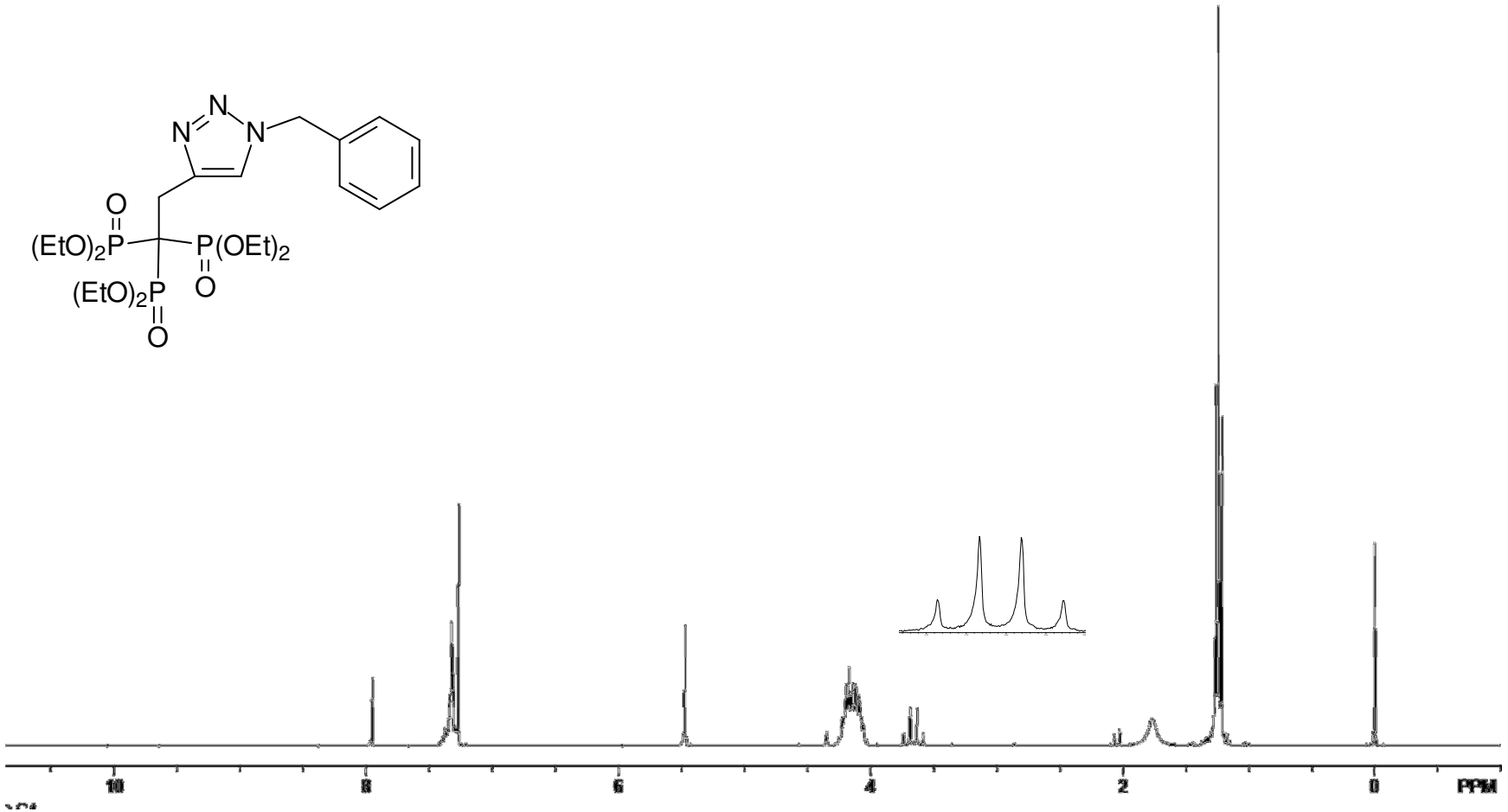


Figure A57. ^{1}H NMR spectrum of trisphosphonate **124**.

