Synthetic studies toward the preparation of phosphate analogs of sphingolipids
by Pranab K Mishra

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry
Montana State University

Abstract:
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SYNTHETIC STUDIES TOWARD THE PREPARATION OF PHOSPHONATE ANALOGS OF SPHINGOLIPIDS

by

Pranab K Mishra

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APPROVAL

of a thesis submitted by

Pranab K. Mishra

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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To

The Two Who Gave Me Life

And

The One Who Touched My Soul

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LIST OF ABBREVIATIONS

DMSO	Dimethyl sulfoxide
DME	Dichloroethane
BTCEAD	Bis(2,2,2-trichloroethyl) azodicarboxylate
DBAD	Di-tert-butyl azodicarboxylate
DEAD	Diethyl azodicarboxylate
Standard P(V)	Condensation product of MVK and triethyl phosphite
MVK	Methyl vinyl ketone

ABSTRACT

Synthetic studies on a model system and a real system toward the syntheses of phosphonate analogs of sphingosine-1-phosphate, sphingomyelins and ceramide 1-phosphate were pursued. In the model system, the pentacovalent oxaphospholene (derived from methyl vinyl ketone and triethyl phosphite) condensed readily with bis(2,2,2-trichloroethyl) azodicarboxylate to form α,β-hydrazido-γ–ketophosphonate in high yields. Upon reduction with $NaBH_4$, this β-hydrazido γ–ketophosphonate produced the desired oxazolidinone as a diastereomeric mixture of 3:1. Treatment of the oxazolidinone with Zn/HOAc/acetone at rt readily cleaved the N-N bond. In the real system, the dienone-P(V) readily condensed with BTCEAD to form diethyl 2-(N,N'-bis(2,2,2-trichloroethoxycarbonyl)hydrazido)-3-oxo-octadecenyl phosphonate in excellent yields. After doing achiral and chiral reduction studies on this condensation product, the desired cis isomer of the oxazolidinone was produced in good yields using R-2-methyl-CBS-oxazaborolidine. N-N bond cleavage was successful on the mixture of isomers of oxazolidinones **42a,b**. Simple hydrolysis followed by proper functionalization of the cleavage product of the desired cis isomer would lead to the desired sphingolipid analogs, but was not done in this work.

INTRODUCTION

1.1: Organophosphates and Organophosphonates:

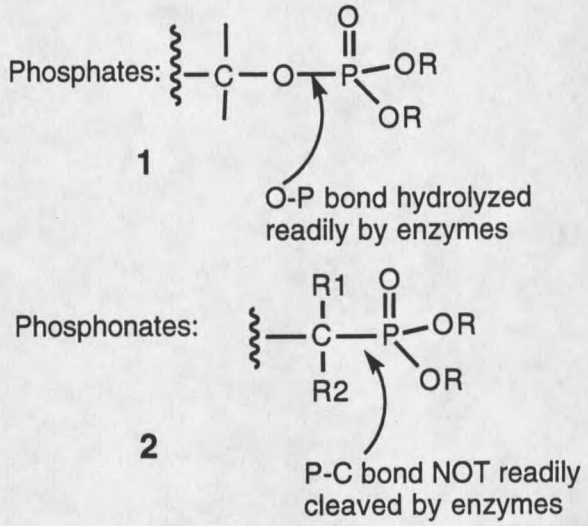
Phosphorus plays one of the most vital and life sustaining roles in all living organisms. Its amazing and diverse behavior is very well displayed by its presence in compounds such as ATP, phospholipids, coenzymes, carbohydrates, proteins and DNA.[1-4] In most of these compounds, phosphorus is present as a phosphate group. The phosphate functionality is known to be the active site in many metabolic processes.[3a] As such, phosphate containing compounds have been of deep interest to organic chemists, as well as medicinal and bio-chemists. These compounds have been found to regulate or control energy production and transfer, signal transduction,[1d,2a,c] calcification,[5d,e] cell proliferation,[5a-c] etc. However, the exact biological functions of many of these organophosphates are not well understood and are still under critical investigation.[1,2,3] While organophosphates are very abundant in nature, interestingly enough naturally occuring phosphonates are rare.

Present in a phosphate group (**1**) (**Figure 1.1**), is an oxygen atom between the carbon and phosphorus. This P-O bond is quite labile and cleaved easily in the body during metabolic processes by certain enzymes. In order to probe a particular physiological process in the body, it could be necessary to replace a labile bond with a non-labile one. In phosphonates, (**2**), the phosphorus atom is directly attached to a carbon atom (**Figure 1.1**). This carbon-phosphorus bond is not hydrolyzable, and therefore does not get cleaved by the metabolic phosphatases enzymes in biological pathways. In that

way, phosphonates could very well act as antimetabolites (agents which could perturb or inhibit a given metabolic process), and could be introduced in the body to investigate biochemical processes.[3,4,6]

Figure 1.1

Phosphonate Analogs of Phosphate-Containing Compounds



1.2: The importance of analogs:

An analog is a compound that is administered into the body of an organism as a substitute for natural metabolites. In subsequent reaction steps, these compounds are capable of specific or nonspecific inhibition of one or more enzymatic processes. Analogs are therefore administered in animal bodies to effect some physiological change or to monitor metabolic or other processes. They are currently becoming increasingly important in the pharmaceutical industries for structure-activity relationship (SAR) studies of biologically active compounds.[4a,d] Such studies can provide insight into the physiological processes that occur in animal bodies.

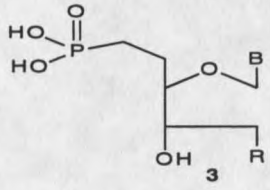
1.3: Phosphonates and their Analogs:

The phosphonic acids and their esters (phosphonates) *have* been shown to behave as analogs for naturally occuring phosphates in regulating or perturbing metabolic processes.[3,4,6] A significant interest has developed over the last three decades in the field of pharmacology and drug design for the preparation and investigation of phosphonic acids and their derivatives as analogs to the naturally occuring phosphates.[3a,4,6] Therefore, phosphonic acids and their derivatives have been in continuous investigation and scrutiny for quite some time. Not surprisingly, the number of phosphonate or phosphonic acid containing drugs is more than 200 on the market or under current development.[1a]

Phosphonates have been found to be useful for treatment of calcification diseases.[5d,e] They exhibit antiviral, antiHIV, antibiotic, and antiacidosis properties.[6,7a] Phosphonate analogs are also being used as important tools for deducing the mechanism of signal transduction across biomembranes.[2a,c] Some of the representative examples are shown in **Figure 1.2**. Compound **3** is a phosphonate containing nucleotide that is not hydrolyzed by phosphatases. These phosphorylated nucleotides can provide important information for understanding signal transduction. They are also useful for phosphorylation of proteins.[7b] Compound **5** has a methylene group which replaces the oxygen atom between the phosphorus and the carbon of the glycerol moiety. This phosphonate analog has been synthesized by Bittman and coworkers,[7d] and has been evaluated for its ability to inhibit leukemic cell growth *in vivo* and *in vitro*. The colonogenic assay indicated that **5** is a potent growth inhibitor of a monocytic leukemic cell (WEHI-3B) (IC_{50} 2.5 M). Furthermore, this compound is

also highly effective in delaying the cell growth of WEHI-3B tumors implanted in mice. Therefore, this phosphonocholine compound is a potential long-lived anticancer agent which could very well be stable *in vivo* since it cannot be hydrolyzed by phospholipase C.[7d] Compound **4** is a phosphonate isostere which is being used as a substrate for host-cell phosphorylating enzymes.[7c] Compound **6** is a phosphonothricin analog which is under medical scrutiny for use as a substitute for better medicinal activity against some gram positive bacteria.[7e]

Figure 1.2

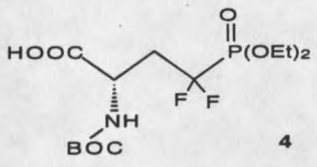


3

Phosphorylated nucleotide

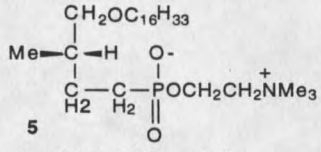
B=cytosine, guanine thymine, uracil

R= H or OH



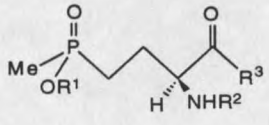
4

Phosphonate isostere analog



5

4-(Hexadecyloxy)-3-(S)-methyl butyl phosphonocholine



1-Phosphonothricin analogs

6

Everyday new organophosphates are being discovered from plant and animal extracts. Scientists are trying to synthesize different phosphonate analogs in order to get a better understanding of these organophosphates' physiological importance.

1.4: Sphingolipids: organophosphates in animal body with unknown characteristics:

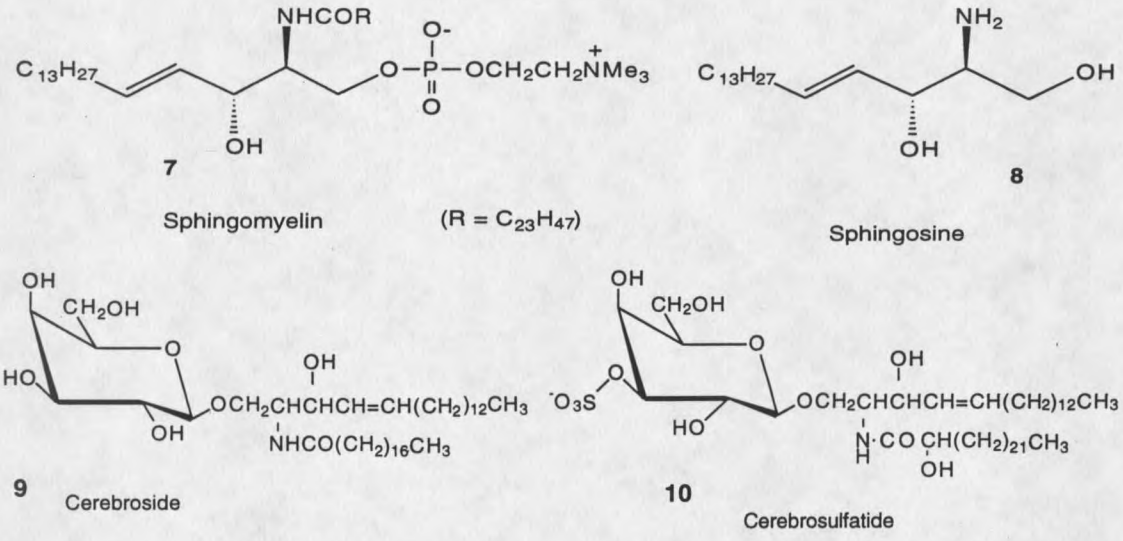
In the human body, there are many organophosphates which are responsible for signal transduction and many other functions. Most of these activities and their biological pathways are still unknown.[2,8] Phosphonate containing analogs of these compounds would be helpful in the search for better understanding of the biological activities these compounds. *Sphingolipids* are such organic biophosphate molecules. Their exact functions are still under detailed investigations.[9,10] The replacement of the scissile P-O bond by a stable P-C bond would produce analogs that are stable to chemical and enzymatic hydrolysis. Moreover, the exact active sites for the enzymes responsible in hydrolyzing these sphingolipids are yet to be discovered. Therefore, both isosteric as well as non-isosteric analogs will be helpful as enzyme inhibitory probes.[3,4,8]

1.5: Historical background:

1.5a. Sphingosines, sphingomyelins and Ceramide 1-phosphate:

In 1876, a London surgeon-chemist, Johann L. W. Thudichum, described the chemical composition of the brain, and was amazed by the presence of cerebrosides or cerebral galactosides and their chemical compositions.[9,10] Three related lipids were among the novel compounds that Dr. Thudichum discovered. He called them sphingomyelin (**7**), cerebroside (**9**) and cerebrosulfatide (**10**), (**Figure 1.3**). In his findings, he included a unique aliphatic alkaloid which was found as the basic moiety of all these lipids, and called it sphingosine (**8**).

Figure 1.3



Although these compounds were initially described and partially characterized by Thudichum, it was not until 1927 that Pick and Bielschowsky[9b] proved their structures to be N-acylsphingosine-1-phosphocholine. It was still not until another fifty years later in 1962 that Shapiro and Flowers[9c] firmly proved that all the biologically available sphingomyelins are of the *D-erythro* configuration.

The 18-carbon sphingosine is the predominant long-chain base found in most mammalian sphingolipids[9d], even though the aliphatic chain length varies in nature. Sphingosines are a group of related long chain aliphatic 2-amino-1,3-diols (**8**) which occur most frequently in animal sphingolipids. Sphingolipids are normally built from long-chain, hydroxylated bases rather than from glycerol. Two such bases are mainly found in animals: sphingosine and dihydrosphingosine (sphinganine). The other base, 4-hydroxy-sphinganine (phytosphingosine), is abundant in both animal and plant kingdoms. When the amino group of sphingosine or sphinganine is acylated

with a fatty acid, the product is a ceramide. The primary hydroxyl group in sphingosine is substituted in one of two ways to give two classes of sphingolipids; with a phosphocholine group it is called a phosphosphingolipid (known as sphingomyelin or ceramide-1-phosphate) and with a carbohydrate (either a mono or oligosaccharide) it is called the glycosphingolipid.

1.5b: The importance of these century old compounds:

Many problems were encountered in the isolation of sphingolipids from the brain. These classes of lipids were largely ignored by the biochemists in the early twentieth century. This resulted in a prolonged time for establishing the complete structures of Thudichum's originally reported sphingolipids. As stated above, these structures were not well established until late 1960's.[9c] Thudichem named these compounds as sphingosines (according to Sphynx of Egypt) probably by being amazed by their structural complexity. The biochemists and medicinal chemists were drawn towards these lipids when it was discovered that problems in biosynthesis of these lipids can cause sphingolipid storage disorders in humans.[10] Highly abnormal levels of various sphingolipids, such as sphingomyelin (Niemann-pick disease), cerebroside (Gaucher's disease and Krabbe's globoid cell leukodystrophy), and an acidic glycosphingolipid called cerebrosulfatide (Tay-Sachs disease), were found in the brains of sick babies.[11] Therefore, the enzymes involved in the metabolism of these lipids came under thorough investigation. The biochemists, medicinal chemists and biologists were very much concerned with the pathogenesis and therapy of patients with these metabolic diseases.[12-17]

1.5c: Sphingolipids - a brief survey:

Sphingolipids are very important membrane lipids found in animal and plant cell walls. Sphingosines are the major constituents of these lipids in animal cells. The most striking feature of the membrane lipids is their enormous diversity. It is very obvious from the analysis of different structures of sphingosine bases that they are very heterogeneous components of sphingolipids, with diverse differences in the structure of the long alkyl chain. The chain length may vary from 14 to 22 carbon atoms, with branching at the ω-1 (iso), ω-2 (anteiso), or internal carbon atoms. The double bond could be at the remote site of the chain or at C-4.

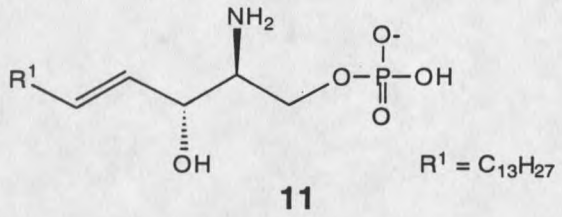
Many different kinds of sphingolipids are known, with a report of more than 300 different structures. The reason for this diversity is not clear, although there is an increasing awareness of the multiple roles of lipids in membranes.[11] Certainly the major role of membrane lipids is to form the bilayer matrix with which the proteins interact. Cerebrosides (ceramide monosaccharides) are important membrane constituents of the brain and central nervous system, and gangliosides (ceramide oligosaccharides that contain sialic acid) have an important role in many tissues as cell-surface receptors. These carbohydrate-containing sphingolipids are found usually in the plasma membrane where they are asymmetrically disposed in such a fashion that their polar head groups are extended into the extracellular environment. Sphingomyelin occurs in both intracellular and plasma membranes. Interestingly they are symmetrically distributed in the bilayer of plasma membrane.

In humans, there are ten known classes of lipid storage diseases in which the degradation of sphingolipids does not take place. This results in

accumulation of sphingolipid, followed by the swelling and malfunction of tissues. It affects the young and usually leads to an early death. The most common symptom in all these diseases is mental retardation. This phenomenon emphasizes the importance of breakdown and resynthesis of sphingolipids in nervous tissue. The retina, spleen and liver could also get affected. The diseases are inherited and are due to genetic defects that result in reduced hydrolytic enzyme activity. During the growth and development of tissues, the cell material is being constantly degraded and resynthesized. Membrane lipids are degraded by lysosomal enzymes.

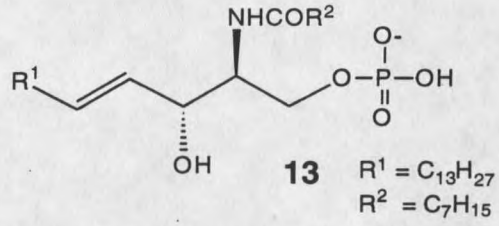
In recent years, a great deal of research has been directed toward better understanding of the biological roles of these naturally occuring compounds. As a result of that, glycosphingolipids have been found to play important roles in a host of biological functions. For example, glyco-sphingolipids have been shown to be involved in such processes as cell growth and differentiation cell-cell recognition and adhesion, oncogenesis, molecular recognition and neuronal repair. Glycosphingolipids are also known to play a role in various lipid storage diseases. The phosphosphingolipids, e.g., sphingosine-1-phosphate **11**, sphingomyelin **12**, and ceramide 1-phosphate **13** (the biosynthetic precursor for sphingomyelin biosynthesis), are key components in mammalian cell membranes, and play a diverse role in signal transduction pathway which is very poorly understood.

Figure 1.4



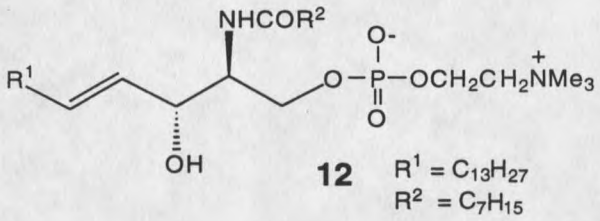
11

Sphingosine-1-phosphate



13

Ceramide-1-phosphate



12

Sphingomyelin

1.5d: Sphingosine-1-phosphate:

Sphingosine-1-phosphate, **11**, is an intermediate product during the degradation of sphingosine by sphingosine kinase to ethanolamine-1-phosphate and a long chain aldehyde (e,g., palmital) by a lyase reaction dependent on pyridoxal phosphate.[18a] Recently, the biological significance of sphingosine-1-phosphate has been reported.[18d,e,f] It has been found that sphingosine-1-phosphate mobilizes the activity of Ca^{2+} in some cells, but the exact physiological significance of sphingosine phosphate still remains unclear.[18f] Very recently, Igarashi and coworkers have demonstrated that sphingosine-1-phosphate inhibits the motility of melanoma cells at a very low concentration (10nM), but at the same concentration sphingosine, N,N-dimethyl-sphingosine, and N,N,N-trimethyl-sphingosine have no inhibitory effect.[18h] Moreover, sphingosine-1-phosphate **11** is far less cytotoxic than

these other three compounds and does not inhibit protein kinase C.[18g] From all these findings, it has been concluded that sphingosine-1-phosphate may act as a potent agent for prevention of tumor cell metasthesis and inflammatory processes. Both of these processes are highly dependent on cell motility. Very few chemical syntheses of sphingomyelin and ceramide-1-phosphate have been reported[18h], and no chemical synthesis of their analogs have yet been reported. The important properties of sphingosine-1-phosphate are described in **Chart 1.**

Chart 1

- Intermediate product during degradation of sphingosine by sphingosine kinase.
- Physiological function in cells is still unclear.
- Does not inhibit protein kinase C.
- It is associated with:
  (a) Ca^{2+} mobilizing activity in certain cells
  (b) Inhibition of motility of melanoma cells at a very low concentration (~10 nM).

1.5e: Sphingomyelins:

Sphingomyelins, the major phospholipid components in nerve cells of mammalian cell walls[10,11] are also present in abundance in lipoproteins.[15] When sphingomyelin metabolism is not functioning properly in an animal, this animal could very well suffer from atherosclerosis, cancer or Niemann-Pick disease, etc. (*vide supra*).[13,14] It has also been well documented that the breakdown product of sphingomyelin is sphingosine. It is this sphingosine that is responsible for inhibiting protein kinase C, which plays a key role in cell regulation and signal transduction.[15] It appears that sphingomyelins have a

participating role in cell regulatory functions and trans-membrane signalling. The major functions of sphingomyelins in mammalian cells are summarized below in **Chart 2.**

Chart 2

Sphingomyelins: Major Functions

- Major phospholipid components in plasma membranes of mammalian cells.
- Important source of lipid second messengers
- Plays a key role in cellular signal transduction pathway.
- It is associated with:
  - (a) Intracellular cholesterol balance
  - (b) Aetherosclerosis
  - (c) Muscular dystrophy
  - (d) Leukæmia
  - (e) Niemann-Pick disease
  - (f) Protein Kinase C Activity

1.5f: Ceramide 1-phosphate:

Ceramide-1-phosphate is the phosphorylated form of ceramide.[13a] This novel phospholipid has been found in rat brain synaptic vesicles[13b] and very recently in human leukemia cells.[13c,d] The hydrolysis of ceramide-1-phosphate apparently takes place by ceramide-1-phosphate phosphatase which is present in liver membranes.[13f] It is not well understood what the exact physiological function of ceramide-1-phosphate is in intercellular transduction. The important functions of ceramide-1-phosphate in the body is represented in **Chart 3.**

Chart 3

- Ceramide-1-phosphate is a biosynthetic precursor to sphingomyelin.
- Galactosyl ceramide is a receptor for HIV binding in cells that lack the CD4 receptor.
- It is found in leukemia cells and rat brain synaptic vesicles.
- It is associated with:
  (a) a key role in cellular signal transduction pathway.
  (b) induction of protein kinase C activity.

1.6: Why membrane sphingolipid analogs?

It has been quite well established for sometime now that glycerophospholipids and their metabolic products (such as diacylglycerol (DAG), inositol triphosphate, eicosanoids, and platelet-activating factor) function in signal transduction and cell regulation.[16] However, the membrane sphingolipid's role in signal transduction has not been well established to date[18], even though these membrane sphingolipids exhibit greater structural diversity and complexity than the glycerolipids (*vide supra*). A lot of questions remain unanswered, such as (i) what is the exact physiological function of ceramide 1-phosphate and sphingosine 1-phosphate in cellular signal transduction processes? (ii) what are the activating factors of sphingomyelin hydrolysis and ceramide generation? (iii) why is there a localization of the signalling pool of sphingomyelin within the cell? (iv) what is the source of sphingosine, the key modulator in this process? (v) what is the mechanism of phospholipid activation of sphingolipid hydrolases? (vi) what is the structure of the active sites in all these enzymes?[13-18]

In spite of the wide range of biological functions exhibited by the phosphosphingolipids, they are actually relatively scarce and difficult to obtain in pure form from biological sources. Therefore, the syntheses of cell permeable analogs of these phosphosphingolipids from easily available starting materials are necessary in order to further explore the functions of these compounds in biological systems.

The use of the naturally occuring sphingomyelins as precursors to these chemically defined sphingomyelins has some disadvantages as well. There is configurational instability at the allylic (C-3) position during acid-catalyzed hydrolysis of the amide-linked chain in 1-butanol at 95° C for 90 min[16a], and in methanol at 70°C for 20 h.[16b] All these published methods have limitations due to the fact that for introduction of the phosphocholine moiety into ceramide at C-1, protection of the allylic hydroxyl group prior to the synthesis is necessary. The common strategy utilized in short for these steps were: (i) blocking of the C-1 hydroxyl group as TBDPS ether, followed by protection of the C-3 hydroxyl group as THP ether, (ii) desilylation, (iii) phosphitylation, (iv) choline insertion, (iv) phosphite oxidation, and deprotection of the THP group.[13-16] In almost all cases, the easily cleavable O-P bond was left intact, which makes these substrates very poor enzyme inhibitors.

1.7: Conclusions:

It is clear from the previous discussions that preparation of phosphonate analogs of sphingolipids would be very helpful to the scientific community in many respects. If synthetically available procedures could be discovered for their developments, these kinds of analogs could very well open up a clear view

of the mysterious behaviors in signal transduction and other processes for the sphingolipid phosphates that are active in the human body.

BACKGROUND

2.1: The phosphonate analogs of sphingolipids:

The McClure laboratory has been engaged in development of new strategies and synthons for the syntheses of natural products and their analogs over the last few years.[20, 21a-g, 23] During this time, one of the projects was focussed on the syntheses of phosphonate analogs of different phosphosphingolipids (**Figure 1.4**), e.g., sphingosine-1-phosphate **11**, sphingomyelin **12** and ceramide 1-phosphate **13**. As discussed before, the idea was to replace the labile P-O bonds in these sphingolipids by a non-labile P-C bond. These analogs are of considerable interest in membrane studies as enzyme inhibitors and as potential therapeutic agents.[4] Very little is known about the active sites of enzymes sphingomyelinase, sphingosine kinase or ceramide kinase.[13a] The metabolic pathways of these three sphingolipids are still under detailed investigation. The phosphorus-carbon bond would be strong enough not to be metabolized by the enzyme. Therefore, these analogs could act as specific and potent inhibitors for the enzyme sphingomyelinase, sphingosine kinase or ceramide kinase, which might help in better understanding of their behaviors in the animal body.

In spite of enormous progress in the fields of synthetic methodology and total synthesis of natural products in the last two decades, viable procedures for the synthesis of enzyme inhibitory analogs of the sphingolipids are still using semisynthetic routes starting from naturally occuring sphingosines.[16g] Sometimes, microbiological processes[16h], often are taken advantage of, providing substantial shortcuts that cannot be attained by classical organic

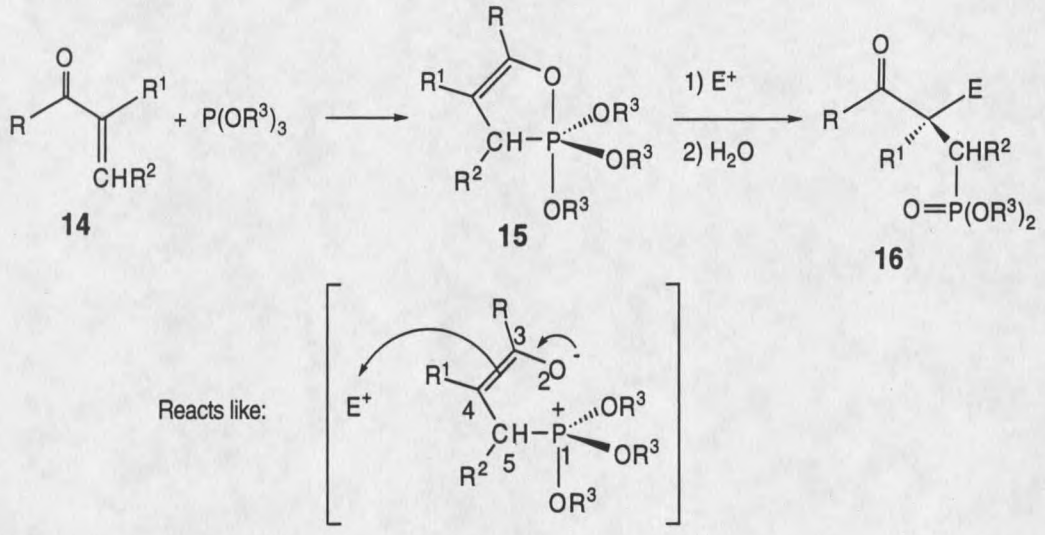
chemistry methods. But still, very few synthetic procedures are currently available for preparation of phosphonate analogs of sphingomyelins and their derivatives. In our synthetic approach, the phosphonate group is introduced in the middle of the sequence of the synthesis and stays untouched in the entire synthetic process as a phosphonate ester. As the molecule takes shape, the facile hydrolysis of these ester groups would enable the production of the desired sphingolipid analogs. The analogs would be one atom shorter than the natural sphingosine derivatives and would thus be the non-isosteric analogs of these sphingolipids. As had been discussed already, very little light has been shed about the binding sites or active sites for sphingomyelinase, sphingosine kinase, or ceramide kinase. Therefore, both isosteric as well as non-isosteric analogs are of importance for SAR studies.[18,19]

2.2: Organophosphorus methodology: a model study for the preparation of Sphingosine analogs.

It was desirable to come up with a synthetic protocol where the C-P bond would be established in place of C-O-P bond, and at the same time the synthetic sequence would be available to classical organic synthetic chemists. In recent years, McClure and coworkers have developed a new organophosphorus methodological approach for preparing highly functionalized phosphonates, **16**, (**Scheme 2.1**) using 2,2,2-trialkoxy-1,2λ^5-oxaphospholene **15** as a nucleophilic source.[20,21a-g] Different kinds of substituted and unsubstituted enones reacted with different trialkyl phosphites to give a series of pentacovalent oxaphospholenes. All these P(V) oxaphospholenes are nucleophilic in nature. As with other carbon

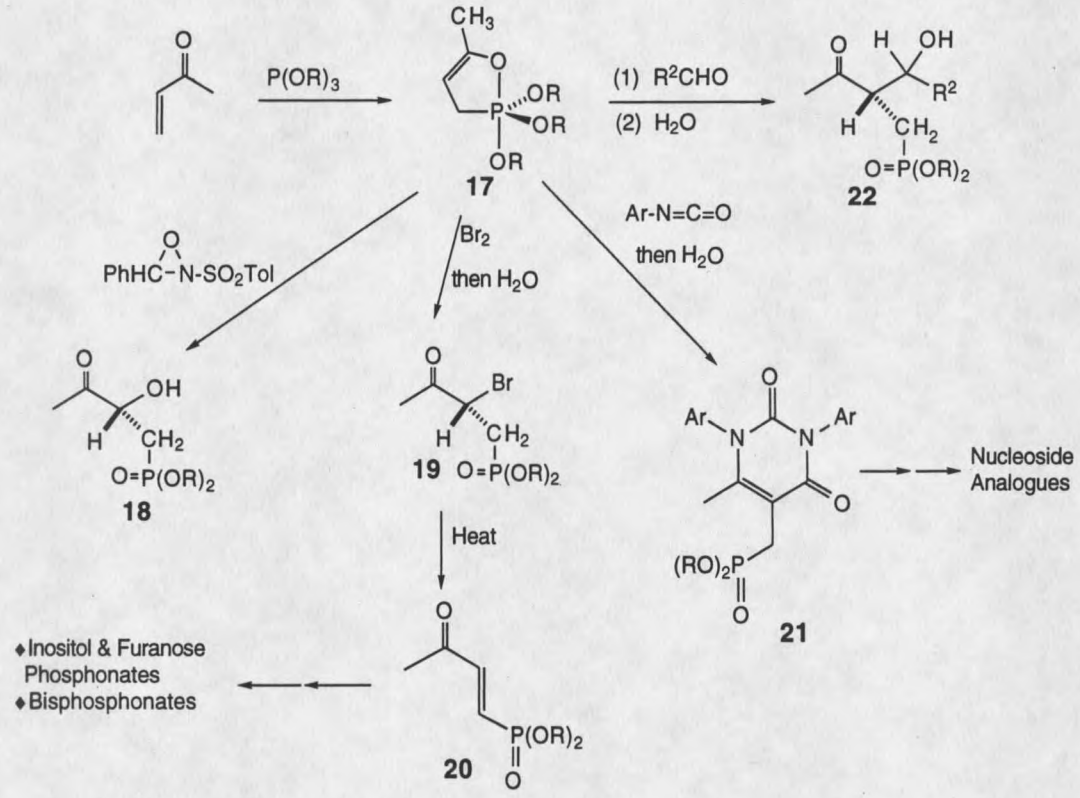
nucleophiles, here also the electron rich carbon at C-4 in **15** attacks different electrophiles like a nucleophile (**Scheme 2.1**).

Scheme 2.1



In model studies utilizing the P(V) **17** derived from methyl vinyl ketone and trialkyl phosphites, many different electrophiles were reacted to produce functionalized phosphonates. Thus, it was shown that **17** reacted with aldehydes under neutral conditions (**Scheme 2.2**) to produce β-hydroxy ketones (**22**) containing an α-phosphonomethyl group.[20,21a,b,c] It was also reported[21d,f,g] that **17** reacted with other electrophilic sources, e.g., bromine, N-bromosuccinimide, oxaziridine, isocyanates, etc., to produce β-heteroatom substituted γ-ketophosphonates or a β-acyl vinyl phosphonate in high yields (**Scheme 2.2**). This methodology is currently being explored to develop new routes to inositols, carbohydrate derivatives, nucleoside analogs, and bisphosphonates, etc.[21]

Scheme 2.2

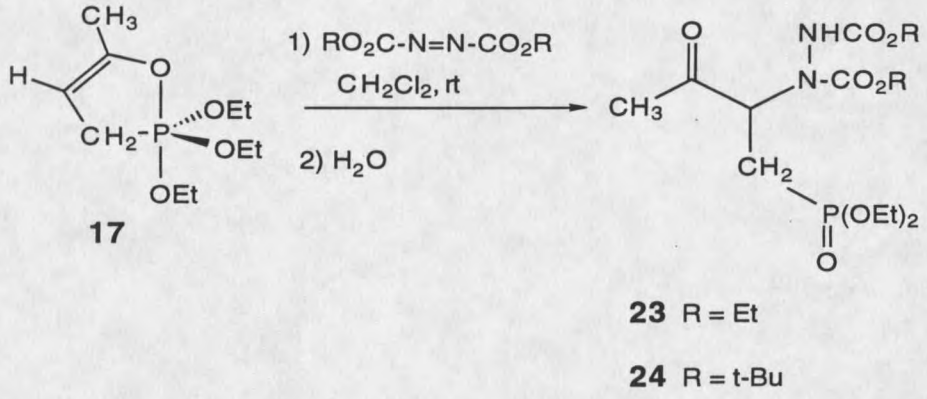


2.3: Initial Attempts to prepare the β-hydrazido-γ-keto phosphonate. A model study toward the preparation of sphingolipid analogs:

McClure and Grote discovered that these oxaphospholenes also react with azo-dicarboxylates to produce β-hydrazido-γ-keto phosphonates in high yields.[21f,g] During these studies, they found that dialkyl azodicarboxylates (R = ethyl and t-butyl) could readily condense with the oxaphospholene **17** in methylene chloride to form the β-hydrazido-γ-keto phosphonates **23**, and **24** (**Scheme 2.3**). This reaction was further optimized by Mishra.[21j] It was found

that the best temperature for these reactions was 0 °C and the solvent of choice was diethyl ether in order to get excellent yields (90-95%).

Scheme 2.3



23 R = Et

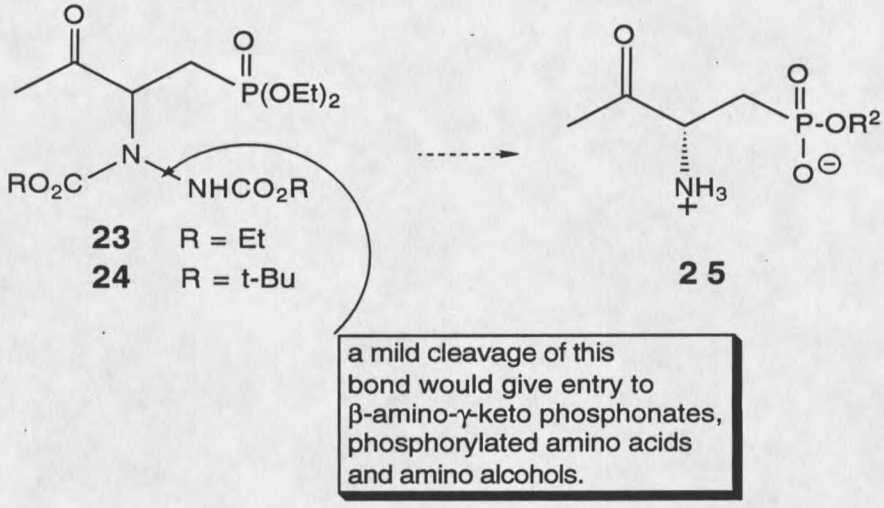
24 R = t-Bu

C. W. Grote, PhD Thesis, 1991, Univ. Of Delaware

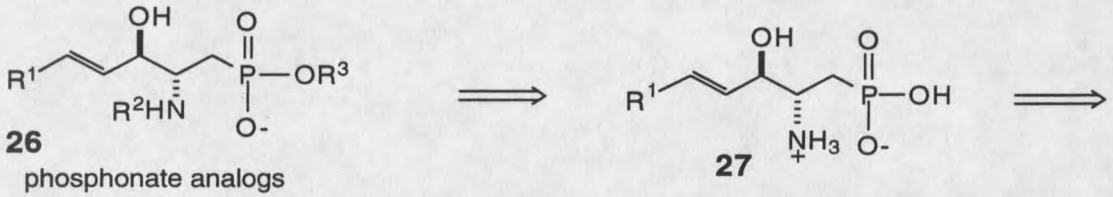
If the N-N bond in the β-hydrazido-γ-keto phosphonates **23** or **24** could be cleaved, then this synthetic sequence would allow the entry into the preparations of different kinds of β-amino-γ-keto phosphonates **25**. Although numerous literature references are available for preparing α-amino-phosphonates,[22] there are fewer options to synthetic chemists for the preparation of β-amino-γ-keto phosphonates, **25**. In particular, no reports exist as such in the literature concerning the reaction of 1,2-λ^5-oxaphospholene **17** with electrophilic nitrogen sources to prepare these kinds of functionalized phosphonates. Thus, the condensation products **23** or **24** could very well be useful as precursors for producing many different kinds of phosphonate derivatives or analogs of amino alcohols or amino acids. Therefore, the most important part of this model study was the key cleavage of the N-N bond,

(**Scheme 2.4**). If the N-N bond cleavage was successful, this methodology would allow the entry into the preparations of different kinds of sphingosine analogs (**Scheme 2.5**).

Scheme 2.4



Scheme 2.5



phosphonate analogs

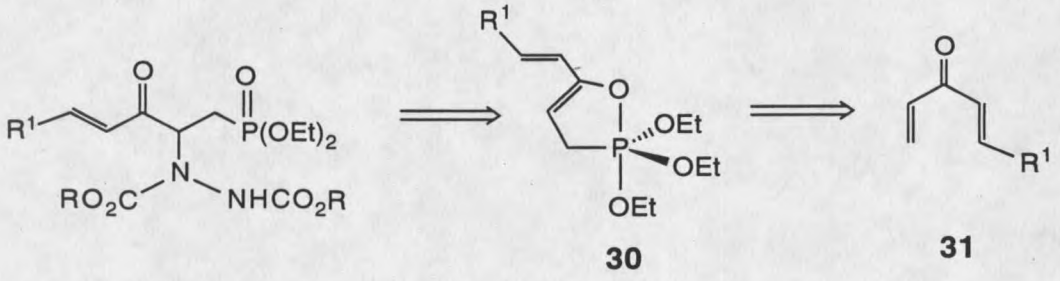
$R^1 = (CH_2)_nCH_3$ for sphingomyelin, sphingosine and ceramide-1-phosphonate

R^2 = H, for sphingosine

$R^2 = CO(CH_2)_nCH_3$ for sphingomyelins and ceramide-1-phosphonate

$R^3 = CH_2CH_2\overset{+}{N}Me_3$,for sphingomyelin, and sphingosine-1-phosphonate

R^3= H, for ceramide 1-phosphonate



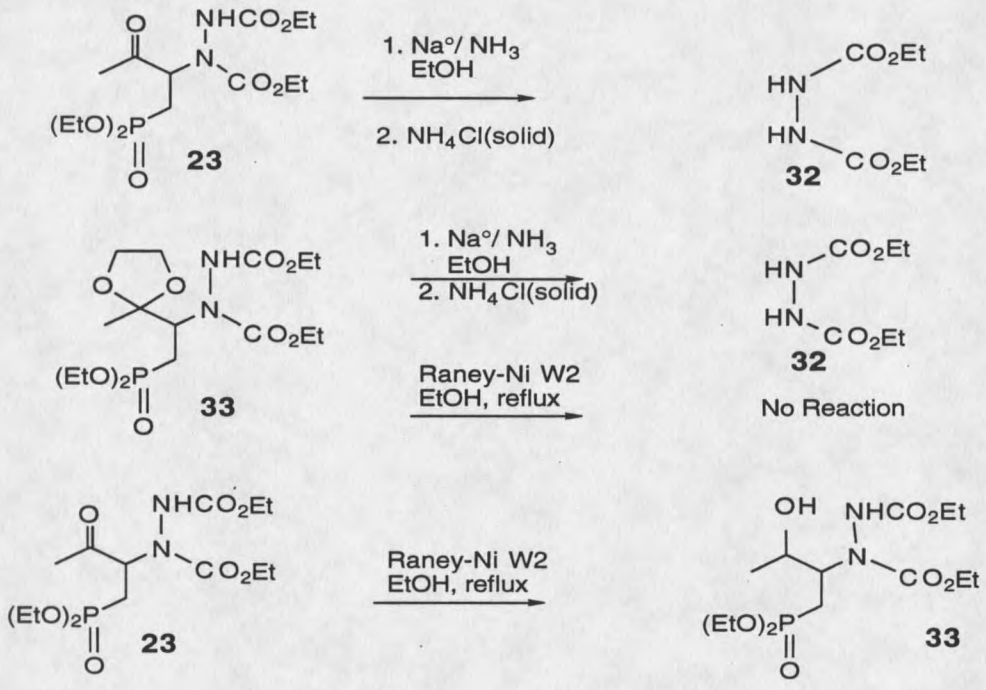
28 R = Et

29 R = t-Bu

Initial attempts pursued by McClure and Grote for the desired cleavage of N-N bond led to some interesting results.[23] The condensation product **24** failed to undergo decarboxylation to produce the hydrazine. The diethyl hydrazide derivative **23** was utilized for the reductive cleavage in both the protected form (as a 1,3-dioxolane), as well as the unprotected ketone by using different cleavage conditions.[24a-d] On the unprotected ketone system, Raney nickel led to the reduction of the ketone to the alcohol. In the protected system the same reagent showed no reaction. Dissolving metal reduction procedures were also attempted on both the compounds. Here the cleavage took place at

the C-N bond α to the ketone (or protected ketone) to give dialkyl hydrazide (**Scheme 2.6**).[23] It has been established in the literature that dissolving metal conditions produce anions on the carbon α to the phosphonate group.[24e]

Scheme 2.6

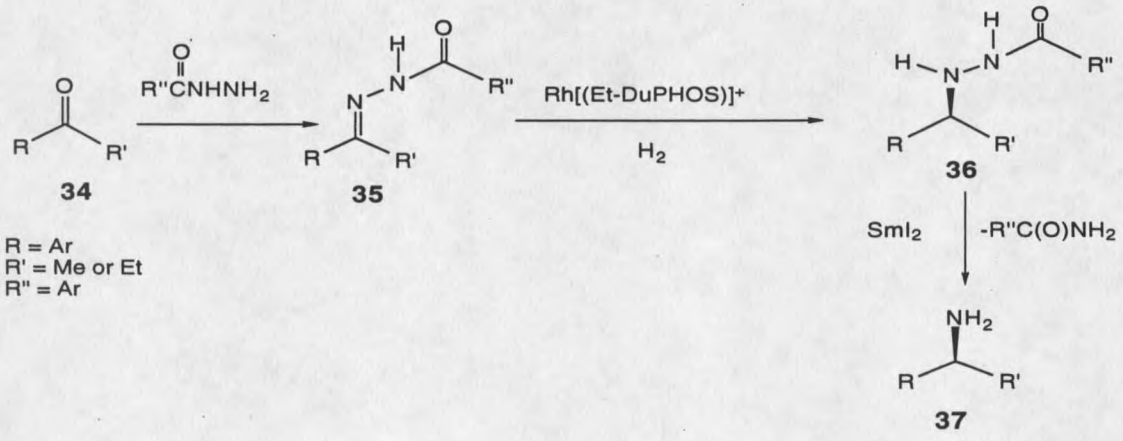


2.4: SmI_2 - Could it induce this N-N bond cleavage?

Since the first introduction by Kagan and co-workers, SmI_2 has become an extremely important reagent for organic synthesis.[25] Samarium (II) iodide can easily be prepared in THF from samarium metal through its oxidation with diiodoethane,[25b] molecular iodine,[25c] or mercuric iodide.[25d] In recent years, samarium diiodide has evolved as a unique single electron transfer reducing agent for promoting sequential transformations.[25e] These transformations do

include both ionic as well as radical intermediates. The first product is SmI_3, but this is subsequently reduced to SmI_2 by samarium metal. This powerful reducing agent, SmI_2, can initiate a variety of coupling reactions and functional group transformations.[25a-h] However, Burk and coworkers[25d] have recently taken advantage of samarium diiodide to cleave the N-N bond in a system as described in **Scheme 2.7**. On the basis of the rich chemistry uncovered for this reagent, Burk hypothesized that this reagent could very well trigger a cleavage of N-N bond in N-benzoyl hydrazine product **36** to prepare chiral amines **37**. The reagent did cleave the N-N bond when 2.2 equivalents were used in the presence of a proton source (here MeOH) within 20 minutes.

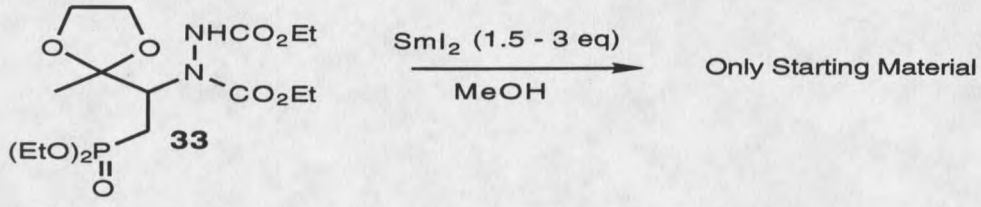
Scheme 2.7



This simple reductive amination by SmI_2 encouraged us to select it as a reagent for our system as well. The carbonyl in the β-hydrazido-γ-keto phosphonate **33** was protected and subjected to SmI_2. The color of the reaction mixture did change to yellow (blue SmI_2 goes to yellow SmI_3), but to our

dismay, nothing happened, and only starting material was recovered. Even 3 equivalents of samarium iodide failed to produce the desired product.