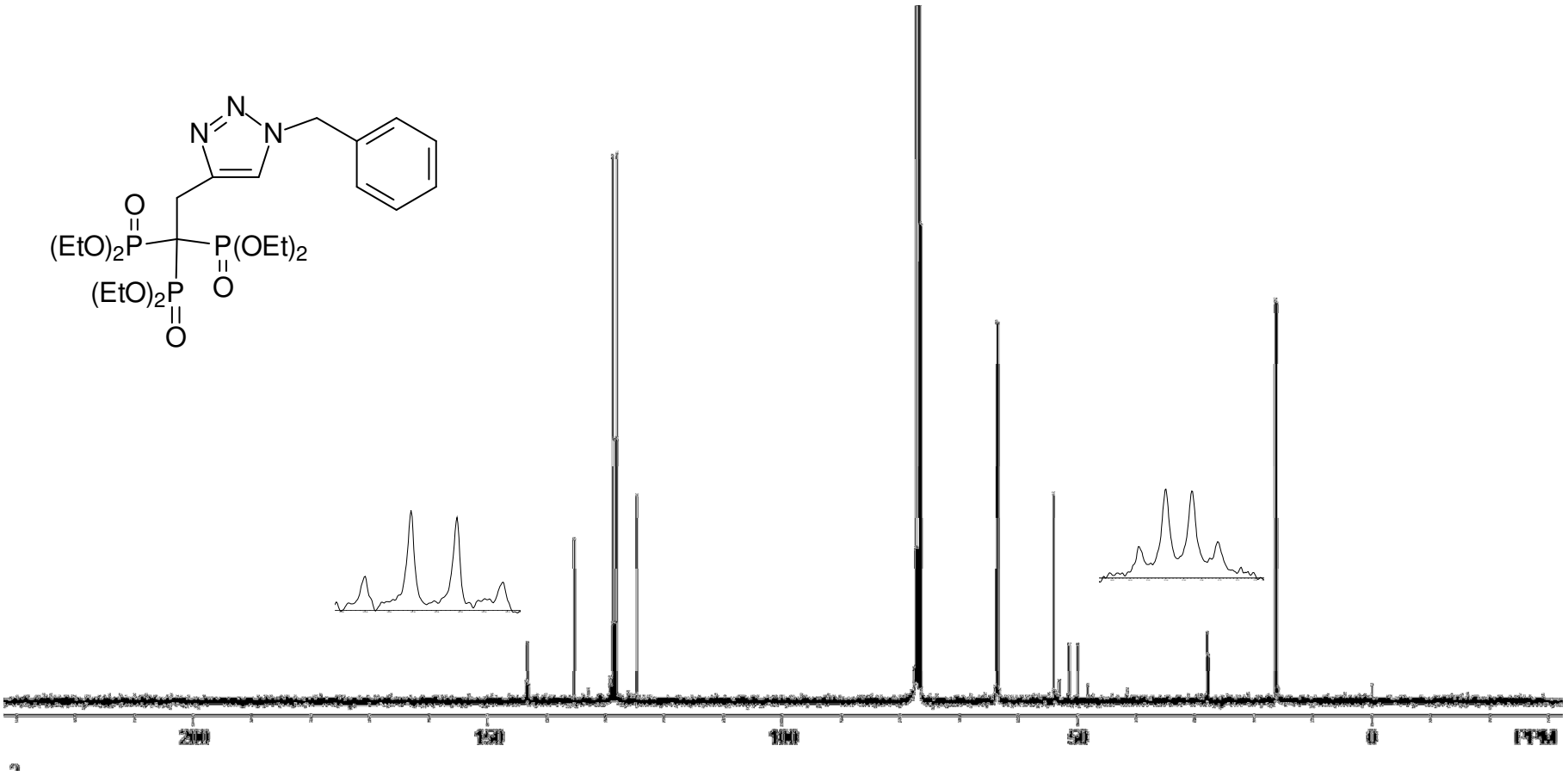


Figure A58. ^{13}C NMR spectrum of trisphosphonate **124**.