Scheme 2.8



2.5: Conclusions:

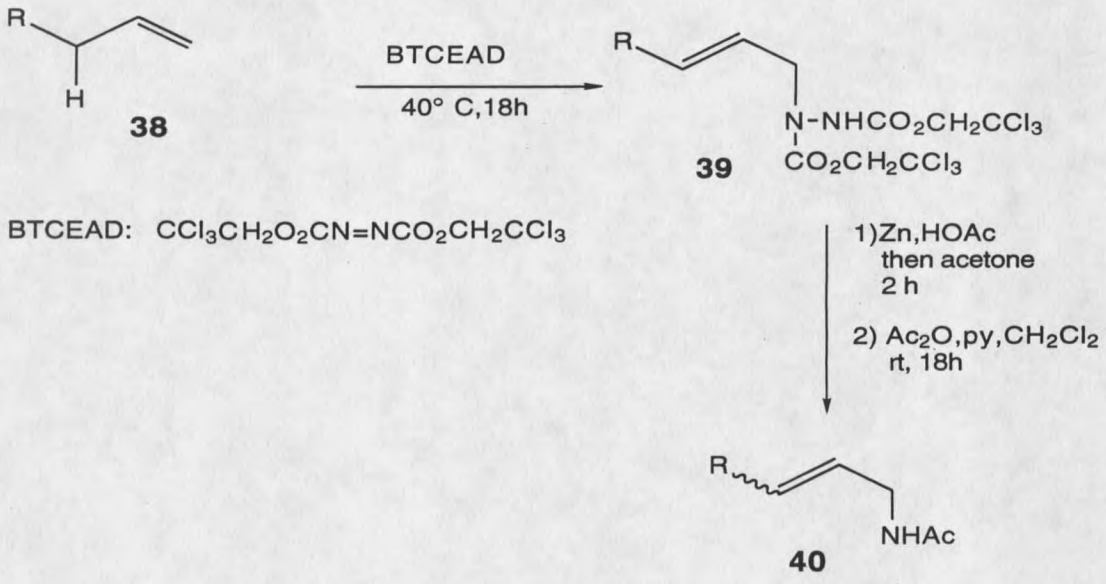
All these studies for cleaving the N-N bond indicated that it might be important to look for a better source for electrophilic nitrogen where the cleavage might be favorable even in the presence of a phosphonate. What the exact character of the phosphonate is in the cleavage is not very clear, although electronically it is probably playing some role. Literature references were not available for these kinds of systems. We decided to pursue the model system further and explore all the possibilities in this regard.

RESULTS AND DISCUSSIONS PART 1

3.1: Search for new electrophilic nitrogen sources:

The demand for a practical electrophilic nitrogen source (NH_2^+) has shown them to be in short supply.[24] For the most part, the choice of such amination reagents is limited to azodicarboxylates or aryl azides. It was clear that while several other cleavage conditions could be tried on the ethyl and t-butyl systems, at the same time other better electrophilic nitrogen sources should be investigated to find more suitable conditions for the desired N-N bond cleavage reaction in the presence of the phosphonate group. Leblanc and coworkers' electrophilic amination protocol using bis (2,2,2-trichloroethyl) azodicarboxylate revealed a very useful procedure for the preparation of different kinds of amines (aromatic as well as aliphatic).[26a] In their report, Leblanc and coworkers reported an N-N bond cleavage on a trichloroethyl hydrazide using very mild Zn/acetic acid/acetone conditions.[26b] They demonstrated that the electronic nature of the ester groups in the azodicarboxylates affect their reactivities. Accordingly, replacement of the ethyl esters in diethyl azodicarboxylate (DEAD) by 2,2,2-trichloroethyl esters (BTCEAD) accelerated a bimolecular ene reaction they were investigating.[26c] Thus, amination of an olefin via an ene reaction with this sufficiently reactive azo compound was pursued by Leblanc. The very mild conditions used for cleavage of the N-N bond was a very efficient way to introduce amine functionality without alteration of the double bond.[26]

Scheme 3.1

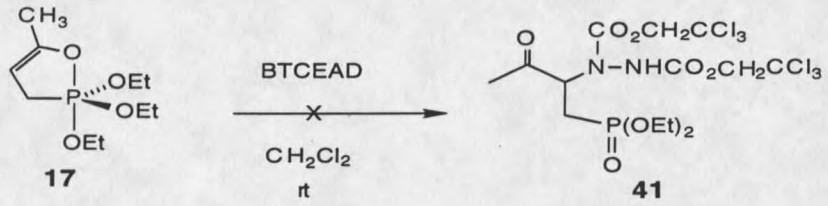


3.2: Attempted Condensation of BTCEAD with P(V) 17:

Because of the presence of the chlorines, it was expected that the behavior of BTCEAD would be somewhat different compared to the other azodicarboxylates eg., DEAD or DBAD, in the first step of the condensation reaction with our oxaphospholene. The condensation of BTCEAD with the standard P(V) **17** was tried using the same conditions as utilized for DBAD or DEAD. For these systems (DBAD, DEAD), the reactions were done in CH_2Cl_2 at room temperature and produced good yields. When the reaction of **17** was tried with BTCEAD using the same conditions as with DEAD or DBAD, no recognizable products were obtained (**Scheme 3.2**). The outcome was very messy no matter what was done to get a reasonable yield of the desired product. One of the main products was always the hydrolysed P(V). However,

there were also always more than 8 spots on the TLC plate. Changing solvents or using additives like HMPA, etc., was ineffectual. It was also obvious that the temperature was playing an important role. Because of the presence of 6 chlorines in the molecule, the BTCEAD was acting as a very hot electrophilic source. The condensation of this extremely hot electrophile with **17** using the conditions shown in **Scheme 3.2** did not lead to any recognizable products. To slow the reactivity, a low temperature NMR study was pursued. It was found that at -78 °C there was no reaction, only starting material was found in the ^{1}H NMR and ^{13}C NMR. When the temperature was raised to -40 °C, a little hydrolyzed P(V) was formed but the rest was still starting material. On further raising the temperature, ultimately to -10 °C, all starting material disappeared and many compounds were formed.

Scheme 3.2

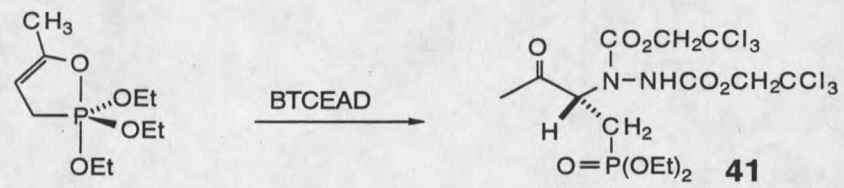


BTCEAD = $CCl_3CH_2O_2CN{=}NCO_2CH_2CCl_3$

Low Temp NMR Study In $CDCl_3$	
Temp	Observation
-78° C	No Reaction Only Starting Material
-40° C	Little Hydrolyzed PV---------Rest Starting Material
-10° C	No Starting Material -------many compounds

A thorough investigation was undertaken to figure out the best reaction conditions for this condensation step. It was clear, however that the reaction was highly sensitive to mode of addition, solvents, temperature and quenching conditions (**Table 3.1**). It emerged from this study that at a lower temperature (-78° C) and with inverse addition in Et_2O, the reaction gave the highest yields (87-93%). It has been shown previously that the reactivity of the 1,2λ^5-oxaphospholene was slowed down considerably in diethyl ether.[20] It was also very obvious from all these studies that slowing the reactivity of the BTCEAD was very important in order to produce clean condensation products in high yield.[21g]

Table 3.1



BTCEAD: $CCl_3CH_2O_2C\text{-}N{=}N\text{-}CO_2CH_2CCl_3$

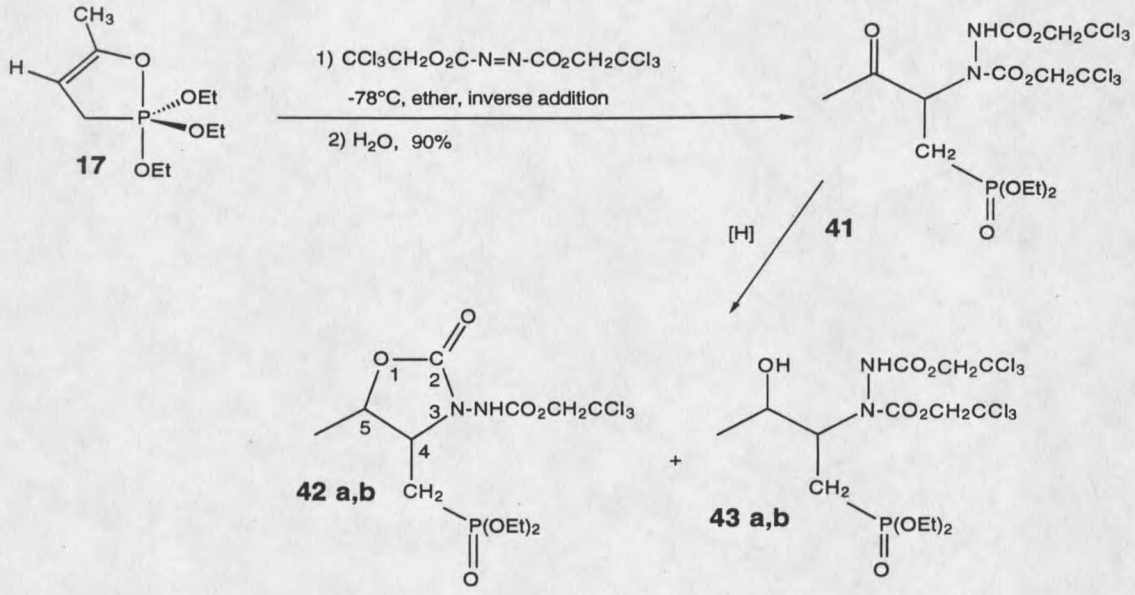
Mode of addition	Temp	Solvents	Yields
Direct addition	0°C-rt	CH_2Cl_2	No Pdt, (mess!)
Direct addition	-78°C	CH_2Cl_2	5%
Inverse addition	0°C-rt	CH_2Cl_2	10%
Inverse addition	-78°C	CH_2Cl_2	20%
Direct addition	0°C-rt	Et_2O	35%
Direct addition	-78°C	Et_2O	55%
Inverse addition	0°C-rt	Et_2O	68%
* Inverse addition	-78°C	Et_2O	87-93% *

3.3: Reduction of the β-keto hydrazide. Preparation of oxazolidinone and N-N bond cleavage:

At this stage and before attempting the N-N bond cleavage, it was decided to reduce the ketone in **41**. Reduction of **41** by $NaBH_4$ in EtOH at -78° C and quenching of the reaction mixture at -78° C produced 30 % of the alcohol **43** and 70% of the oxazolidinone **42** (see **Scheme 3.3**). Attack of the incipient alkoxide on one of the hydrazide diester carbonyls produced the oxazolidinone **42,** and provided internal protection of the alcohol. When the hydrazide was

reduced at 0° C - rt, the only isolated compound was oxazolidinone **42** in very high yield. Both the alcohol and the oxazolinone were a 3:1 mixture of diastereomers.

Scheme 3.3



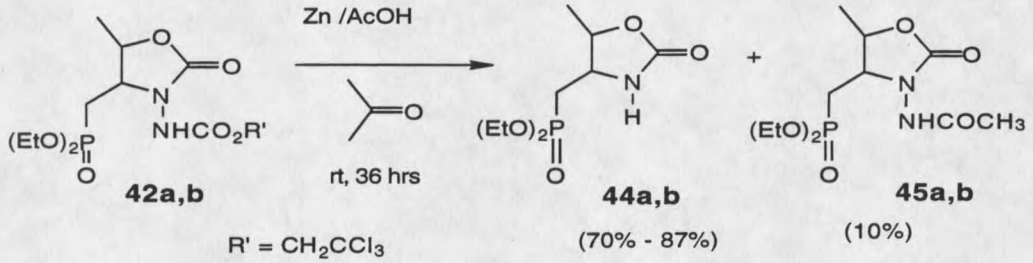
$NaBH_4$, -78°C EtOH	50%	30%
$NaBH_4$, 0°C -> r.t EtOH.	82%	0%

The structure of the oxazolidinone **42** was established by performing ^1H NMR homonuclear decoupling experiment. The multiplet for 1 proton at 4.48 ppm resolved to a quartet upon irradiation of the methine multiplet at 3.82 ppm. The multiplet at 4.48 ppm also resolved to a doublet upon irradiating the methyl doublet at 1.49 ppm. Therefore, the multiplet at 4.48 ppm was due to the methine on C5. Also, upon irradiation of the methylene next to the phosphorous, the multiplet due to the methine at C4 collapsed to a doublet.

At this point, we were not able to separate the diastereomers of **42**. Therefore, we decided to cleave the N-N bond in the mixture of oxazolidinones.

The oxazolidinone **42** would provide an internal protection for the alcohol, and after the N-N cleavage, the ring could be opened to produce the desired amino alcohol. Following Leblanc's protocol, *the cleavage was achieved under Zn/ HOAc/ acetone conditions on **42*** (see **Scheme 3.4**). The cleavage product, **44**, was isolated in very good yield (83%) by proper optimization of the reaction conditions. More than 5 equivalents of Zn dust were necessary, and the acetic acid had to be extremely dry. Distillation of acetic acid from P_2O_5 resulted in the formation of a small amount of acetic anhydride. One of the side products isolated was the acetylated product **45** (10%). Each step of the reaction was followed by TLC and the different intermediates were isolated (see Section 3.6).

Scheme 3.4



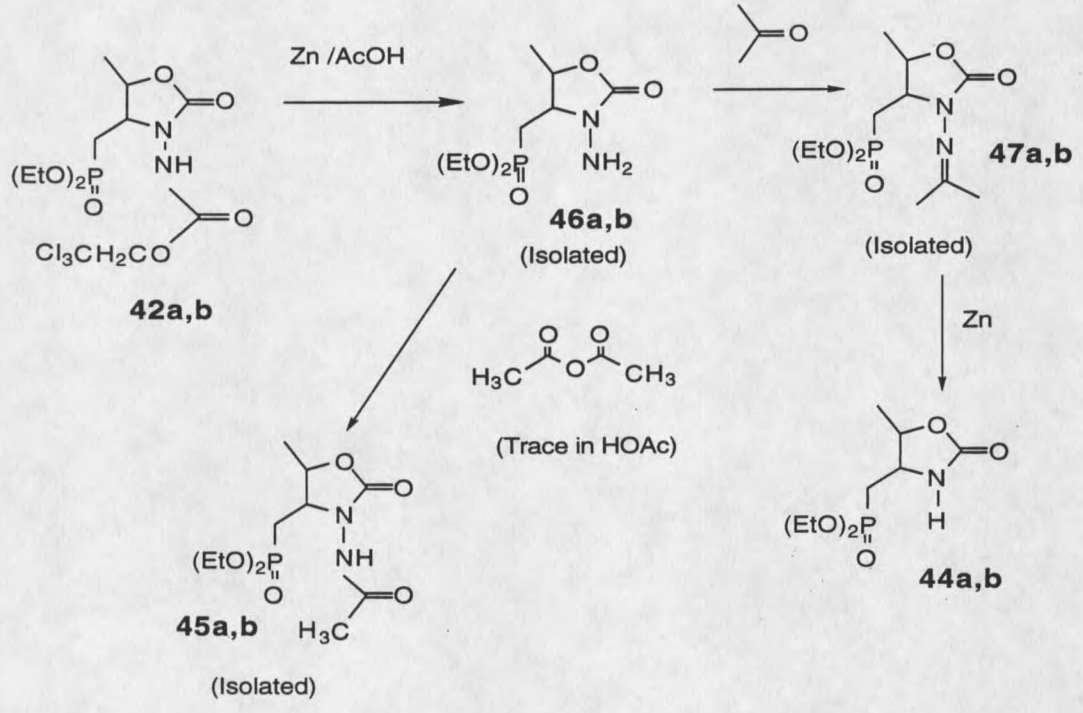
3.4: Mechanistic Interpretations. Isolation of different intermediates from reduction of N-N bond:

At this stage, it was decided to probe the mechanism of this cleavage step. According to Leblanc and coworkers[26a], the reaction goes via hydrolysis of the hydrazides and subsequent formation of a hydrazine. Upon addition of acetone, this hydrazine becomes a hydrazone. The hydrazone derivative then gets cleaved by Zn.

To explore the mechanism in our system, oxazolidinone **42a,b** was first submitted to only Zn/HOAc. The reaction was very closely followed by TLC. A spot close to the base line appeared, which upon isolation and characterization

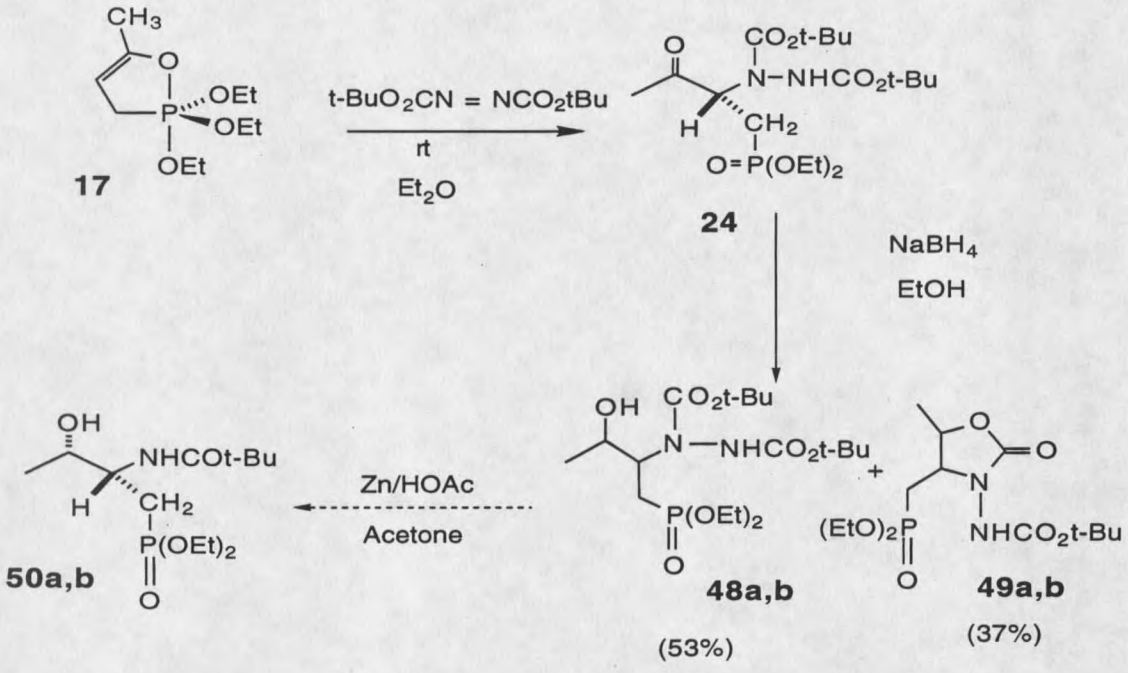
was shown to be the hydrazine derivative **46a,b** (see **Scheme 3.5**). This hydrazine reacted further with added acetone to produce the hydrazone **47a,b**. This hydrazone then reacted with Zn in acetic acid to produce the desired cleavage product **44**. Thus, the isolation of the reaction intermediates did support the earlier mechanism postulated by Leblanc and coworkers.[26a]

Scheme 3.5



The t-butyl derivative of the β-keto hydrazide was then again taken into consideration. Upon reducing this keto hydrazide with $NaBH_4$, 40% of the oxazolidinone **49a,b** was isolated, with the alcohol **48a,b** being the major product (55 %) (**Scheme 3.6**).

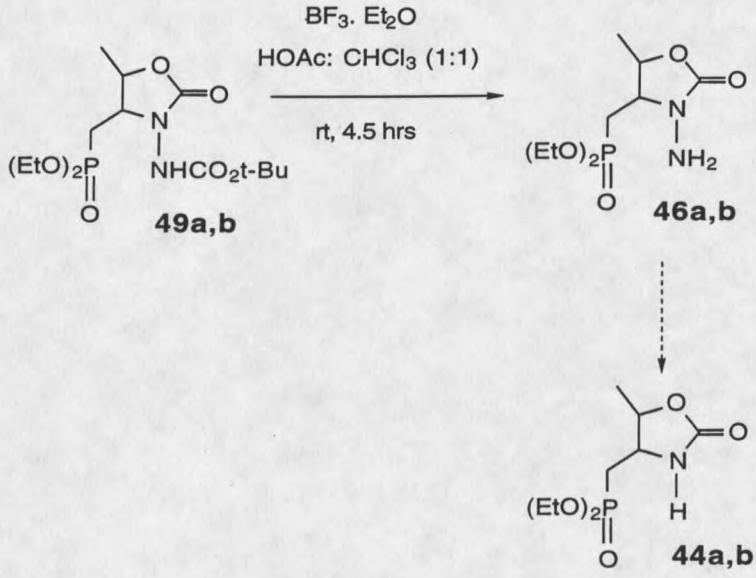
Scheme 3.6



ratio of isomers = 4:1 for both alcohol and oxazolidinone

It was decided to submit this oxazolidinone to $BF_3.Et_2O$/HOAc under the reaction conditions shown in **Scheme 3.7** to hydrolyze the ester group. The reaction did produce the expected hydrazine **46ab** in high yield. It was conceivable from this analogy that another route to β-amino-γ-keto phosphonates could be from t-butyl ester derivative as well (**Scheme 3.7**).