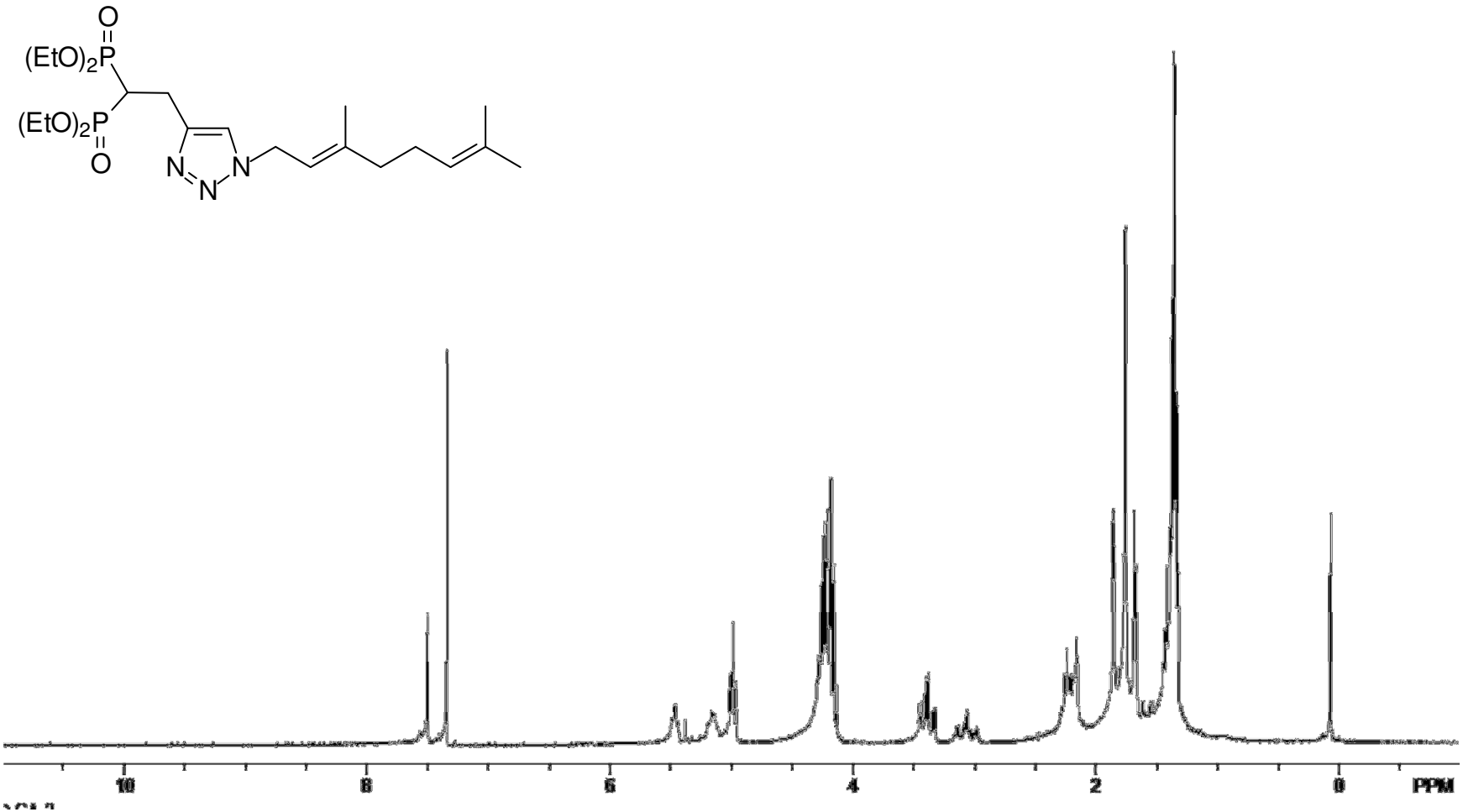


Figure A59. ^{1}H NMR spectrum of bisphosphonate **128.**

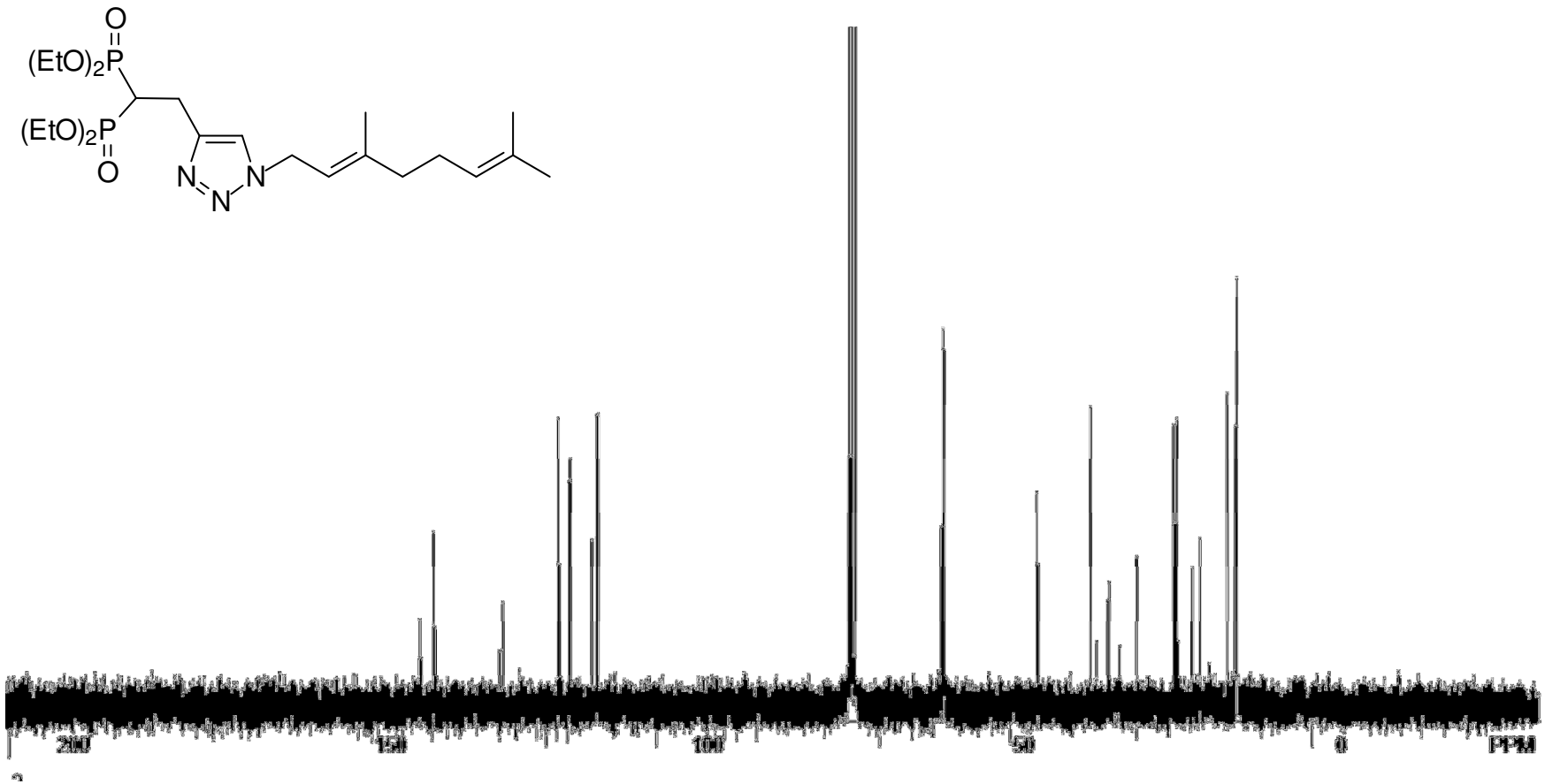


Figure A60. ^{13}C NMR spectrum of bisphosphonate **128.**