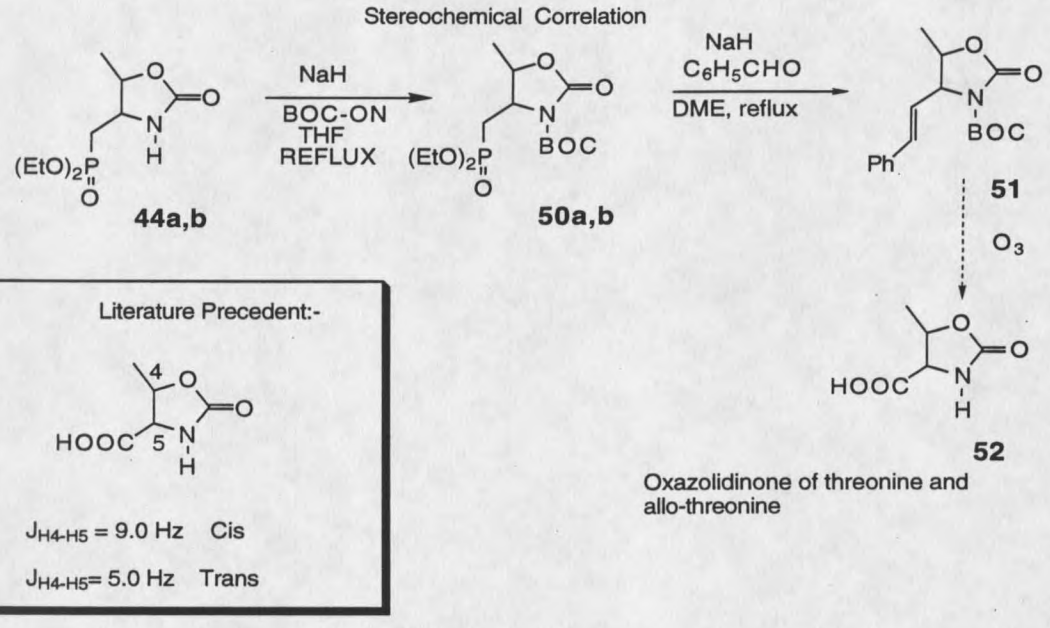
Scheme 3.7



3.5: Stereochemical Correlation of the oxazolidinone 44:

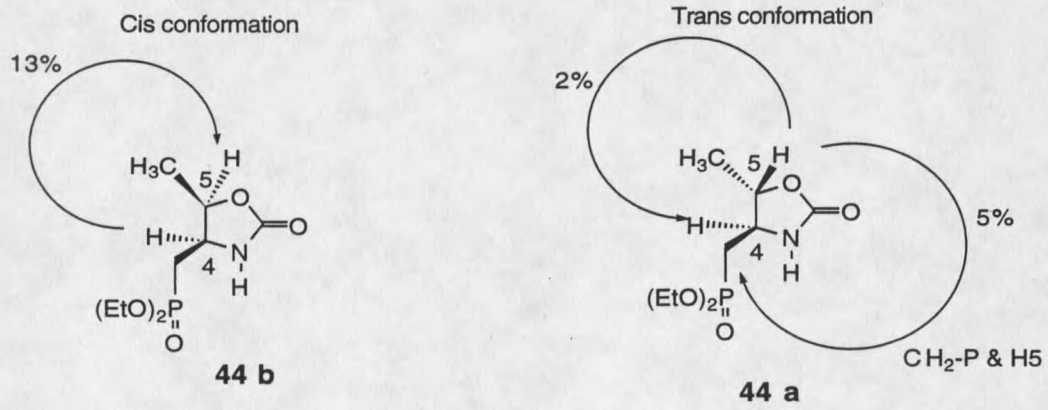
Determination of the relative stereochemistry in **44** was taken into consideration at this point. While doing homonuclear decoupling studies and NOE experiments, it was decided to make threonine and allo-threonine from **44**, and then compare our synthetic material with the available literature data of these natural products (**Scheme 3.8**). The first step of protection of the nitrogen by a BOC group went smoothly in almost quantitative yield. But the subsequent HEW step to produce **51** was problematic. The reaction produced an extremely low yield (<10%) even after heating the reaction. At this point, this approach was abandoned seeing that extremely good NOE and homonuclear decoupling data were available.

Scheme 3.8



The advantages of ^1H NMR homonuclear decoupling and NOE studies were utilized to establish the structure of both diastereomers of **44**. Stereochemical assignments for diastereomers **44a** and **44b** were verified via NOE studies.[21h,i] On irradiation of the H on C4 in **44b**, there was a 13% NOE enhancement for the H on C5. In **44a,** by irradiating the H on C4, there was only 2% NOE enhancement for H on C5. Moreover, in **44a**, irradiating the H on C5 produced a 5% NOE enhancement on the CH_2 next to the phosphorus. But for **44b**, no such NOE was observed. Threrefore, **44b** was the cis isomer, and **44a** was the trans isomer. By the decoupling studies, the coupling constants were determined across the C_4-C_5 bond in the major isomer of **44a** to be J_{4-5} = 5.9 Hz and $J_{4,5}$ = 7.2 Hz in the minor isomer. According to the literature, J_{4-5} cis > J_{4-5} trans for oxazolidinones.[27] Therefore, it was clear to us that our major product is the trans compound, **44a** (Figure 3.1).

Figure 3.1



Minor Isomer: $J_{4\text{-}5} = 7.2$

Major Isomer: $J_{4\text{-}5} = 5.9$

3.6 Conclusions:

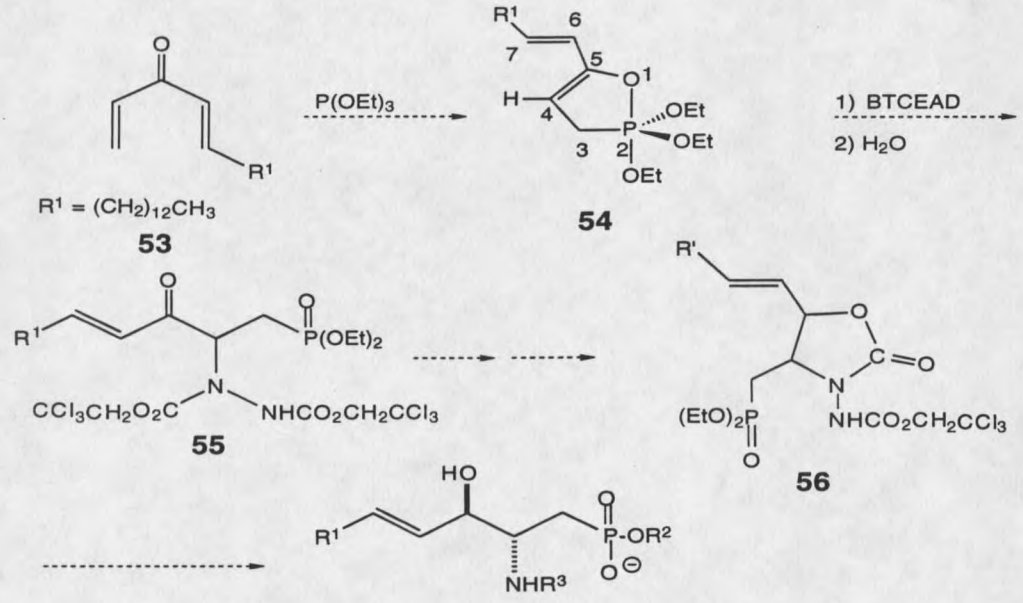
As shown above, we were finally successful in achieving the key N-N bond cleavage in the oxazolidinone **42**, derived from condensation of the model P(V), **17**, with BTCEAD. Therefore, we decided to pursue this strategy in the "real" system.

RESULTS AND DISCUSSIONS PART 2

4.1: Preparation of the dienone needed for the synthesis of sphingolipid analogs:

After the model study was successful, it was decided to pursue the real system needed for the synthesis of the sphingolipid analogs. The proposed synthetic scheme is shown in **Scheme 4.1**. The idea was to make the dienone using any well defined synthetic route. One of the double bonds in the dienone **53** would be unsubstituted and so would be less hindered. It was presumed that this unsubstituted double bond would react faster with triethyl phosphite than the substituted one because of its easy accessibility. By using one equivalent of triethyl phosphite, the desired dienone - P(V) **54** should be able to be produced. Condensation of this P(V) **54** with BTCEAD should then produce the α,β-unsaturated β-keto hydrazide **55**. This β-keto hydrazide **55** would then be pursued for reduction to produce the oxazolidinone **56**. The reductive cleavage of the N-N bond would then be attempted on this oxazolidinone.

Scheme 4.1



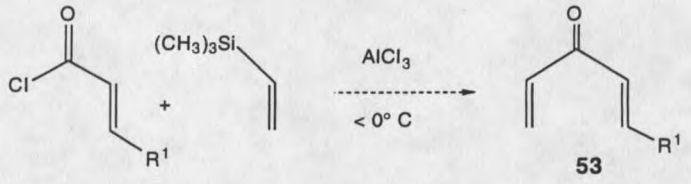
26a: Sphingosines: $R^1 = (CH_2)_{12}CH_3$; $R^2, R^3 = H$

26b: Sphingomyelin: $R^1, R^2 = (CH_2)_{12}CH_3$; $R^3 = COCH_2CH_2\overset{+}{N}Me_3$

26c: Ceramide 1-phosphate: $R^1 = (CH_2)_{12}CH_3$
$R^2 = H$, $R^3 = COCH_2CH_2\overset{+}{N}Me_3$

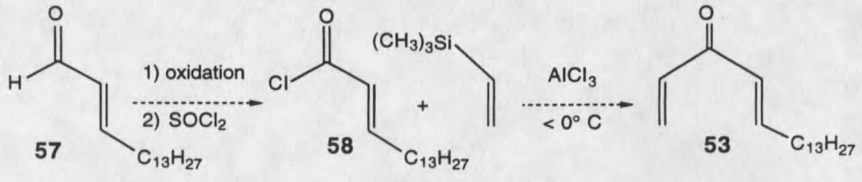
The first challenge was to make the desired dienone **53**. Dienones undergo Nazarov's cyclization[28] very easily. However, Paquette and coworkers[28c,d] have shown that these dienones could be made in high yield by reacting β-substituted α,β–unsaturated acid chlorides with vinyl silanes in the presence of aluminum trichloride as described in **Scheme 4.2** .

Scheme 4.2



It was apprehended that the desired acid chloride could be prepared from an aldehyde by oxidation to the acid, and then reaction with oxalyl chloride or sulfonyl chloride would produce the desired acid chloride. Whatever could be the approach, the question of non-isomerization of the double bond was crucial. The proper transposition of this double bond was essential for the synthesis.

Scheme 4.3

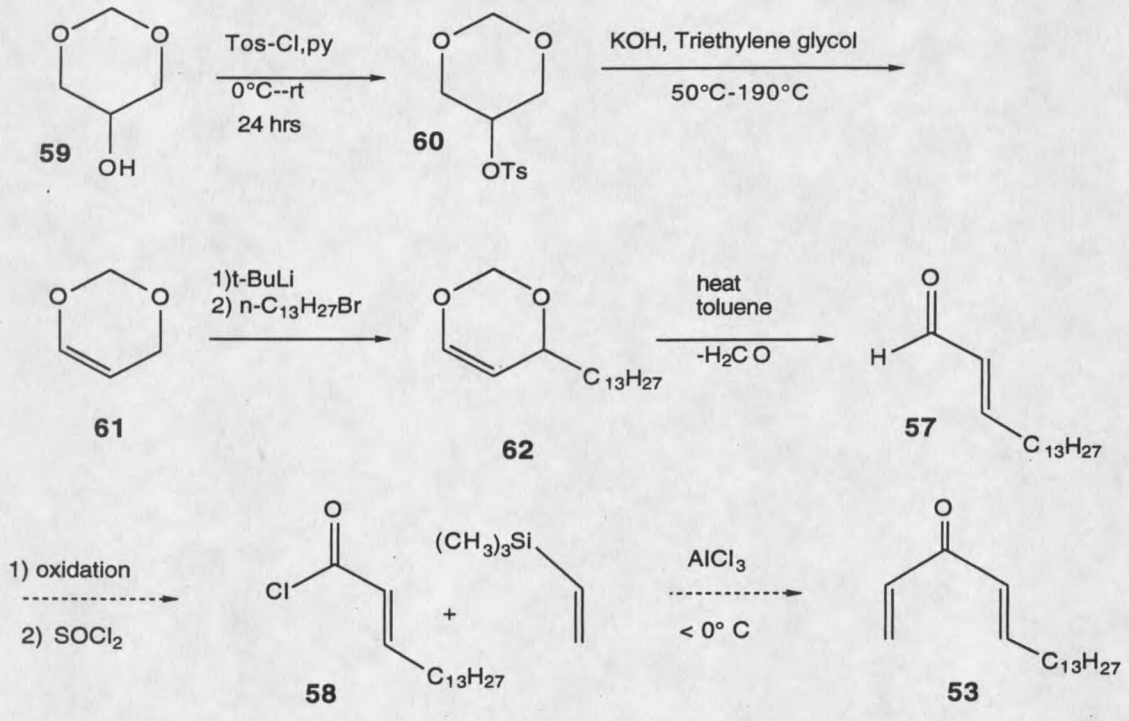


The desired aldehyde, **57**, was prepared following a Schollkopf's literature procedure[29] with a few modifications, and was accomplished with higher yields in most steps (see **Scheme 4.4**). Preparation of **60** went very smoothly. The next step of elimination of the tosylate for preparation of **61** was accomplished very successfully by using 5 equivalents of predried KOH. The KOH was dried on the pump for 48 hrs before using to get rid of all the water that is present generally in commercially available KOH. This simple modification increased the yield to 87% from the reported 60% for this step. It appeared that the volatile

elimination product **61** (boiling point 49 °C) could very well be stored in the freezer for months without decomposition in sealed flasks for future use. Therefore, (2E)-hexadecenal **57** could be prepared in multigram scale. For installation of the fatty acid chain, the dioxene **61** was then lithiated with t-butyllithium (1.02 eq) and alkylated with 1-bromotridecane to afford the dioxene **62**. Heating the dioxene **62** in toluene initiated the retro-Diels-Alder reaction to produce the hexadecenal **57** in almost quantitative yield by elimination of formaldehyde. The yield was more than 95 % for preparation of **62** and the reaction time was 16 hrs at -78° C. It is important to mention here that in all cases, a trans orientation between the two vinyl protons at C4 and C5 was maintained. No isomerization of the double bond was noticed.

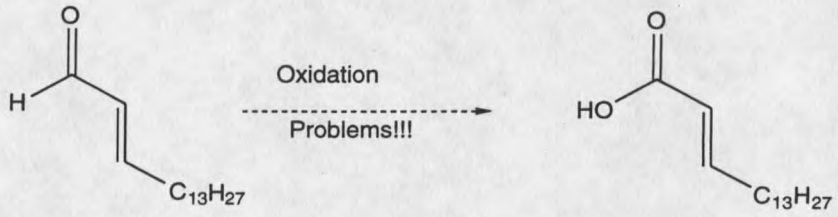
Schollkopf and coworkers purified the aldehyde by distillation under very low pressure at high temperature.[29] But, by running quick flash chromatography, the isolation was much easier and the yield was greater (90-95%). The aldehyde **57** is quite stable, and could be taken to the next step without any decomposition.

Scheme 4.4



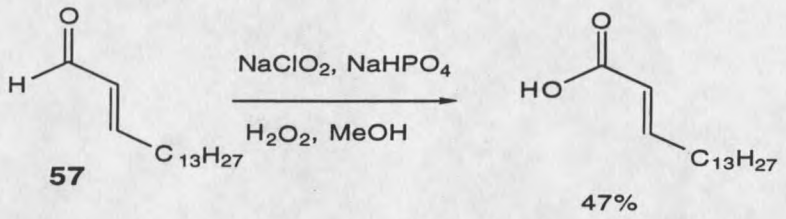
The next step was to make the acid chloride **58**. The oxidation level of aldehydes is at the intermediate level between alcohols and carboxylic acids. So many of the reagents that can effect the conversion of alcohols to carboxylic acids could also be useful for oxidizing aldehydes to carboxylic acids or their derivatives. But for the α–β unsaturated aldehydes, the oxidation is not always simple.[30] There is always a problem of *isomerization* of the double bond. The reagents which are widely used for non-conjugated systems are of very little or no use in conjugated systems.

Scheme 4.5



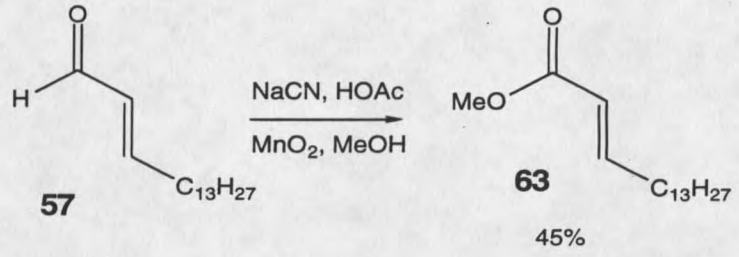
The attention was focussed on a suitable oxidizing agent sodium chlorite.[30c] The cheap sodium chlorite is a readily available useful reagent for conversion of α,β–unsaturated aldehydes to carboxylic acids. The reaction always needs the presence of a chlorine scavenger such as sulphamic acid, resorcinol, or 2-methylbutene. 2-Methylbutene was selected as the reagent of choice for the chlorine trap which has so far been established as the best one for this purpose. The yield for this step was only moderate (<47%), even with the addition of H_2O_2. The peroxide helps to remove the hypochlorite anion from the reaction mixture formed as a by-product. But hydrogen peroxide can only be used when there is no chance of side reactions. In the system where the hypochlorus acid could react faster with the aldehyde than with the chlorine scavenger, the yields could be very low because of formation of different side products. This is probably what occurred in this case with **57**. Another chlorine scavenger, DMSO, can help in these situations, but failed to produce any fruitful results in this system.

Scheme 4.6



Another approach to the dienone was based on the use of the Weinreb amide[30d] derived from the ester. For conjugated aldehydes, one of the best method for preparation of a methyl ester from an aldehyde is based on manganese dioxide as reported by E. J. Corey.[30b] The α,β-unsaturated aldehyde is treated with manganese dioxide in the presence of cyanide ions in methanol and acetic acid. The cyanide ion converts the conjugated aldehyde into a cyanohydrin which then is *in situ* converted into an acyl cyanide by MnO_2. This acyl cyanide reacts with methanol to form the methyl ester of the carboxylic acid (**Scheme 4.7**). Following this procedure, the conversion of the aldehyde to methyl ester was attempted. Although the yields reported by Corey and coworkers were more than 80%, in our system it failed to produce a good yield (<45%). As reported by Corey and coworkers, in this reaction, the double bond geometry remained untouched. This particular feature of this methodology distinguishes it from oxidation of these kinds of substrates with other reagents such as silver dioxide, where *Z/E* isomerization is very common. However, due to the low yield of the ester, **63**, we did not pursue this route further.

Scheme 4.7

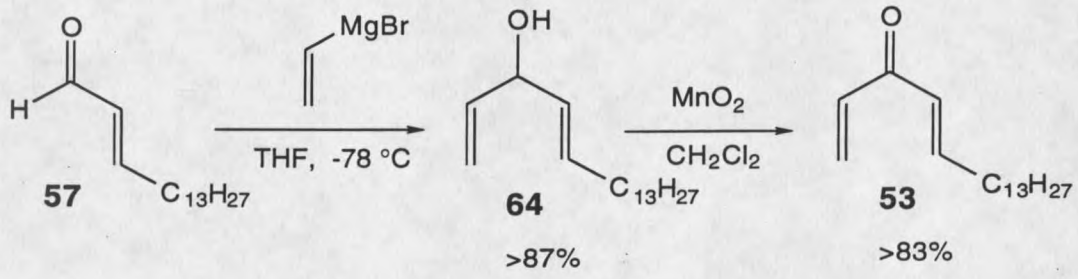


57 **63**

45%

Literature precedence[30a] came to our attention during this time that for conjugated aldehydes with the substitution at the β carbon, Grignards could add in a 1,2 fashion at low temperature. Therefore, it was decided to try to add vinyl magnesium bromide in a 1,2 manner to **57** to prepare the alcohol **64** (**Scheme 4.8**). The reaction was pursued by lowering the temperature to -78°C and adding the aldehyde to the precooled Grignard reagent. The reaction temperature was monitored, and the best results were obtained when the reaction temperature was warmed up to -30°C over a 12 hr period of time. The solution was then recooled to -78°C to quench with cold saturated ammonium chloride solution. This technique resulted in high yield of the desired alcohol **64** (84%), with rest of the material being the unreacted aldehyde. No other side products were observed.

Scheme 4.8



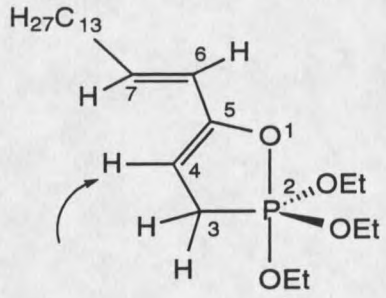
With the alcohol **64** in hand, preparation of dienone **53** from the alcohol was accomplished very easily by using 10-20 mole excess of manganese dioxide in CH_2Cl_2 in high yields. The unreacted alcohol was recycled. Quick flash chromatography on silica gel was safe enough to give a very high yield of this dienone (>83%) without any Nazarov product. Precautionary measures, e.g., thorough base washing of the glassware, were important for this isolation.

4.2: Preparation of the desired P(V) from the dienone:

Once the dienone was in hand, we pursued the synthesis of the desired dienone - P(V) **54**. The β-substitution on one of the double bonds of the dienone made one double bond more accessible for nucleophilic attack by the triethyl phosphite. Use of more than 1 equivalent $P(OEt)_3$ could be deleterious and might lead to other products by second attack at the substituted double bond. Therefore, we assumed that only one equivalent of $P(OEt)_3$ would be best to make the desired dienone - P(V) **54**. Unfortunately, this approach did not work. The proton attached to carbon 4 in the internal double bond of the P(V) has a very characteristic chemical shift and multiplicities (see **Figure 4.1**). By continuous monitoring of the progress of P(V) formation via ^1H, ^{31}P and ^{13}C NMR, it was found that 1 equivalent of triethyl phosphite led to many different

compounds along with the desired P(V). The desired P(V) **54** started forming after 6 hrs, but began to decompose to side products after 12 hrs. Although the standard P(V) **17** (from methyl vinyl ketone) could easily be distilled from any by-products, it is worth mentioning here that when distillation was tried under high vacuum on the dienone P(V) **54** mixture, the compound decomposed to form a black tar.

Figure 4.1



54

As the investigation continued, it became clear that 2 or more equivalents of triethyl phosphite were needed for this reaction to produce the desired product **54** in very high yields with high purity. The reaction was complete within 24 hrs, and the excess triethyl phosphite could easily be removed under vacuum to give very pure dienone-P(V) **54**.

4.3: Condensation of the Dienone P(V) with BTCEAD:

4.3a: Use of the "Standard" conditions:

The next step was to condense this P(V) **54** with BTCEAD to produce **55** in good yield. This was one of the steps that was troublesome in the model system (*vide supra*) (**Table 3.1**). We expected this condensation reaction to go

much more easily as the correct reaction conditions for the model system had already been determined. In the model system (**Scheme 3.3**), the best yields were obtained by inverse addition of the P(V) **17** to the bis(-2,2,2-trichloroethyl) azodicarboxylate reagent in diethyl ether at -78°C followed by quenching at -78°C. Unfortunately, the same reaction conditions failed to produce any desired condensation product with the dienone P(V) **54**. Different reaction conditions and solvents with different modes of addition were pursued with caution and perseverance to get to this key compound. At first, the condensation was tried in diethyl ether at temperatures lower than -78°C. However, many products were produced (TLC). Even a very low temperature like -116°C did not make any difference. The different solvents and reaction conditions tried, along with additives such as HMPA, are illustrated in **Table 4.1**. All these reaction conditions with different solvent systems failed to produce a good yield of the desired product **55**. Attention was then turned to the use of Lewis acids in this reaction.