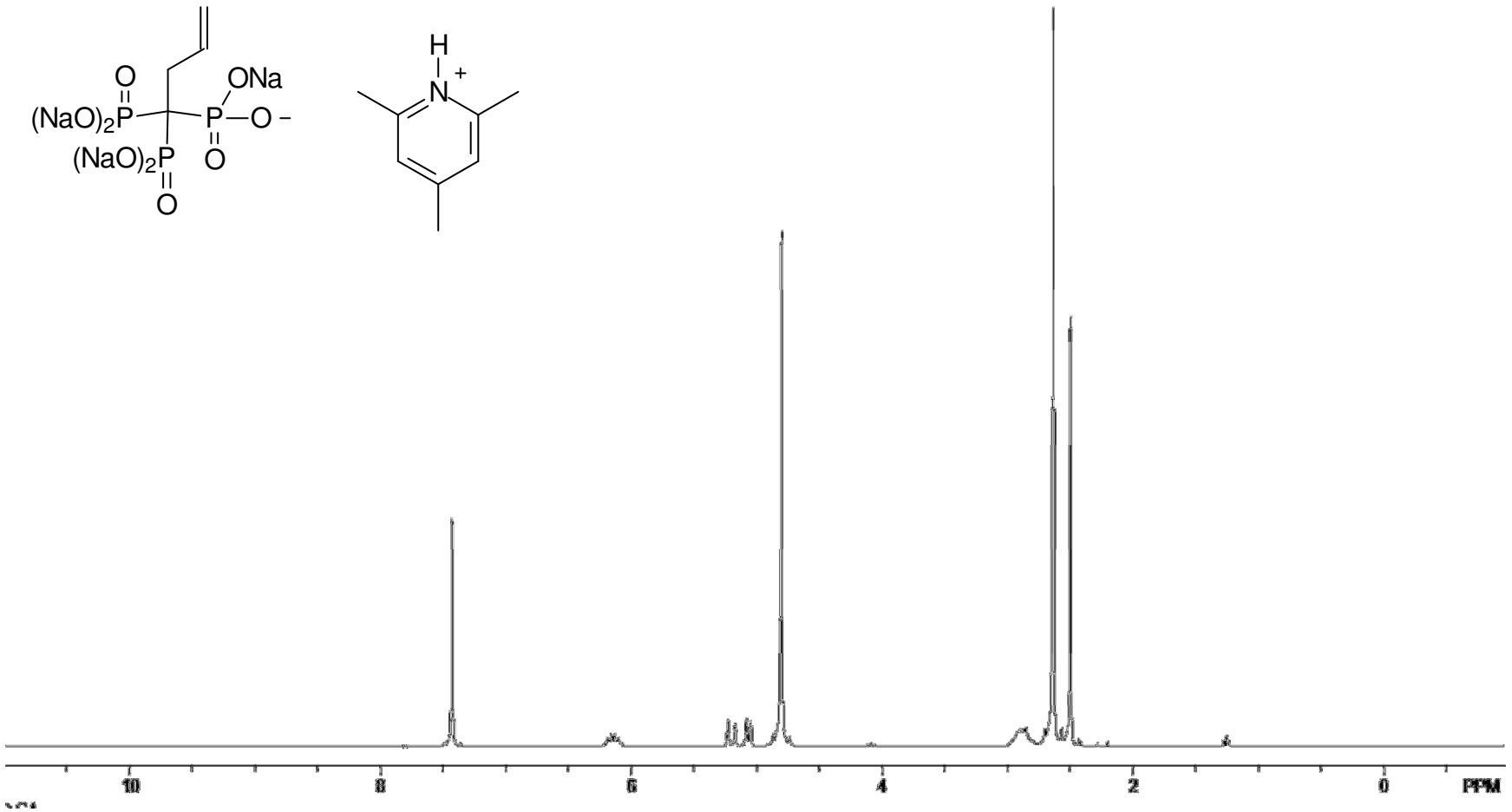


Figure A61. ^{1}H NMR spectrum of trisphosphonate **130.**

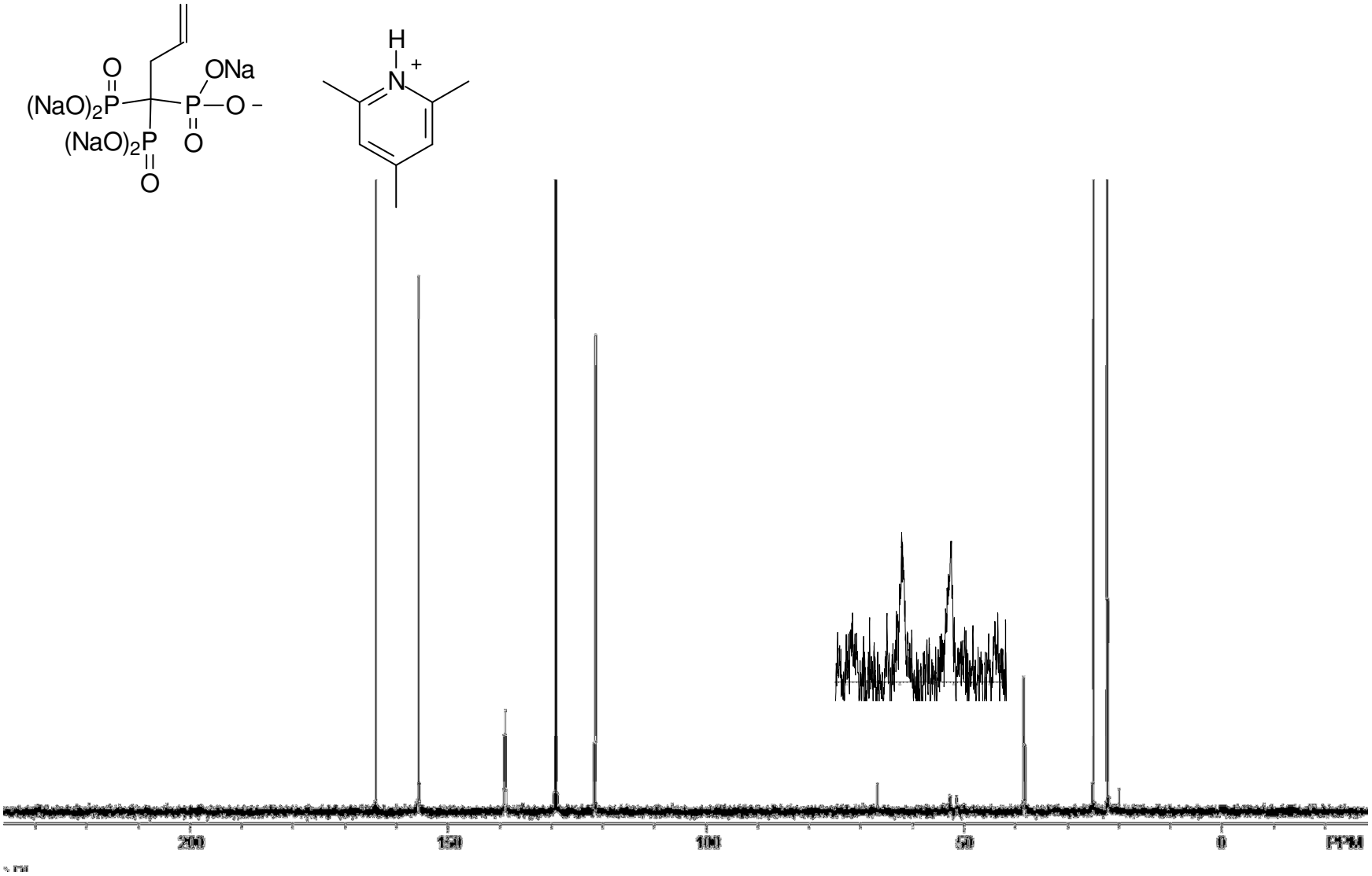


Figure A62. ^{13}C NMR spectrum of trisphosphonate **130**.