Table 4.1

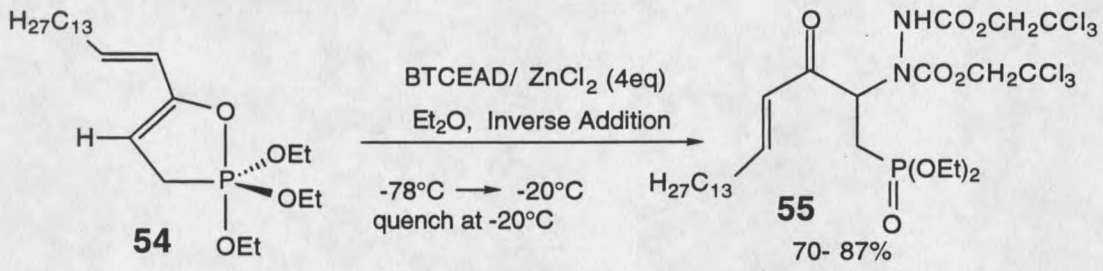
Solvents	Type of addn	Temperature	Hydrolysis pdt (**67**)	Yields (**55**)
Et_2O	direct	rt	no desired pdt	12%
Et_2O	inverse	rt	no desired pdt	15%
Et_2O	direct	-78°C	no desired pdt	16%
Et_2O	inverse	-78°C	no desired pdt	22%
CH_2Cl_2	direct	-78°C	no desired pdt	0%
CH_2Cl_2	inverse	-78°C	no desired pdt	5%
Et_2O:CH_2Cl_2 1:1	direct	-78°C	no desired pdt	5%
Et_2O:CH_2Cl_2 1:1	inverse	-78°C	15%	10%
THF	direct	-78°C	no desired pdt	13%
THF	Inverse	-78°C	12%	10%
THF:Hexane 1:1	direct	-78°C	no pdt	17%
THF:Hexane	inverse	-78°C	10%	10%
THF:CH_2Cl_2 1:1	direct	-40°C	no pdt	13%
THF:CH_2Cl_2 1:1	Inverse	-40°C	20%	18%
DCE	direct	-30°C	no pdt	17%
DCE	inverse	-30°C	no pdt	15%

Unless otherwise stated, all the reactions were run in dry and distilled solvents under argon. The direct addition means addition of the BTCEAD solution to the dienone-P(V) solution in the respective solvents. The inverse addition means the addition of dienone-P(V) solution to BTCEAD solution. One of the side products isolated was always the hydrolysis product **67** in 5-22 % yields.

4.3b: Second trial for preparing the BTCEAD-Dienone P(V) condensation product 55 using Lewis acids:

We next investigated the condensation of the dienone P(V) **54** with BTCEAD in the presence of Lewis acids. By utilizing many side-by-side reactions with different Lewis acids, we found a procedure (**Scheme 4.9**) where we could make more than 70-87% yield of the desired condensation product β-hydrazido-γ-keto phosphonate **55**. It appeared that by employing 4 equivalents of $ZnCl_2$, it was possible to get a very good amount (up to 87%) of the desired product.

Scheme 4.9



The procedure utilized is as follows: a freshly prepared $ZnCl_2$ solution in Et_2O was transfered via cannula into a round bottom flask with diethyl ether. Solid BTCEAD was then quickly added to the reaction vessel, and the reaction mixture was allowed to stir for 90 minutes at room temperature. This $ZnCl_2$-BTCEAD complex mixture was then cooled to -78°C, and the dienone-P(V) **54** was added (neat) via cannula, with the last part being rinsed with minimum amount of diethyl ether. The reaction mixture was stirred for 12 hrs at -78°C, slowly warmed to -20 °C over 16 hrs and was recooled to -78 °C to quench with cold saturated ammonium chloride solution. Usual workup followed by flash column chromatography provided the desired product in yields up to 87%.

Optimization of the reaction conditions for this reaction was accomplished by screening many different Lewis acids as well as different temperatures and solvents (**Table 4.2**).

Table 4.2

Lewis acids	Type of addn	Temperature	Solvents	Yields
$TiCl_4$ (2 eq)	direct	-78°C	CH_2Cl_2	No Pdt
$TiCl_4$ (4 eq)	inverse	-78°C	CH_2Cl_2	22%
$TiCl_4$ (2 eq)	direct	-78°C	Et_2O	No Pdt
$TiCl_4$ (4 eq)	inverse	-78°C	Et_2O	35%
$BF_3.Et_2O$ (2 eq)	direct	-78°C	CH_2Cl_2	No Pdt
$BF_3.Et_2O$ (4 eq)	inverse	-78°C	CH_2Cl_2	No Pdt
$BF_3.Et_2O$ (2 eq)	direct	-78°C	Et_2O	No Pdt
$BF_3.Et_2O$ (4 eq)	inverse	-78°C	Et_2O	No Pdt
$ZnCl_2$ (2 eq)	direct	-78°C	CH_2Cl_2	25%
$ZnCl_2$ (4 eq)	Inverse	-78°C	CH_2Cl_2	32%
$ZnCl_2$ (2 eq)	direct	-78°C	Et_2O	58%
$ZnCl_2$ (4 eq)	inverse	-78°C to-20°C	Et_2O	87%
$MgBr_2.Et_2O$ (2 eq)	direct	-78°C	CH_2Cl_2	No Pdt
$MgBr_2.Et_2O$ (4 eq)	Inverse	-78°C	CH_2Cl_2	28%
$MgBr_2.Et_2O$ (2 eq)	direct	-78°C	Et_2O	37%
$MgBr_2.Et_2O$ (4 eq)	inverse	-78°C	Et_2O	45%

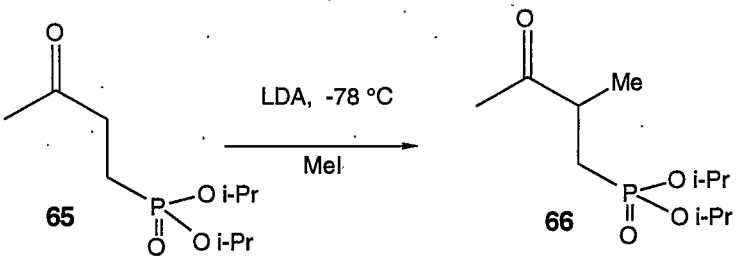
Unless otherwise stated, all the reactions were run in dry and distilled solvents under argon. The 1 molar solutions of the Lewis acids were prepared freshly each time before use. The direct addition means addition of the Lewis acid complexed BTCEAD solution to the dienone-P(V) solution in the respective solvents. The inverse addition means the addition of dienone-P(V) solution to the Lewis-acid complexed BTCEAD. For complexation the Lewis acid and BTCEAD was stirred at room temperature for 90 minutes.

The exact role of $ZnCl_2$ as the Lewis acid is not clear, although it appears that it slows down the condensation reaction by coordinating with the azodicarboxylate reagent. An NMR study was separately performed to see if the coordination of BTCEAD is good enough to show in different chemical shifts for both carbon as well as protons, but the chemical shifts did not change appreciably. The $ZnCl_2$ was also added to the dienone P(V) **54** to possibly induce any chemical shift changes. This also did not lead to any conclusive results, as the chemical shifts did not change. In both these cases, the $ZnCl_2$ solution was made in $CDCl_3$.

The structure of the condensation product **55** was not obvious from proton and carbon NMR due to signal overlap. A 2-D ^{1}H -^{13}C heterocorrelation experiment was performed on compound **55** to establish the structure conclusively. The two double bond carbons were found at 150.3 ppm and 125.1 ppm, whereas both the protons attached to the double bond resonated at 7.08-6.81 ppm (dm, J = 15.2 Hz). This coupling constant established the trans configuration of the double bond of the newly formed condensation product. This trans configuration was furthermore confirmed by the reduced products **56** and **81** (*vide supra*, see **Scheme 4.17**). It was obvious from these experiments, *that the signals due to both protons of the double bond are sitting on top of each other* in compound **55**, which made the proton NMR spectrum very difficult to interpret. These shifts are not very common, and it appeared from the structure of **55** that the reason of this downfield shift of one of the protons might be because of the deshielding due to the proximity in space of the carbamate groups or phosphorus groups. Interestingly enough, the same deshielding effect was also seen in the t-butyl adduct **68**.

A separate experiment was undertaken to establish the structure as well. The proton NMR of the hydrolysis product (**67**) of dienone - P(V) **54** is very different for the two vinyl protons. It was decided to see if this compound could be selectively enolized at the position β to the phosphorus in order to react it with BTEAD. McClure and Jung did enolize[20] the hydrolysis product **65** of standard P(V) **17** at the position beta to the phosphorus using 9-BBN triflate. These boron enolates are not very nucleophilic in nature, so LDA was utilized here on **65** and **67**. Very careful reaction conditions did produce the desired enolate of **65** which was further alkylated with MeI (**Scheme 4.10**). The hydrolysis product **65** was dissolved in THF, cooled to -78°C, and LDA (1 eq) was added drop by drop at -78°C. The reaction temperature was raised to -10°C over 1 hour period of time and then cooled to -78°C to quench with MeI. Methylation occured beta to the phosphorus in very high yield (78%) to produce **66**.

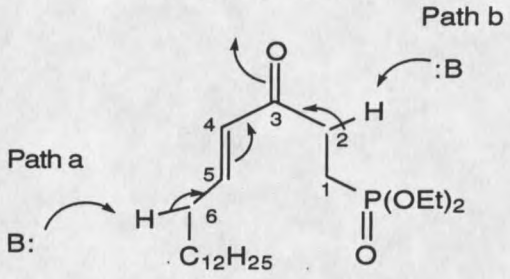
Scheme 4.10



Similar reaction conditions were tried with the dienone-P(V) hydrolysis product **67** (**Scheme 4.11**). Here, there are two possibilities of proton abstraction (**Figure 4.2**). Protons gamma to the enone carbonyl (at C6) are also very acidic. Therefore, while the enolization might take place via path b, there is a chance of competition between the proton at C2 vs C6. It was

necessary to try the enolization at C2 to correctly establish the structure of the condensation product **55**.

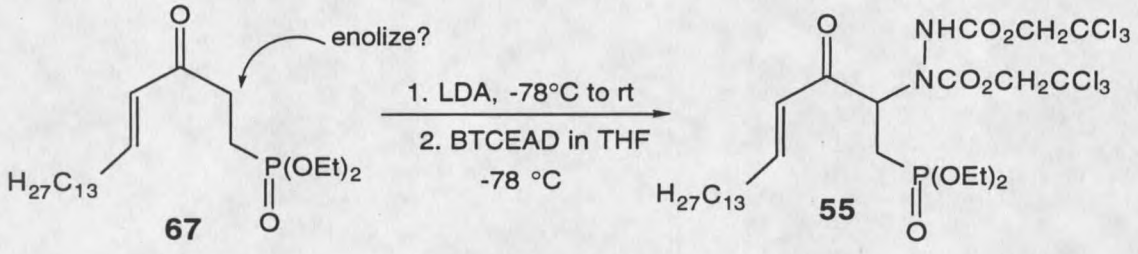
Figure 4.2



67

Therefore, the enolization followed by alkylation with BTCEAD was tried. The successful enolization of **67** with LDA followed by quenching with BTCEAD produced the same product **55** (81%) as obtained from the earlier condensation reactions shown in **Scheme 4.9**. Thus the exact structure of the condensation product was established.

Scheme 4.11



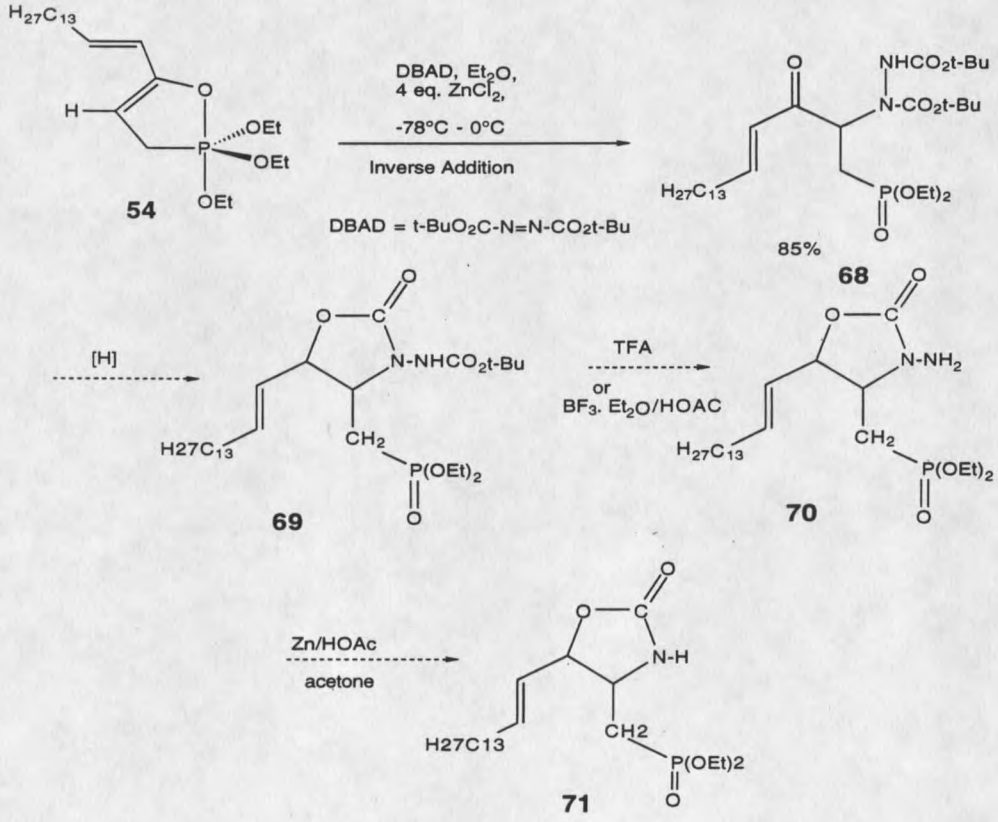
4.4: Other electrophilic amination sources that were investigated:

4.4a: Condensation product with DBAD:

While we were investigating the correct conditions for preparing the condensation product **55** from BTCEAD, we decided that it was important to see

if the same P(V) **54** could condense with other azo-di-carboxylates e.g., DBAD or DEAD. We reasoned that the hydrolysis of the BOC groups could easily produce the 3-amino-oxazolidinone **70** as seen in the model system, (see **Scheme 3.7**) by using TFA, or BF_3-Et_2O in acetic acid. The reduction steps could then be carried out from this 3-amino-oxazolidinone as a substrate (**Scheme 4.12**). After enough studies, it was ultimately possible to prepare the condensation product **68** in high yield with DBAD using Lewis acids. The best reaction condition was to use 4 equivalents of $ZnCl_2$ and diethyl ether. The reaction failed to produce any product without Lewis acids.

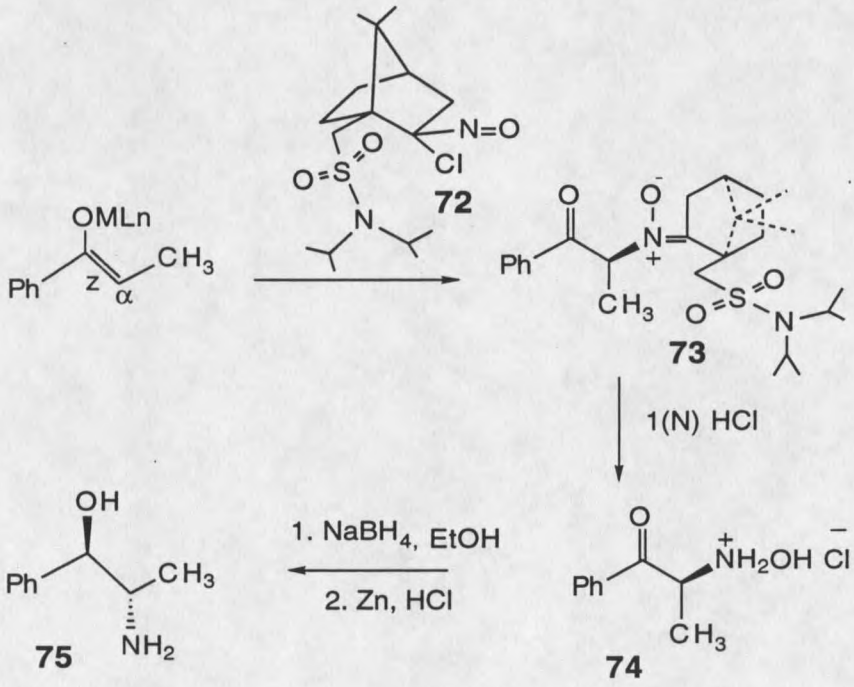
Scheme 4.12



We did not pursue the rest of the steps in **Scheme 4.12** yet. But the most crucial step of preparation of the condensation product has been figured out. Therefore, it is certainly a very promising route for the preparation of sphingolipid analogs as well.

Very recently, Oppolzer and coworkers have reported[31] that chiral α-chloro-α-nitroso reagents **72** are capable of aminating prochiral ketone enolates with high enantiofacial differentiation (**Scheme 4.13**). Acidic hydrolysis of the resulting nitrones **73** then provided the optically pure β-keto-N-hydroxyl ammonium salts **74**, regenerating the chiral ketone of **72**. The potential of this amination reduction sequence for diastereo- and

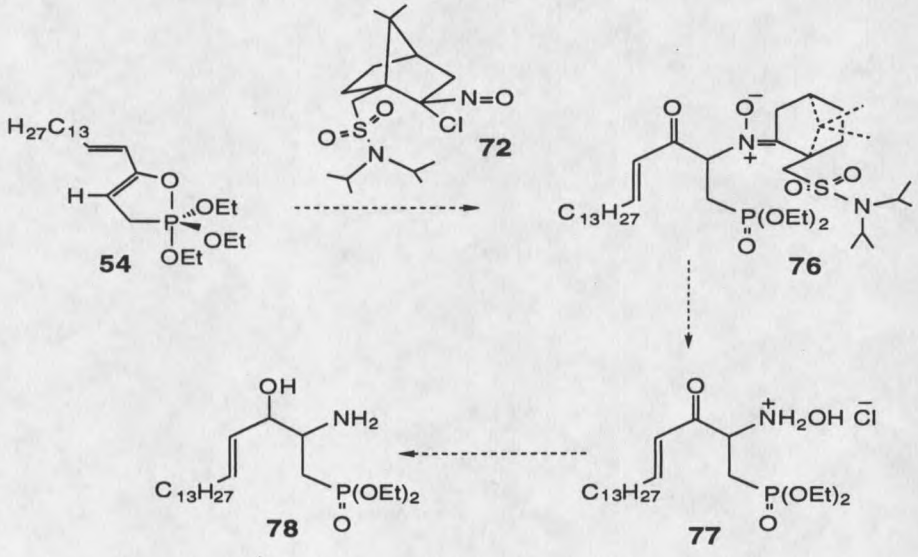
Scheme 4.13



enantioselective syntheses of biologically interesting β-aminols attracted our attention. It was decided to prepare the desired racemic chloro-nitroso reagent (**72**) in pure form and try this condensation step with our dienone P(V) (**54**).

One question in this approach was whether the P(V) was nucleophilic enough to form the nitrone or not (**Scheme 4.14**).

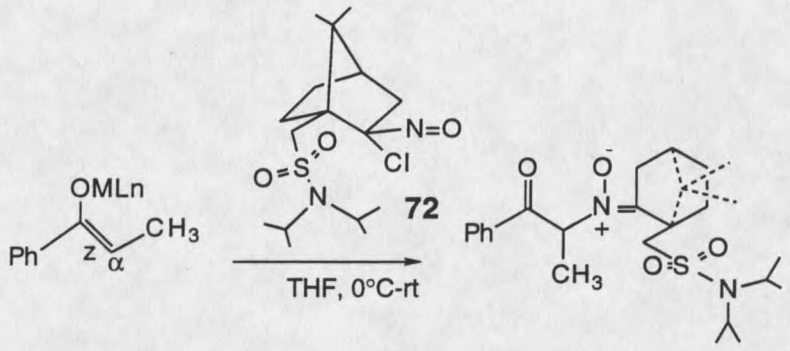
Scheme 4.14



The α-chloro-α-nitroso reagent **72** was made following the literature procedure.[31] Condensation of this reagent with dienone oxaphospholene (**54**) was not successful, and only the hydrolysis product **67** was isolated in each case. Even the presence of Lewis acids like $ZnCl_2$ did not produce any product.

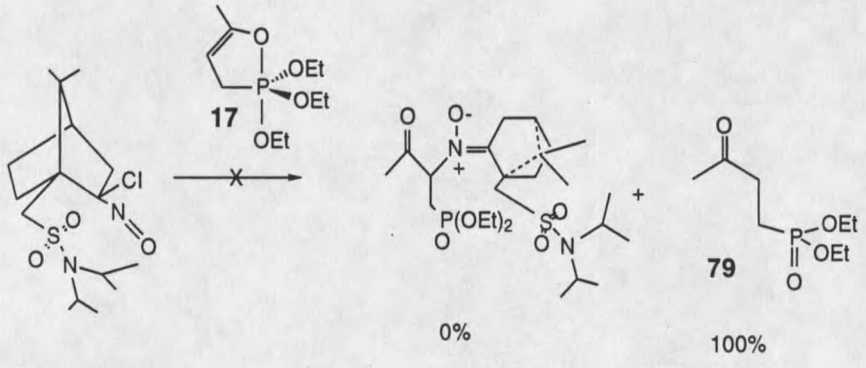
To check the efficacy of the prepared reagent and its purity, the same substrate and reaction conditions utilized by Oppolzer and coworkers (**Scheme 4.15**) were tried. On reaction with the lithiated species of propiophenone, the desired nitrone was produced in high yield which could easily be purified by flash chromatography.

Scheme 4.15



Once the purity and reactivity of the prepared chloro-nitroso reagent was beyond question, it was tried to react with standard P(V) **17**. Here also, only hydrolysis products were produced even in presence of different Lewis acids.

Scheme 4.16



4.5: Reduction of the β-keto hydrazide 55:

The next step was the reduction of the carbonyl in condensation product **55** for preparing the oxazolidinone. In stereoselective synthesis, sometimes a chemical reaction can be actively directed by substrate functionality. This is substrate control, wherein the stereochemical bias of the molecule might be able to direct a particular outcome of a reaction. In our system, a chemo- as

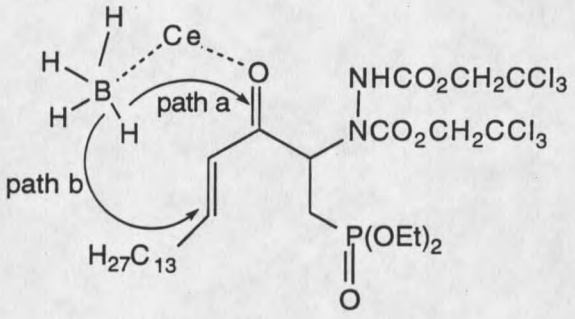
well as stereoselective reduction was required for the carbonyl. 1,2, and not 1,4 reduction from one face of the carbonyl would only be able to produce the correct geometry of the oxazolidinone that is important for the synthesis of our final target.

Reduction of carbonyl derivatives with α, β-unsaturation poses a potential problem.[32] Conjugate reduction is common for conjugated ketones even where the β carbon is relatively unhindered. If reduction proceeds via coordination of the metal (such as cerium) to the carbonyl oxygen, 1,2-reduction occurs via path a (**Figure 4.3**). But if the β-carbon is the most electrophilic side, hydride delivery could take place through a seven-center transition state where the double bond could be easily reduced to give a saturated ketone (path b). In our system, the unsaturation is not the only problem, as there are carbamates attached to the α-carbonyl carbon. Carbamates are amide type derivatives, but contain an electron-withdrawing ester group on the nitrogen.

In some systems, sodium borohydride can give a 1,2 reduction product in an α, β-unsaturated enone. This is specially effective when the terminal carbon of the conjugated system is sterically hindered. It is also an excellent reagent for reducing ketones in presence of esters, hydroxyl or amide groups alpha to the carbonyl, or with a carbohydrate residue or halogens in the alpha position. Addition of cerium salts to sodium borohydride leads to a very selective reagent which always reduce 1,2 in an α, β-conjugated carbonyl system.[32b] The selectivity of cerium salts and sodium borohydride in the 1,2-reduction can be explained by a chelated intermediate **80**. In **80** the borohydride moiety is "held" by the carbonyl carbon. The transfer of the hydride to the alkene is

difficult unless the complexation is reversible. If the complexation is irreversible, then the hydride transfers to the carbonyl (1,2 addition). If the complex formation is reversible, then the hydride can transfer to the alkene and 1,4 reduction predominates.

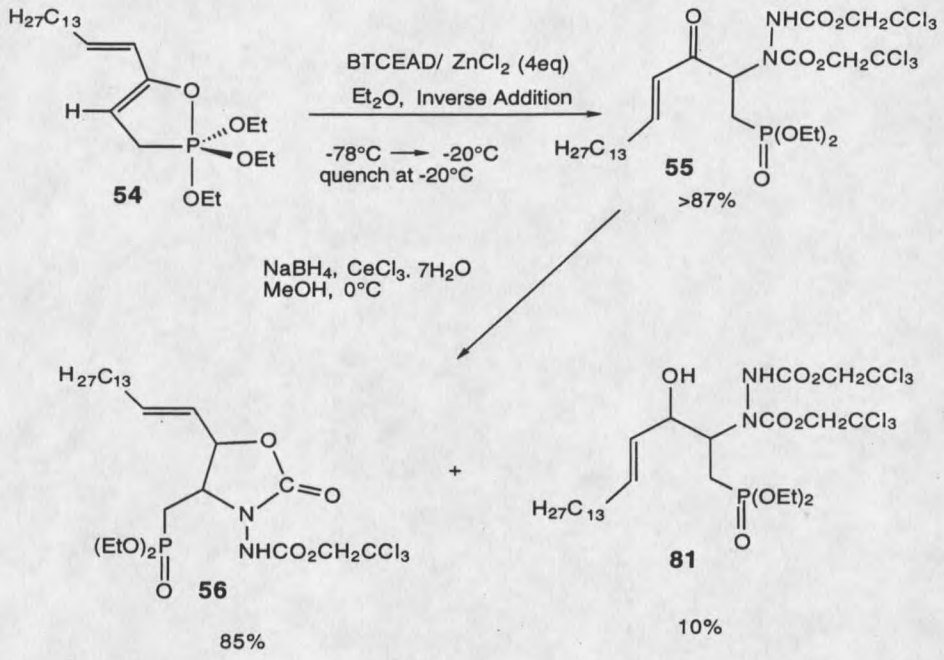
Figure 4.3



80

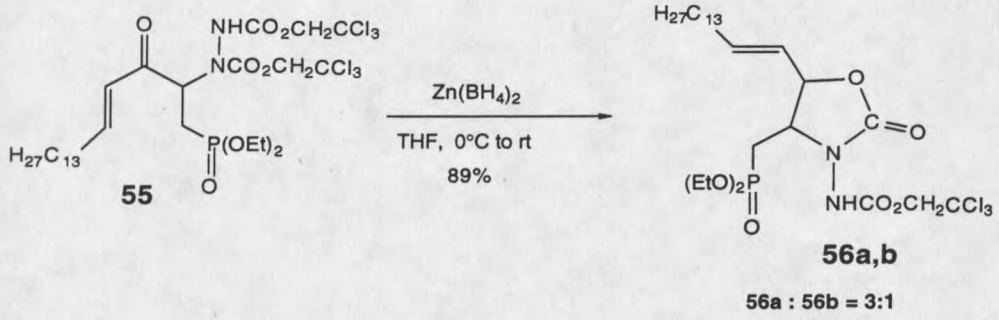
As a first approach to achieve our objective, Luche[32b] reaction conditions (**Scheme 4.17**) were utilized to investigate the selectivity of this reagent on our substrate. Not only reduction of the carbonyl, but attack of the incipient alkoxide onto one of the esters was necessary for the formation of the oxazolidinone **56**. Luche conditions failed to give complete conversion to the oxazolidinone. The oxazolidinone **56** was 85 % of the total products and the alcohol **81** was produced in 10% yield. The ratio of diastereomers in oxazolidinone was found to be 2 : 1.

Scheme 4.17



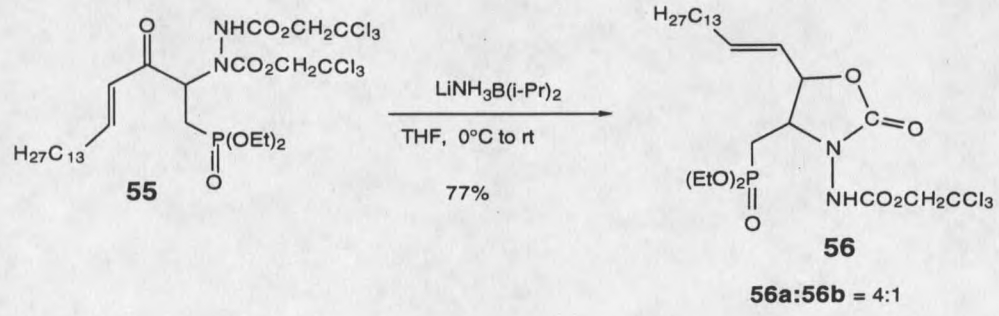
Changing the metal ion can have a very significant effect on the course of the reduction. Zinc borohydride[33], produced by reacting zinc chloride with sodium borohydride in diethyl ether, is most useful in the reduction of α,β–unsatuated systems for selective 1,2-reduction. Use of $ZnBH_4$ on our compound **55** produced the oxazolidinone, **56**, in 89% in a 3:1 diastereomeric ratio (**Scheme 4.18**).

Scheme 4.18



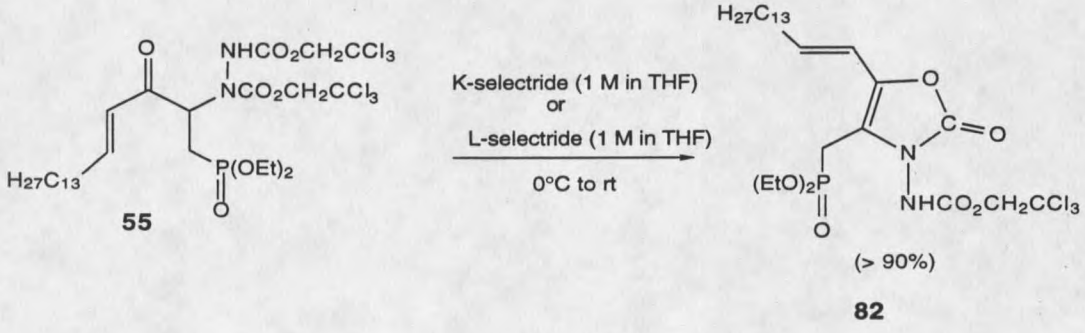
Lithium amino borohydrides have also been introduced very recently, again as a new class of powerful, selective air-stable reducing agents.[34] In α,β–conjugated systems, these reagents produce exclusively 1,2-reduction products only. Lithium (N,N-diisopropyl-amino)borohydride was prepared according to the literature[34] and was employed in the reduction of **55** to test for any difference in selectivity. The reaction were performed at 0°C under argon. Unfortunately, only the ususal trans isomer **56a** of the oxazolidinone **56** was obtained as the major isomer (**Scheme 4.19**).

Scheme 4.19



Greater diastereoselectivity could also be achieved by attaching sufficiently larger groups to the boron atom. Thus, reaction of tri-sec-butyl borane with potassium hydride or lithium trimethoxyaluminum hydride can produce potassium tri-sec-butyl borohydride (K-Selectride) or lithium tri-sec-butylborohydride (L-Selectride). Literature sources report that the reduction by these Selectrides is 1,2- in enones if there is substitution at the β-position to the carbonyl.[35] Selectrides were investigated in our system because of this reason. It was thought that while the cation (Li^+) would coordinate with the carbonyl and the ester group, the hydride transfer might take place for the most part from one side (**Scheme 4.20**).

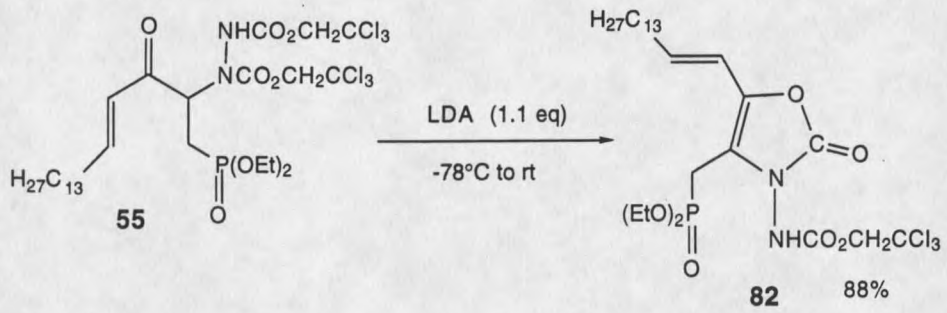
Scheme 4.20



Therefore, treatment of **55** with either Selectride was pursued. However, the only product isolated was the unsaturated oxazolidinone **82**. These products proved that the proton α to the carbonyl and β to the phosphorus is so acidic that even a very mild base like K-Selectride could deprotonate it. To establish this mechanism, the condensation product **55** was enolized with LDA (1 eq), and it was found that the enolization did take place at the position β to

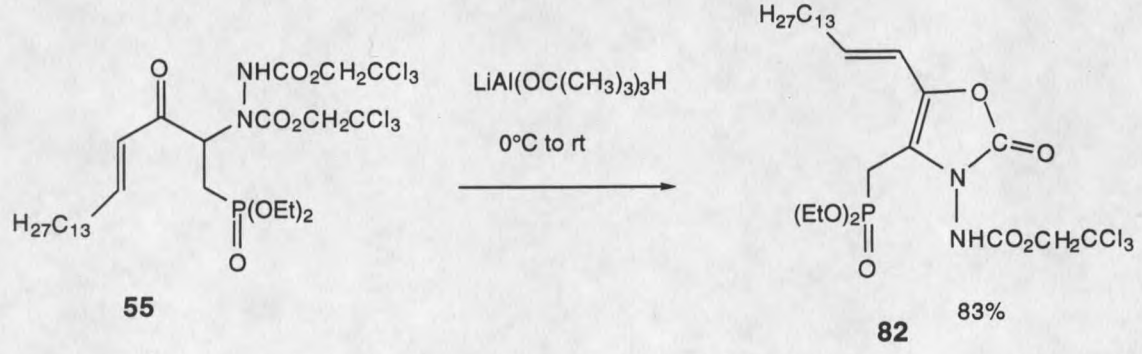
the phosphonate and α to the carbonyl. The incipient enolate ion attacked at one of the ester groups to produce **82** in 88% yield (**Scheme 4.21**).

Scheme 4.21



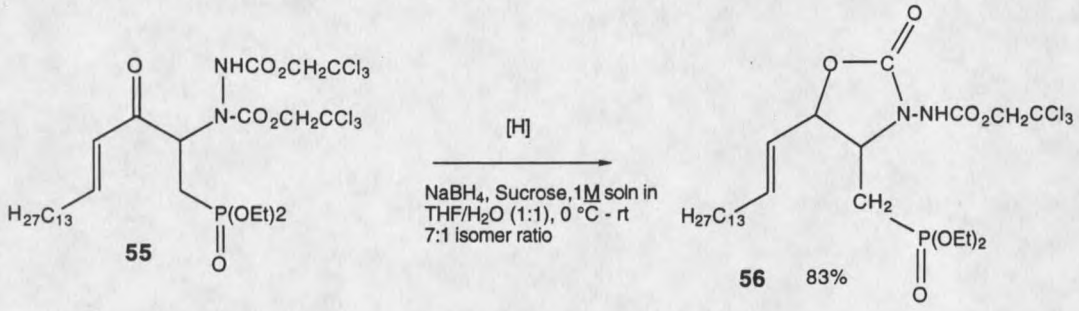
$LiAlH(Ot\text{-}Bu)_3$ is one of the most commonly used alkoxyaluminum hydrides, among the different alkoxyaluminum hydride reagents that can reduce the conjugated carbonyl primarily in a 1,2 manner without touching other functionalities. Marshall used this reagent in synthesis of globulol. But very surprisingly, this reagent produced only **82** in our system (**Scheme 4.22**).

Scheme 4.22



A literature report on the regioselective and stereoselective reduction of α,β-unsaturated ketones in glycosidic aquous media was investigated in our system. Plusquellec and coworkers[36c] regioselectively reduced α,β-unsaturated ketones to the corresponding allylic alcohols in essentially quantitative yields in aquous media in presence of glycosidic surfactants or amphiphilic carbohydrates. They reasoned the excellent stereodifferentiation was due to the hydrophobic interactions between the amphiphilic carbohydrates and the lipophilic substrates. Following this reduction procedure, the successful reduction of the α,β-unsaturated β-keto hydrazide was obtained (**Scheme 4.23**) in a greater selectivity (7:1) with the oxazolidinone **56** being the only product. The reaction was performed in a 1M solution of sucrose-THF (1:1) with $NaBH_4$ as the reducing agent. Different reaction conditions were investigated in order to obtain a better ratio of isomers. It was found performing the reaction at 0°C produced the best yield (83%) with an isomer ratio of 7 :1.

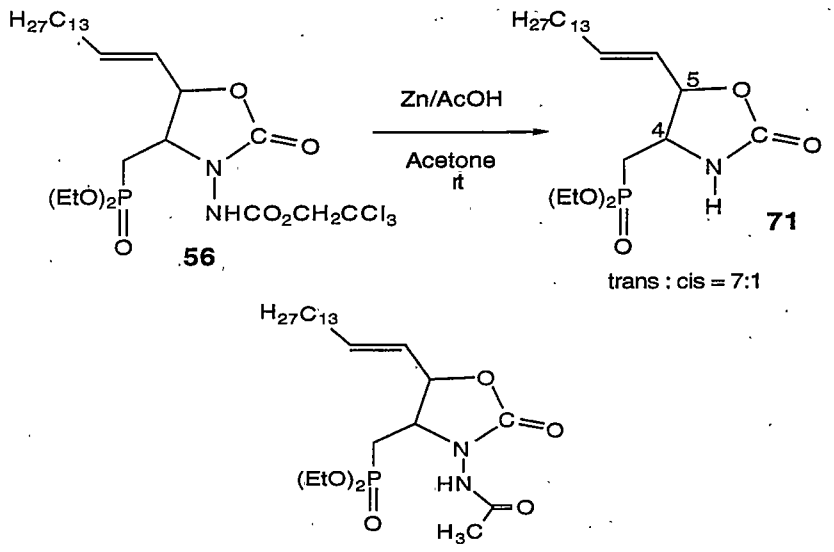
Scheme 4.23



4.6: Cleavage of the N-N bond in the real system; preparation of the final molecule:

While the various reduction methods to obtain the right isomer in very high selectivity were being screened, it was very important to determine the stereochemistry of the two isomers before we could proceed any further. As it was obvious from the NMR spectra, the only way we could get the coupling constants in between C4 and C5 in the oxazolidinone intermediate **56** was by reducing the N-N bond. Hence, the disatereomeric mixture (7:1) of the oxazolidinone **56a,b** was submitted to the N-N bond cleavage conditions. It was found that cleavage does occur to produce **71a,b** (yield = 77 % to date) along with a minor amount of **83a,b** (yield = 10 %) without any double bond isomerization or reduction of the double bond (**Scheme 4.24**). The coupling constants of the two isomers were 6.8 Hz for the major isomer and 7.5 Hz for the minor isomer. According to the literature reports as discussed previously, the coupling constant for the *cis* isomer is always greater than the *trans*. So the major isomer from all the achiral reducing agents was the trans isomer. Now that we know the relative stereochemistry it was the time to find out if chiral reducing agents would improve our selectivity.

Scheme 4.24



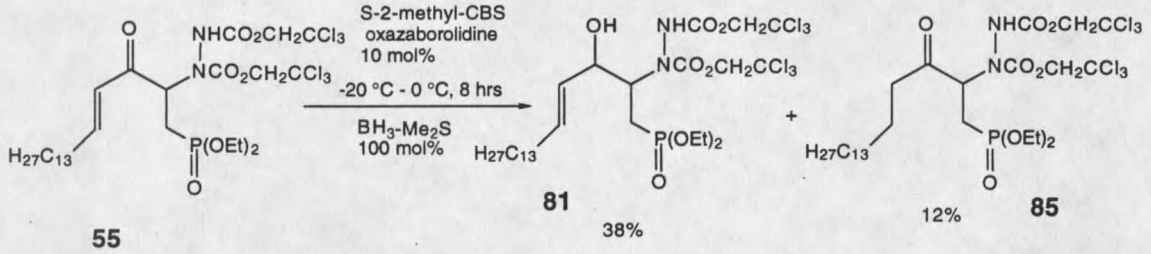
4.7: Chiral reducing agents:

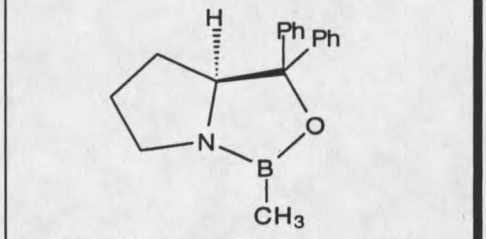
4.7a: CBS reagent:

In the search for chiral reducing agents, S-2-methyl CBS oxazaborolidine, **84**, was investigated to see if the correct isomer could be produced in higher yield. Corey has described a new method for the catalytic enantioselective reduction of ketones to chiral secondary alcohols.[37] The catalyst is a chiral oxazaborolidine such as **84a,b** (10-100 moles) and normally the stoichiometric reducing reagent is borane (usually 0.6 mol/mol of ketone). The catalyst provides excellent enantioselectivity and the chiral catalyst is easily recoverable. The reaction time is very short and the yields are usually good. Therefore, CBS reagent in 10 mol% to 100 mole % was applied to our system. Different reaction conditions were tried and the results were quite interesting (**Scheme 4.25**). At lower temperature, cyclization did not happen and the

alcohol was produced in a good yield as only one isomer. Moreover, the double bond also was reduced to produce the saturated carbonyl (12% yield) **85**.

Scheme 4.25



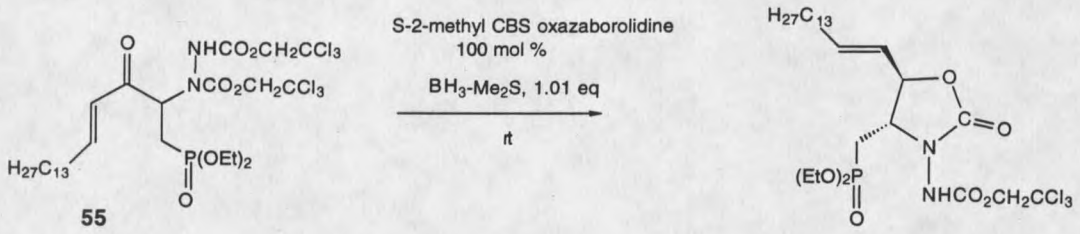


S-2-methyl-CBS-oxazaborolidine

84a

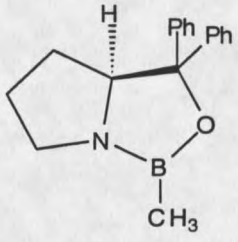
It was apparent from our previous studies that at lower temperature the formation of oxazolidinone is slow and unfavorable. So the reaction was then attempted at room temperature. Fortunately enough, this time we were able to get only one isomer of the oxazolidinone (*trans*). No allylic alcohol was formed, and the double bond was not reduced. The yield was low (40 %) with the material balance being unreacted starting material. It appeared at this point that the stereochemical bias is actually favoring the formation of only one isomer, and that by using the other antipode of the CBS catalyst we might be able to get the right oxazolidinone isomer **56b** that we need.

Scheme 4.26



55

56a

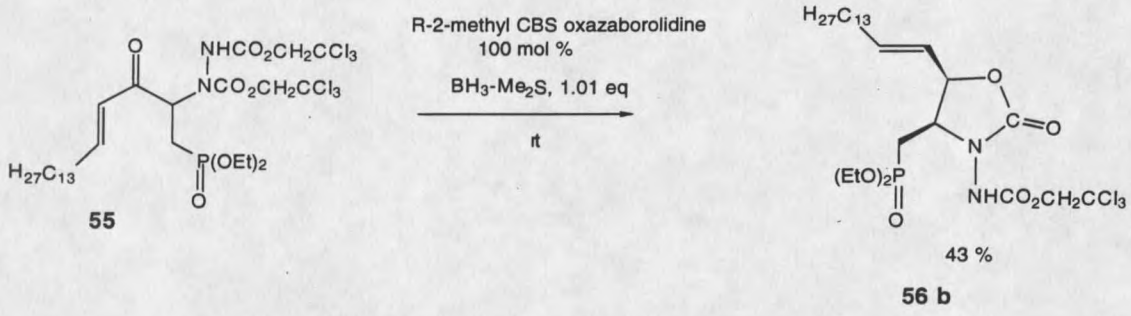


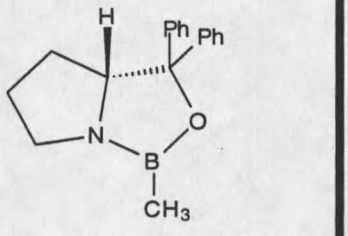
S-2-methyl-CBS-oxazaborolidine

84a

Therefore, R-2-methyl CBS oxazaborolidine was then tried on **55** using the same reaction conditions as before (rt, 100 mol% catalyst, 1.01 eq BH_3, Me_2S) (**Scheme 4.27**). The reaction did produce the correct cis isomer **56b** in 43 % yield (to date). Thus, by careful observations and synthetic approaches, we have been able to develop a useful synthetic approach to the oxazolidinones which should be very useful to produce the non-isosteric-phosphonate analogs of the sphingolipids mentioned earlier.