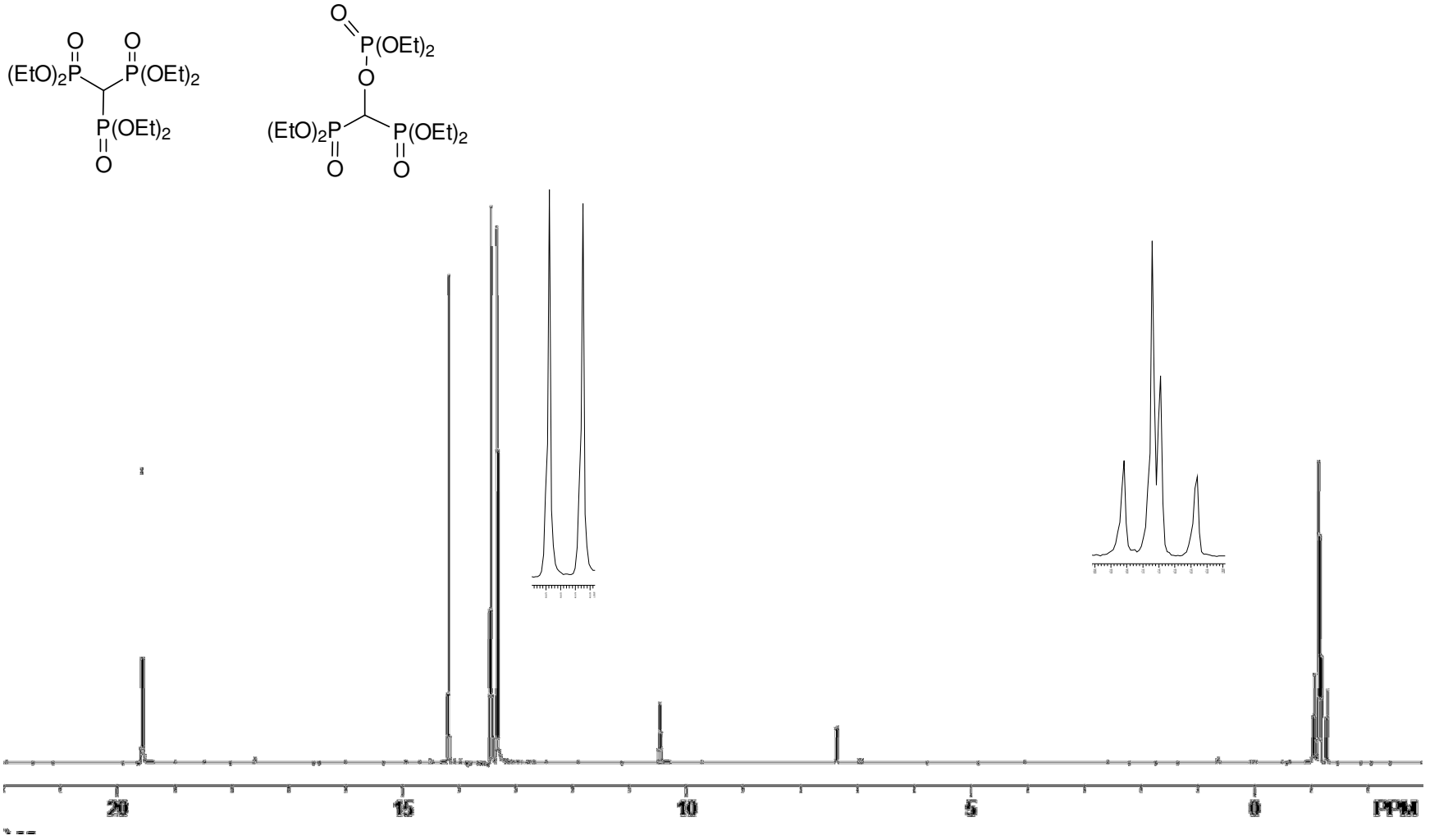


Figure A63. ^{31}P NMR of compounds **75** and **132**.

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