Scheme 4.27

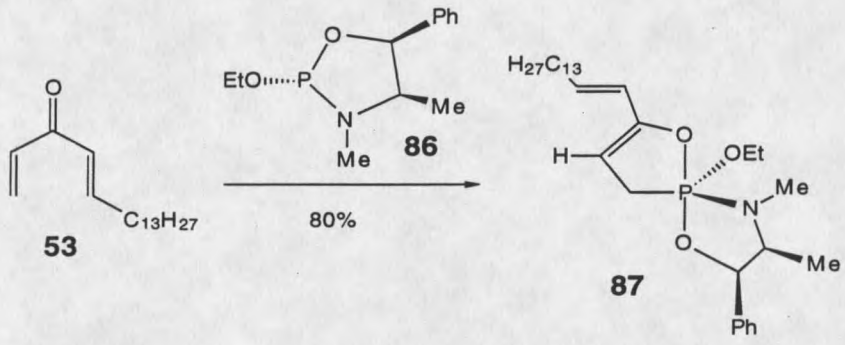




4.8: Future Plans: preparation of the final molecule:

As we had been losing almost half of the material in the reduction of **55** with chiral CBS reagents to **56a** or **56b**, we plan to investigate the use of chiral ligands on the P(V) in order to produce the condensation product **55** in chiral form. Thus, ephedrine and other chiral ligands on the phosphorus to form chiral P(III)'s are being investigated in the McClure group. Preliminary results show that chiral P(V) **87** has been formed from ephedrine P(III) **86** and the dienone **53** in very high yield. Research is in progress at this point to make the desired condensation product **55** with high enantiomeric purity.

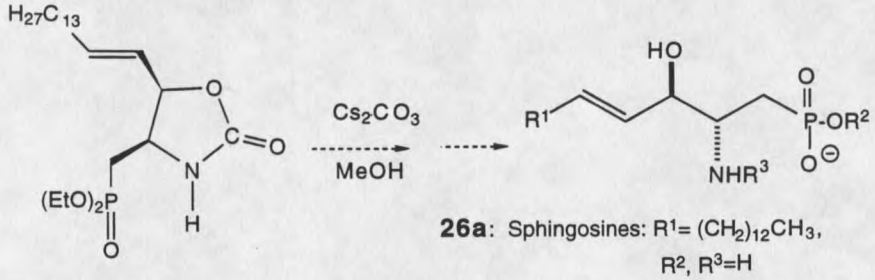
Scheme 4.28



As most of the important methodologies have been figured out (*vide supra*), the preparation of the desired molecule chirally would be much easier and effective.

To finish the synthesis, it is anticipated that the desired cleavage of N-N bond product of **56b** would be easily hydrolyzed[39,40] to give the desired sphingolipid analogs after proper functionalization (see **Scheme 4.29**).

Scheme 4.29



26a: Sphingosines: $R^1 = (CH_2)_{12}CH_3$, $R^2, R^3 = H$

26b: Sphingomyelin: $R^1, R^2 = (CH_2)_{12}CH_3$, $R^3 = COCH_2CH_2\overset{+}{N}Me_3$

26c: Ceramide 1-phosphate: $R^1 = (CH_2)_{12}CH_3$ $R^2 = H$, $R^3 = COCH_2CH_2\overset{+}{N}Me_3$

EXPERIMENTAL SECTION

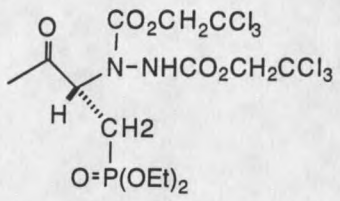
General:

All phosphites were treated with sodium prior to distillation. Methyl vinyl ketone was treated with solid K_2CO_3 and $CaCl_2$ prior to distillation. Et_2O was distilled from sodium benzophenone ketyl. CH_2Cl_2 was distilled from CaH_2. $CDCl_3$ and acetic acid were distilled from P_2O_5 under argon. Acetone was treated with 4 Å molecular sieves prior to distillation. All reactions were carried out under dry argon atmosphere in oven-dried round bottom flasks. Methyl iodide and deuterated chloroform were purified by filtering through basic alumina just before use. The acetic acid was distilled from benzene followed by distillation from CrO_3. The acid was further dried over $CuSO_4$ and purified by fractional distillation. Commercially available zinc dust was further purified by washing with 2% HCl followed by washing with water and acetone. It was then dried in an oven for 48 h and dried under high vacuum (0.1 mm) for 24 h. Proton, carbon and phosphorus NMR spectra were obtained on either Bruker AM-250, DRX-250 (250 MHz), or AM-300, DPX-300 (300 MHz) spectrometers as solutions in $CDCl_3$. Proton and carbon NMR chemical shifts are reported in ppm downfield from TMS (or relative to internal $CHCl_3$). ^{31}P spectra are reported in ppm from an external reference of 85% H_3PO_4. Proton NOE data were acquired on either a Bruker AM-500 (500 MHz) spectrometer or DRX-250 (250 MHz) or DPX-300 (300MHz) with low decoupling irradiation power (40L, or 70 dB) and long irradiation times. Infrared spectra were recorded on a Bruker IFS 25 IR. High resolution mass spectra were obtained on a VG 70E-HF double-focusing mass spectrometer operating at a resolution of 5000. FAB spectra was obtained by using glycerol as a matrix, EI data were obtained by

using 70 ev, CI data were obtained by using NH_3 as a reagent gas at 0.3 torr and source temperature of 150° C. Column chromatography was performed on silica gel, Merck grade 9385, 230-400 mesh, 60 Å using a step gradient of CH_3OH in CH_2Cl_2 or EtOAc/hexane unless otherwise noted. Preparative thin layer chromatography was on a 1 mm thick silica gel plate manufactured by Merck. The solvent mixtures used for column chromatography were volume/volume mixtures. R_f values indicated refer to thin layer chromatography on Analtech 2.5 x 10 cm, 250μ analytical plates coated with silica gel GF. High pressure liquid chromatography was done on a Rainin-60Å semi preparative silica gel column. Elemental analyses data were obtained from Robertson Microlit Laboratories, Inc, Madison , NJ.

Preparation of $Zn(BH_4)_2$ (0.14 M) Into a oven dried round bottom flask, $ZnCl_2$ (2 g, 14.7 mmol) was heated to melting under vacuum (1 mm). Then the reaction flask was cooled to rt under nitrogen. Dry Et_2O (25 mL) was added, and the mixture was refluxed for 90 min to dissolve all the $ZnCl_2$. $NaBH_4$ (1.20 g, 31.7 mmol) was dissolved in Et_2O (75 mL) in a seperate flask. The $ZnCl_2$ solution was then added drop by drop via cannula at rt to this ethereal solution of $NaBH_4$ while stirring. The reaction mixture was allowed to stir at rt for 12 more h. The solution was then transferred via cannula into a clean dry rbf give a 0.14 M solution of $Zn(BH_4)_2$. The solids were discarded.

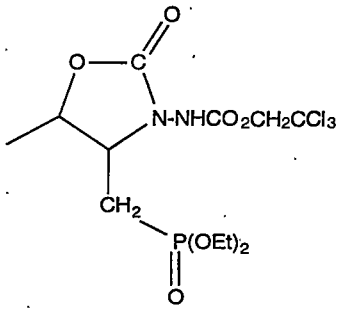
Preparation of SmI_2 (0.1 M) solution: Samarium metal (413 mg, 2.74 mmol) and iodine (508 mg, 2 mmol) were added to a dry round bottom flask under argon. Dry THF (20 mL) was added to the flask, and the mixture was refluxed for 1 hour to give a deep blue solution of SmI_2 (0.1 M).



41

(±)-Diethyl 2-(N,N'-bis(2,2,2-trichloroethoxycarbonyl)hydrazido)-3-oxobutylphosphonate (41). The oxaphospholene **17** (0.968 g, 4.098 mmol) was transferred via cannula into a flame dried flask under Ar and dissolved in of dry diethyl ether (15 mL). In a separate flame dried flask under Ar was placed the bis(2,2,2-trichloroethyl) azodicarboxylate (BTCEAD) (1.875 g, 4.917 mmol), and dissolved in dry diethyl ether (15 mL). The BTCEAD solution was cooled to -78 °C and the oxaphospholene was added via cannula to the BTCEAD solution over a period of 5 min. After stirring at -78 °C for 12 h, the reaction mixture was hydrolyzed by treatment with pH 7 buffer (15 mL), and allowed to stir for another 2 h at rt. The hydrolyzed product was extracted with EtOAc (3 x 30 mL) and washed with water (3 X 10 mL). The combined organic extracts were dried over $MgSO_4$, and the solvent was removed under reduced pressure to give a crude oil. Purified product **41** (2.158 g, 3.51 mmol, 91%) was isolated via flash column chromatography using 75 g silica gel, eluting with 40% EtOAc/Hex. R_f 0.33 (50%EtOAc/hex). ^1H NMR: 4.73 (4H, m), 4.08 (5H, m), 2.34 (3H, s), 2.24 (2H, m), 1.31 (6H, m). ^{13}C NMR: 202.3, 154.2, 153.8, 94.6, 94.4, 75.8, 75.1, 62.5 (d, J_{P-C} = 6.3 Hz), 62.2 (d, J_{P-C} = 6.8 Hz), 58.2, 26.8, 22.4 (d, J_{P-C} = 145.6 Hz), 16.3. ^{31}P: 28.1 ppm. IR (neat, cm^{-1}): 3178, 1761, 1725, 1399. HRMS (CI) calcd for $C_{14}H_{22}N_2O_8PCl_6$ $(M+H)^+$ 586.9245, found

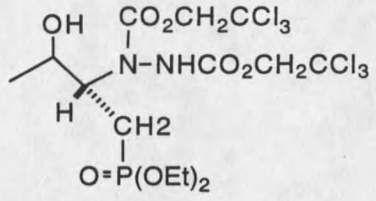
586.9247. Anal. Calcd. for $C_{14}H_{22}N_2O_8PCl_6$: C, 28.66; H, 3.75; N, 4.77; P, 5.29; Cl, 35.83. Found: C, 28.90; H, 3.70; N, 4.71; P, 5.16; Cl, 35.38.



42a,b

(4*S, 5*R**) Diethyl [(3-(N'-(2,2,2-trichloroethoxycarbonyl)hydrazido) -5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate (42a), and (4*R**, 5*R**) diethyl [(3-(N'-(2,2,2-trichloroethoxycarbonyl)-hydrazido)-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate (42b):** The ketone **41** (0.727 g, 1.24 mmol) was dissolved in dry EtOH (25 mL) in a dry round bottom flask, and cooled to 0 °C. To this cooled solution was added solid $NaBH_4$ (0.140 g, 3.72 mmol) in one portion. The ice bath was then removed and the reaction mixture was allowed to stir at rt overnight. The reaction was quenched after 9 h with saturated aq. NH_4Cl (10 mL), extracted with Et_2O (3 x 20 mL), dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. After flash column chromatography (2.5% MeOH/CH_2Cl_2), R_f 0.35 (5% MeOH/CH_2Cl_2), the diastereomeric mixture of oxazolidinones **42a,b** was isolated as a clear oil (0.678 g, 93%, 1.15 mmol). Attempts to separate the diastereomers were not successful at this point. The ratio of isomers **42a** : **42b** = 3:1 by ^1H NMR integration. Major diastereomer, **42a**: ^1H

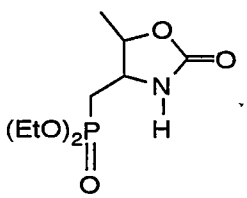
NMR: 8.40 (1H, bs, NH), 4.82 (2H, m), 4.45 (1H, m), 4.12 (4H, m), 3.85 (1H, m), 2.4-1.9 (2H, m), 1.45 (3H, d, J = 6.1 Hz), 1.30 (6H, m). ^{13}C NMR: 155.4, 153.8, 94.7, 76.3, 74.9, 62.2, 59.2, 28.8 (d, J_{P-C} = 140.2 Hz), 19.9, 16.2. Minor diastereomer, **42b**: ^{1}H NMR: 8.38 (1H, bs, NH), 4.82 (2H, m), 4.35 (1H, m), 4.12 (5H, m), 2.4 - 1.9 (2H, m), 1.36 (3H, d, J = 6.5 Hz), 1.30 (6H, m). ^{13}C NMR: 155.4, 153.8, 94.7, 74.9, 73.9, 62.2, 55.4, 24.7 (d, J_{P-C} = 143.1 Hz), 19.9. 16.2. ^{31}P: (**42a**) 24.8 ppm; (**42b**) 25.7 ppm. HRMS (CI) calcd for $C_{12}H_{20}N_2O_7PCl_3$ (mixture of **42a,b**) $(M+H)^+$ 441.0152, found 441.0150. Anal. Calcd. for $C_{12}H_{20}N_2O_7PCl_3$ (mixture of **42a,b**): C, 32.72; H, 4.54; N, 6.36; P, 7.04; Cl, 23.86. Found: C, 32.63; H, 4.60; N, 6.15; P, 6.20; Cl, 21.91.



43a,b

(4*S, 5*R**) Diethyl [2-(N-N'-bis-(2,2,2-trichloroethoxycarbonyl) hydrazido)-3-hydroxybutyl]phosphonate (43a), and (4*R**, 5*R**) diethyl [2-(N-N'-bis-(2,2,2-trichloroethoxycarbonyl)hydrazido)-3-hydroxybutyl]phosphonate (43b):** The trichloroketohydrazide **41** (2.17g, 3.7 mmol) was dissolved in EtOH (60 mL) and cooled to -78 °C. To this cooled solution was added solid $NaBH_4$ (0.451 g, 11.9 mmol). The reaction was allowed to stir at -78 °C for 10 h, and then quenched at -78 °C with sat. aq. NH_4Cl (15 mL). The mixture was extracted with CH_2Cl_2 (3 x 30 mL), dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure.

After gravity column chromatography (1.5-2.5% MeOH/CH_2Cl_2), the 3:1 mixture of alcohols, **43a,b**, (0.65g, 1.11 mmol, 30%) and the diastereomeric mixture of oxazolidinones **42a,b** (1.01 g, 2.29 mmol, 62%) were isolated as clear oils. Attempts to separate the diastereomers of the alcohol were unsuccessful. R_f (alcohol **43a,b**) 0.32 (2.5% MeOH/CH_2Cl_2). ^1H NMR (mixture of **43a** and **43b**): 4.85 - 4.62 (4H, m), 4.40 (1H, m), 4.20-3.90 (4H, m), 3.51 (1H, m), 2.22 - 1.75 (2H, m), 1.40 - 1.26 (6H, m), 1.22 (3H, d, J = 5.7 Hz). ^{13}C NMR: (major isomer, **43a**) 156.6, 154.1, 94.6, 75.5, 68.2 (d, J_{P-C} = 18.4 Hz), 62.4 (d, J_{P-C} = 7.0 Hz), 59.0 , 25.5 (d, J_{P-C} = 143.7 Hz), 18.7, 16.1. (Minor isomer, **43b**): 156.6, 154.1, 94.5, 74.9, 68.0 (d, J_{P-C} = 18.40), 62.1 (d, J_{P-C} = 6.5 Hz), 58.9, 25.1 (J_{P-C} = 146.3 Hz), 18.7, 16.1. ^{31}P (mixture of **43a** and **43b**): 28.1. IR (mixture of **43a** and **43b**, neat, cm^{-1}): 3464, 1733, 1652, 1405, 1221, 1026. LRMS (CI) calcd for $C_{14}H_{23}O_8N_2PCl_6$ $(M + H)^+$ 590.9, found 590.9. HRMS by EI on the mixture of **43a** and **43b** was performed on two fragment ions. HRMS (EI) for $C_{12}H_{18}O_7N_2PCl_6$ (M - OCH_2CH_3) 542.8962, found 542.8982. HRMS (EI) for $C_{12}H_{21}O_7N_2PCl_3$ (M - OCH_2CCl_3) 441.0147, found 441.0151.

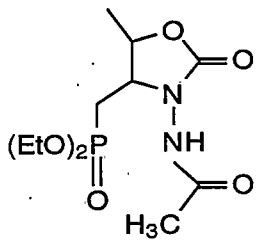


44a,b

(4*S,5*R**) Diethyl [(5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate (44a), and (4*R**, 5*R**) diethyl [(5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate (44b):** The 3:1 diastereomeric mixture of oxazolidinones **42a,b** (300 mg, 0.68 mmol) was dissolved in HOAc (3 mL), and

Zn dust (1.5 g, 23.3 mmol) was added at rt under Ar over a period of 5 min. After about 4 h, acetone (0.60 mL) was added to the reaction mixture. The reaction mixture was allowed to stir at rt for another 32 h. The reaction mixture was then quenched with 10% aq. $NaHCO_3$, and tested with litmus paper to be slightly basic. The mixture was extracted with EtOAc (3x20 mL), dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. After gradient HPLC chromatography (10 mL/min, 1.5 - 4% MeOH/CH_2Cl_2), the N-N cleavage products, **44a,b**, (105 mg, 0.42 mmol, 62%, R_f 0.30 (3% MeOH/CH_2Cl_2)) were isolated, along with the hydrazines **46a,b** (27 mg, 0.101 mmol, 15%, R_f 0.18 (5% MeOH/CH_2Cl_2)), the hydrazones **47a,b** (13 mg, 0.042 mmol, 6%, R_f 0.32 (5% MeOH/CH_2Cl_2)) and the N'-acetylated hydrazines **45a,b** (32 mg, 0.103 mmol, 14%, R_f 0.28 (5% MeOH/CH_2Cl_2)) as intermediates. The reaction could be coaxed to completion (78-83% of **44a,b**) by addition of another 10-20 eq. of Zn dust and 2 mL of HOAc after the initial 4h, followed by addition of acetone (0.20 - 1.0 mL) after a further 12 h. Stirring for another 16-20 h converted the intermediates into the products **44a,b**. The diastereomers **44a** and **44b** could easily be separated by HPLC chromatography in 5.5% MeOH/CH_2Cl_2 (4 mL/min). In the best run, 0.425 g (0.964 mmol) of the oxazolidinones **42ab** were converted to the following amounts of isolated cleaved products: **44a** (0.15 g, 0.59 mmol, 62.2%), **44b** (0.05 g, 0.199 mmol, 20.7%) and **45a,b** (0.025 g, 0.081 mmol, 8%). Major diastereomer, **44a**: ^1H NMR: 6.20 (1H, bs, NH), 4.30 (1H, app. quint., J = 6.1 Hz), 4.05 (4H, m), 3.61 (1H, app. quint., J = 6.6 Hz), 1.94 (2H, dd, J_{P-H} = 18.1 Hz, J_{H-H} = 6.7 Hz), 1.35 (3H, d, J = 6.2 Hz), 1.26 (6H, t J = 7.1 Hz). ^{13}C NMR : 158.0, 78.8 (J_{P-C} = 14.3 Hz), 62.0 (J_{P-C} = 6.5 Hz), 54.5 (J_{P-C} = 3.1 Hz), 31.2 (J_{P-C} = 139.9 Hz), 19.5, 16.1. ^{31}P: 26.6.

IR (neat, cm $^{-1}$): 3258, 1749, 1241, 1040. Minor diastereomer **44b**: ^{1}H NMR: 5.80 (1H, bs, NH), 4.78 (1H, app. quint., J = 6.4 Hz), 4.08 (5H, m), 1.86 (2H, dd, J_{P-H} = 18.6 Hz, J_{H-H} = 6.6 Hz), 1.33 (9H, m). ^{13}C NMR: 158.04, 75.2 (J_{P-C} = 18.1 Hz), 62.3 (J_{P-C} = 6.1 Hz), 51.0 (J_{P-C} = 5.0 Hz), 27.0 (J_{P-C} = 142.1), 16.4, 15.0. ^{31}P: 28.5. IR (neat, cm $^{-1}$): 3276, 1760, 1238, 1050. HRMS (CI) calcd for $C_9H_{18}NO_5P$ (**44a,b** mixture) $(M+H)^+$ 252.1000, found 252.1000. Anal. Calcd for $C_9H_{18}NO_5P$ (**44a,b** mixture): C, 43.02; H, 7.17; N, 5.57; P, 12.35. Found: C, 43.13; H, 7.36; N, 5.84; P, 12.26.

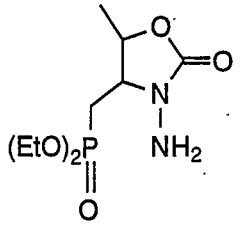


(Isolated)

45a,b

(4*S, 5*R**) Diethyl [(3-acetamido-5-methyl-2-oxazolidinon-4-yl) methyl]phosphonate (45a), and (4*R**, 5*R**) diethyl [(3-acetamido-5-methyl-2-oxazolidinon-4-yl)methyl]-phosphonate (45b):** This compound was always isolated as a minor intermediate during the preparation of **44a,b** due to the contamination of acetic anhydride in acetic acid. Major isomer **45a**: ^{1}H NMR: 8.80 (1H, bs, NH), 4.43 (1H, m), 4.04 (4H, m), 3.82 (1H, m), 2.20 - 1.87 (2H, m), 1.96 (3H, s), 1.47 (1H, d, J = 6.1 Hz), 1.26 (6H, m). ^{13}C NMR: 169.6, 155.6, 76.1, 61.9, 58.9, 28.5 (J_{P-C} = 139.9 Hz), 20.2, 19.6, 16.0 (d,

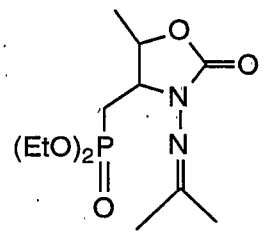
$J_{P\text{-}C}$ = 5.5 Hz). Minor Isomer **45b**: ^{1}H NMR: 8.78 (1H, s, NH), 4.70 (1H, m), 4.24 (1H, m), 3.94 (4H, m), 2.20 - 1.87 (2H, m), 1.96 (3H, s), 1.30 (1H, d, J = 6.5Hz), 1.26 (6H, m). ^{13}C NMR: 169.5, 156.1, 73.5, 61.9, 58.9, 24.3 (d, $J_{P\text{-}C}$ = 143.2 Hz), 20.2, 19.6, 16.0 (d, $J_{P\text{-}C}$ = 5.5 Hz). IR (mixture of **45a,b**, neat, cm^{-1}) 3234, 1782, 1690, 1236, 1052, 1017. ^{31}P: (**45a**) 25.2 ppm; (**45b**) 26.1 ppm. HRMS (CI) calcd for $C_{11}H_{21}N_2O_6P$ (mixture of **45a,b**): $(M+H)^+$ = 309.1216, found 309.1228. Anal. Calcd for $C_{11}H_{21}N_2O_6P$ (**45a,b** mixture): C, 42.85; H, 6.81; N, 9.09; P, 10.06. Found: C, 42.46; H, 6.68; N, 8.83; P, 9.76.



46a,b

(4*S, 5*R**) Diethyl [(3-amino-5-methyl-2-oxazolidinon-4-yl)methyl] phosphonate (46a), and (4*R**, 5*R**) diethyl [(3-amino-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate (46b):** The mixture of the oxazolidinones **42a,b** (0.423 g, 0.96 mmol) was dissolved in HOAc (2 mL). To this stirred solution was added Zn dust (1.5 g, 23.3 mmol) over 5 min. The reaction mixture was stirred overnight for 12 h. The reaction mixture was then quenched with 10 % aq. $NaHCO_3$, tested with litmus paper to be basic, and extracted with EtOAc (5 x 3 mL). The organic layer was dried with $MgSO_4$ and solvent removed in vacuo to give a crude oil (250 mg). The hydrazines **46a,b** were isolated as a mixture of diastereomers via flash column chromatography (210 mg, 0.786 mmol, 82%). R_f 0.32 (10% MeOH/CH_2Cl_2). A small amount of

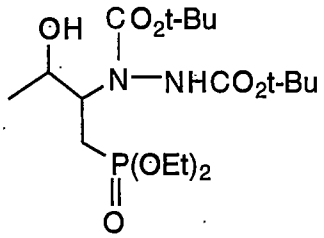
the N'-acetylated hydrazines, **45a,b**, was also isolated (45 mg, 0.144 mmol, 15%), R_f 0.28 (5% MeOH/CH_2Cl_2). Major isomer **46a**: ^{1}H NMR: 4.50 - 4.45 (1H, m), 4.20 - 3.95 (4H, m), 3.80 - 3.55 (1H m), 2.32 - 1.85 (2H, m), 1.45 (3H, d, J = 6.2 Hz), 1.25 (6H, m). ^{13}C NMR: 155.5, 69.5, 62.4 (d, J_{P-C} = 6.8 Hz), 59.2, 28.7 (d, J_{P-C} = 140.4 Hz), 20.0, 16.2. Minor isomer **46b**: ^{1}H NMR: 4.72 (1H, m), 4.30 - 4.20 (1H, m), 4.20 - 3.95 (4H, m), 2.32 - 1.85 (2H, m), 1.35 (3H, d, J = 6.7 Hz), 1.25 (6H, m). ^{13}C NMR: 155.5, 68.4, 62.4 (d, J_{P-C} = 6.8 Hz), 55.5, 24.7 (d, J_{P-C} = 142.5 Hz), 20.0, 16.2. ^{31}P NMR: (**46a**) 24.8; (**46b**) 25.8. HRMS (CI) calcd for $C_9H_{19}N_2O_5P$ (mixture of **46a,b**): $(M+H)^+$ 267.1109, found 267.1108.



47a,b

(4*S, 5*R**) Diethyl [(3-(N'-isopropylideneamino)-5-methyl-2-oxazolidinon-4-yl)methyl]-phosphonate (47a), and (4*R**, 5*R**) diethyl [(3-(N'-Isopropylideneamino)-5-methyl-2-oxazolidinon-4-yl) methyl]phosphonate (47b).** During the preparation of **44a,b**, an aliquot was taken out after the addition of acetone to the reaction mixture to monitor the reaction by isolation of the intermediates. The aliquot was diluted with CH_2Cl_2 (10 mL) and filtered through celite. The reaction mixture was then neutralized with saturated $NAHCO_3$ and was extracted with EtOAc (3 x 15 mL). After flash column chromatography, **47a,b** were isolated as intermediates. Major isomer **47a**: ^{1}H NMR: 4.42 (1H, app. quint., J = 6.1 Hz), 4.09 (4H, m), 3.87 (1H, m),

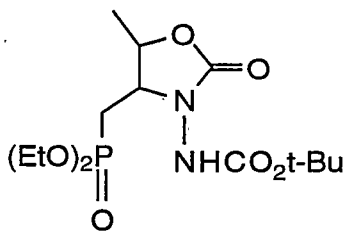
2.25-1.85 (2H, m), 2.01 (6H, s), 1.51 (3H, d, J = 6.2 Hz), 1.30 (6H, m). ^{13}C NMR: 169.4, 155.9, 76.3 (d, J_{P-C} = 7.7 Hz), 62.2 (d, J_{P-C} = 5.8 Hz), 55.3, 25.3 (d, J_{P-C} = 143.1 Hz), 20.6, 16.3 (d, J_{P-C} = 5.8 Hz), 15.2. Minor isomer **47b**: ^1H NMR: 4.87 (1H, app. quint., J = 6.9 Hz), 4.33 (1H, app. quint., J = 7.5 Hz), 4.09 (4H, m), 2.25 - 1.91 (2H, m), 2.01 (6H, s), 1.38 (3H, d, J = 6.6 Hz), 1.30 (6H, m). ^{13}C NMR: 169.6, 156.0, 73.8 (d, J_{P-C} = 7.7 Hz), 62.2 (d, J_{P-C} = 5.8 Hz), 55.3, 25.3 (d, J_{P-C} = 145.0 Hz), 19.8, 16.3 (d, J_{P-C} = 5.8 Hz), 15.2. HRMS (FAB) calcd for $C_{12}H_{23}N_2O_5P$ (mixture of **47a,b**) $(M+H)^+$ 307.1424, found 307.2048.



48a,b

(2*S, 3*R**) Diethyl [2-(N-N'-bis(t-butoxycarbonyl)hydrazido)-3-hydroxybutyl]phosphonate (48a), and (2*R**, 3*R**) diethyl [2-(N-N'-bis(t-butoxycarbonyl)hydrazido)-3-hydroxybutyl]phosphonate (48b):** A flame dried, one-necked, 100 mL round bottom flask was charged with the t-butyl-keto hydrazide **24** (1.21 g, 2.86 mmol) under argon. Dry ethanol (30 mL) was added and the reaction flask was cooled down to -78 °C. $NaBH_4$ (0.32g, 8.58 mmol) was added quickly at this temperature, and the reaction mixture was slowly allowed to warm to rt over 9.5 h and stirred at rt for another 2 h. The reaction mixture was then cooled to 0 °C, quenched with cold NH_4Cl (15 mL),

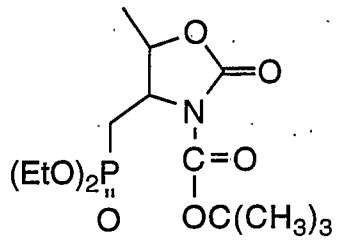
and extracted with ethyl acetate (4 x 30 mL). The organic layers were combined together, dried over $MgSO_4$, filtered, and solvent was removed in vacuo. Purification via gradient flash column chromatography using 1.5-3% $MeOH/CH_2Cl_2$ produced oxazolidinones **49a,b** (17%) and alcohols **48a,b** (73%). Ratio of diastereomers **48a: 48b** = 4:1. Attempts to separate the diastereomers were unsuccessful. R_f in 3.5 % $MeOH/CH_2Cl_2$ =0.35, ^1H NMR: 4.20 (1H, m), 3.9 (4H, m), 3.3 (1H, m), 1.8 (2H, m), 1.45 (18H, m), 1.20 (6H,m), 1.18 (3H, d, J = 5.98 Hz), ^{13}C NMR: 157.7, 154.5, 81.6, 81.1, 67.9 (d, J_{P-C}=19.7 Hz), 61.9(d, J_{P-C} = 6.7 Hz), 61.2(d, J_{P-C} = 6.41 Hz), 56.8(d, J_{P-C} = 6.42 Hz), 27.9, 25.4 (d, J_{P-C} = 144.47 Hz), 18.8, 15.9 (d, J_{P-C} = 6.3 Hz), 15.8 (d, J_{P-C} = 6.48 Hz). ^{31}P NMR: (major isomer): 27.7 ppm, (minor isomer): 27.3 ppm. HRMS (M^+) calculated for $C_{18}H_{37}N_2O_8P$ = 441.2363, found 441.2365.



49a,b

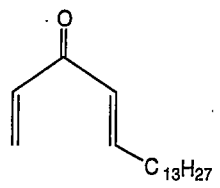
(4*S, 5*R**) Diethyl [(3-(N'-(t-butoxycarbonyl)hydrazido)-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate (49a), and (4*R**, 5*R**) diethyl [(3-(N'-(t-butoxycarbonyl)-hydrazido)-5-methyl-2-oxazolidin-4-yl)methyl]phosphonate (49b).** A flame dried, one-necked, 100 mL round bottom flask under argon was charged with the t-butyl-keto hydrazide **24** (2.38

g, 5.65 mmol). Dry ethanol (50 mL) was added, and the reaction flask was cooled to 0 °C. $NaBH_4$ (0.57g, 15.2 mmol) was then added in one portion. The icebath was removed and the reaction mixture was allowed to stir at rt for 11 h. The reaction mixture was then cooled to 0 °C, was quenched with cold NH_4Cl (25 mL), and extracted with ethyl acetate (3 x 30 mL). The organic layers were combined together, dried over MgSO4, filtered and solvent removed in vacuo. Purification via gradient flash column chromatography using 1.5-3.5% $MeOH/CH_2Cl_2$ produced oxazolidinone R_f = 0.32 in 5%$MeOH/CH_2Cl_2$ (37%) and alcohol (53%). ^1H NMR: 4.5 (1H, m), 4.2- 4 (4H, m), 3.8 (1H, m), 2.3-1.9 (2H, m), 1.5 (3H, d, J = 6.9 Hz), 1.42 (9H, s), 1.2-1.38 (6H, m). ^{13}C NMR: 155.9, 154.2, 81.9, 75.9, 62.8 (d, J_{P-C} = 6.8), 58.9, 28.5(d, J_{P-C} = 140.1Hz), 27.9, 19.9, 16.2. ^{31}P NMR: (major Isomer): 24.4 ppm, (minor isomer): 24.8 ppm. IR (neat, cm^{-1}): 3172, 2962, 2926, 2853, 1794, 1750, 1445, 1365, 1235. HRMS (FAB) calcd for $C_{14}H_{27}N_2O_7P$ (mixture of **49a,b**) $(M+H)^+$ 367.1562, found 367.2134.



50a

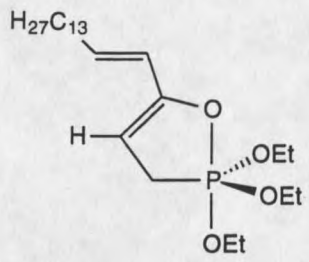
(4*S, 5*R**) Diethyl [(3-(N-butoxycarbonyl)-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate (50a):** NaH (21 mg, 0.89 mmol) was placed into a dry round bottom flask. The trans diastereomer **42a** of oxazolidinones (149 mg, 0.59 mmol) was dissolved in THF (10 mL), and transferred via cannula over the NaH at rt under Ar. The reaction mixture was stirred at rt under argon for 1 h, and BOC-ON was added (219 mg, 0.89 mmol). Copious amounts of white yellow precipitate fell out of solution. After 10 min, the TLC showed the absence of starting material. The reaction mixture was quenched by ice-cold NH_4Cl (6 mL), extracted with ethyl acetate (3 x 20mL), dried over $MgSO_4$, filtered, and the solvent was removed in vacuo. After flash column chromatography on silica gel (2.5% MeOH/CH_2Cl_2), the BOC-protected product **50a**, (174 mg, 0.49 mmol, 83%), R_f = 0.31 (5% MeOH/CH_2Cl_2)) was isolated. **50a**: ^1H NMR: 4.55 (1H, m), 4.04 (1H, m, J = 6.1 Hz), 3.95 - 3.41 (1H, m), 2.06(2H, dd, J_{P-H} = 18.3 Hz, J_{H-H} = 6.5 Hz), 1.55 (9H, s), 1.44 (3H, d, J_{H-H} = 6.6 Hz) 1.39 (6H, t, J = 7.1 Hz). ^{13}C NMR : 150.5, 148.8, 83.8, 74.5, 61.8 (d, J_{P-C} = 6.1 Hz), 56.9, 29.2 (J_{P-C} = 137.4 Hz), 27.6, 20.2,15.9 (d, J_{P-C} = 3.2 Hz) ^{31}P: 26.8.



53

(4E)-1,4-octadecen-3-one (53):

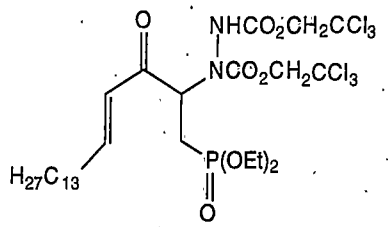
To the alcohol **64** (1.3 g, 4.9 mmol) in a flame dried round bottom flask was added CH_2Cl_2 (30 mL) and MnO_2 (2.5 g, 24.5 mmol) was then added to the reaction flask in one portion. The reaction mixture was then stirred at rt for 24 h. More than 60% conversion was completed within first 5 h. To bring the reaction to completion, another 3-5 eq of MnO_2 was added, and the reaction mixture was allowed to stir for 12 h with continuous monitoring via TLC. The solution was then filtered through a sintered glass funnel over celite. After evaporation of solvent and, gradient flash column chromatography (R_f = 0.54 in 5%EtOAC/Hex) with 1-2.5 % EtOAc/Hex **53** (1.07 g, 83%, 4.06 mmol) was isolated as a clear oil. ^{1}H NMR: 6.91 (1H, dt, J = 15.7, 6.9 Hz), 6.58 (1H, dd, J = 17.4, 10.5 Hz), 6.33 (1H, dm, J = 15.7 Hz), 6.25 (1H, dd, J = 17.4, 1.3 Hz), 5.78 (1H, dd, J = 10.5, 1.3 Hz), 2.25-1.43 (2H, m), 1.42-1.25 (22H, m), 0.85 (3H, t, J = 6.2 Hz). ^{13}C NMR: 189.7, 149.0, 135.0, 128.2, 127.9, 32.7, 31.9, 29.7, 29.5, 29.4, 29.3, 29.2, 28.1, 22.7, 14.0. IR: 1730, 1627, 1606, 1467, 1059. HRMS (EI) calcd for $C_{18}H_{32}O$ = 264.2453, found 264.2444.



54

2,2,2-triethoxy-2,2-dihydro-5-((E)-pentadec-1-enyl)-1,2λ^5-oxa-phospholene (54):

To the dienone **53** (460 mg, 1.74 mmol) in a flame dried round bottom flask, freshly distilled triethyl phosphite (580 mg, 3.48 mmol) was added via syringe. The reaction mixture was stirred at rt vigorously under argon for 24 h. The excess of triethyl phosphite was then removed under high vacuum to give pure dienone oxaphospholene **54** as a clear oil in almost quantitative yield. Any further purification attempt by distillation under high vacuum led to quick decomposition of the compound. ^{1}H NMR: 5.98 (1H, dt, J = 15.3, 6.9 Hz), 5.75 (1H, d, J =15.4 Hz), 4.68 (1H, dm, $J_{P\text{-}H}$ = 47.4 Hz), 3.87 (6H, m), 2.61 (2H, d, $J_{P\text{-}H}$ = 18.6 Hz), 2.08 (2H, m), 1.37 (2H, m), 1.30 (29H, m), 0.85 (3H, t,J = 6.14). ^{13}C NMR: 151.7 (d, $J_{P\text{-}C}$ = 16.9 Hz), 131.2, 122.3 (d, $J_{P\text{-}C}$ = 3.9 Hz), 94.2 (d, $J_{P\text{-}C}$ = 5.6 Hz), 62.2 (d, $J_{P\text{-}C}$ = 10.6 Hz), 32.7, 32.3, 31.9, 29.6, 29.5, 29.4 (d, $J_{P\text{-}C}$ = 162.5 Hz), 29.3, 29.2, 28.2, 16.6 (d, $J_{P\text{-}C}$ = 7.2 Hz), 14.1. ^{31} P: 23.8 ppm, IR (neat, cm^{-1}): 2930, 1730, 1695, 1627, 1606, 1467, 1261, 1220, 1159, 1059. HRMS (EI) calcd for $C_{24}H_{47}O_4P$ = 430.3211, found 430.3218.

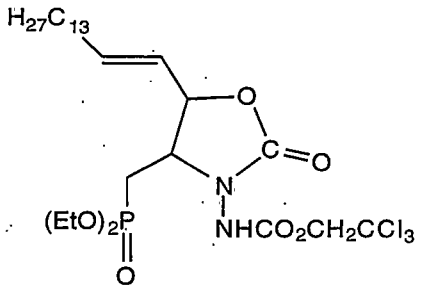


55

(±) (4E)-Diethyl [2-(N,N'-bis(2,2,2-trichloroethoxycarbonyl)hydrazido)-3-oxo-octadecenyl]phosphonate (55):

Dry diethyl ether (60 mL) was transferred into a flame dried round bottom flask and $ZnCl_2$ solution (23.2 mL, 23.2 mmol, 1.0 M Et_2O) was added. The solution was stirred for 10 minutes at rt, and solid BTCEAD (2.65 g, 6.97 mmol) was added quickly. The reaction mixture was stirred for 90 minutes at rt. The reaction mixture was then cooled to -78°C and the dienone P(V) (2.5 g, 5.81 mmol) was added neat via cannula with the final amount being added as diethyl ether (1 mL) rinse. The reaction mixture was stirred at -78°C for 12 h, slowly warmed to -20 °C over 16 h. The reaction misture was recooled to -78 °C, and quench with cold saturated ammonium chloride solution (25 mL). After warming to rt and stirring for 2.5 h, the reaction mixture was extracted with ethyl acetate (5 x 50 mL). The organic phase was dried over $MgSO_4$, filtered, and solvent removed in vacuo. Purification via column chromatography produced **55** as a clear oil (3.94g, 5.05 mmol, 87%) yield. It is important to note that less than 4 eq of $ZnCl_2$ led to lower yield, but more than 4 eq $ZnCl_2$ did not change the yield. ^1H NMR: 7.08 - 6.81 (2H, m), 4.94 - 4.44 (4H, m), 4.22 - 3.88 (4H, m), 2.81 - 2.02 (4H, m), 1.52 - 1.03 (28H, m), 0.83 (3H, t, J = 6.2 Hz). ^{13}C NMR: 192.4, 153.7, 150.8, 150.3, 125.1, 94.5 (d, J_{P-C} = 10.4 Hz), 75.6, 75.0, 62.3, (d, J_{P-C} = 22.6 Hz), 32.6, 31.8, 29.5, 29.4, 29.3, 29.2, 29.1, 17.9, 23.8, 23.2, 22.7 (d, J_{P-C} =

146.1 Hz), 21.9, 16.3, 14.0. IR (neat, cm $^{-1}$): 3154, 2915, 2848, 1771, 1704, 1621, 1397. ^{31}P NMR: 29.5 ppm. HRMS calculated for $C_{28}H_{47}N_2O_8PCl_6$ = 780.1201, mass found 780.1148.

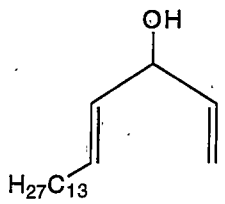


56a,b

(4*S, 5*R**) Diethyl [(3-(N'-(2,2,2-trichloroethoxycarbonyl)amino)-5-((E)-pentadec-1-enyl)-2-oxazolidinon-4-yl)methyl]phosphonate (56a), and (4*R**, 5*R**) diethyl [(3-(N'-(2,2,2-trichloroethoxycarbonyl)-amino)-5-((E)-pentadec-1-enyl)-2-oxazolidinon-4-yl)methyl]phosphonate 56b:**

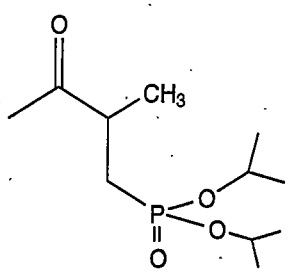
To the dienone-P(V) BTCEAD condensation product **55** (210 mg, .27 mmol) a freshly prepared solution of sucrose (10 mL, 1.0 M, 1:1 THF/H_2O) was added at rt. The reaction mixture was stirred for 1/2 h, cooled to 0 °C, and solid $NaBH_4$ (101 mg, 2.69 mmol) was added portion wise over 2 minutes. The icebath was removed, and the reaction mixture was allowed to stir for another 14 h at rt. The reaction mixture was recooled to 0 °C and quenched with satd. aq. ammonium chloride solution (7 mL). The reaction mixture was extracted with EtOAc (3 x15 mL), the organic layers combined, dried over $MgSO_4$, filtered, and the solvent

was removed in vacuo. Purification via gradient flash chromatography using EtOAc/ Hex (10%-50%) yielded the two oxazolidinone isomers a clear oil in 84 % overall yield (7 :1 ratio ratio of diastereomers). **56a** ,Trans isomer= (0.125 mg, 0.20 mmol, 88%), R_f = 0.42 (50 % EtOAc/Hex). ^{1}H NMR: major isomer: 8.03 (1H, bs), 5.90 (1H, dt, J = 15.3, 6.6 Hz), 5.47 (1H, dd, J = 7.8, 7.4 Hz), 5.09 (1H, m), 4.75 (2H, m), 4.30 (1H, m), 4.07 (4H, m), 2.09-2.01 (4H, m), 1.32-1.22 (28 H, m), 0.84 (3H, t, J = 6.2 Hz). ^{13}C NMR (major isomer): 155.5, 153.5, 139.4, 124.4, 94.6, 80.1 (d, J_{P-C} = 20.2 Hz), 75.1, 62.5 (d, J_{P-C} = 6.4 Hz), 57.9, 32.3, 32.1, 31.8, 29.6, 29.5, 29.4, 29.3, 29.1, 28.5, 28.4 (d, J_{P-C} = 145.2 Hz), 22.6, 16.4 (d, J_{P-C} = 5.5 Hz), 14.1. ^{31}P NMR: 26.2 ppm. IR (neat, cm^{-1}): 3174, 2924, 2853, 1789, 1755, 1100, 1050. **56b**, cis isomer = (0.18 mg, 0.028 mmol, 12%). R_f = 0.46 (50 % EtOAc/Hex),^{1}H NMR: 8.02 (1H, bs), 5.89 (1H, dt, J = 15.3, 7.7 Hz), 5.48 (1H, dd, J = 15.3, 7.7 Hz,), 5.09 (1H, m), 4.89 - 4.62 (2H, m), 4.30 (1H, m), 4.25 - 3.94 (4H, m), 2.08 - 2.00 (4H, m), 1.32-1.22 (28H, m), 0.84 (3H, t, J = 6.3 Hz). ^{13}C NMR (cis isomer): 155.8, 153.8, 140.1, 121.2, 94.7, 78.3 (d, J_{P-C} = 10.7 Hz), 75.1, 62.5 (d, J_{P-C} = 6.4 Hz), 62.3 (d, J_{P-C} = 6.1 Hz), 55.8, 32.3, 32.1, 31.8, 29.6, 29.5, 29.4, 29.3, 29.1, 28.6, 26.1 (d, J_{P-C} = 142.7Hz), 22.6, 16.4 (d, J_{P-C} = 5.5 Hz), 14.1. ^{31}P NMR: 27.5 ppm . IR (neat, cm -1): 3175, 2935, 2851, 1749, 1671, 1099, 1054. 3174, 2924, 2853, 1789, 1755, 1100, 1050. HRMS (EI) calculated for $C_{26}H_{46}O_7N_2Cl_3P$ (mixture of **56a** and **56b**) = 634.2108, found 634.2115.



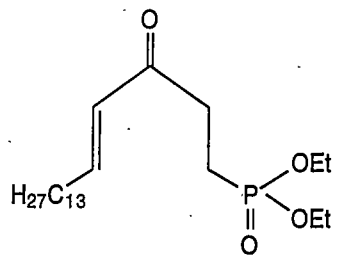
64

(±) (4E)-3-hydroxy-octadec-1,4-dienol (64). Vinyl magnesium bromide (26.2 mL, 1.0 M in THF) was taken into a dry round bottom flask and the flask was cooled to -78 °C. The aldehyde **57** (5.2 g, 21.84 mmol) was dissolved in dry THF (100 mL), cooled to 0 °C, and was added to the the precooled Grignard reagent via cannula. After the addition was complete, the reaction mixture was stirred for another 6 h at -78 °C. The reaction mixture was then warmed up to -30 °C over a 12 h period of time. The reaction mixture was then recooled to -78 °C and quenched at -78 °C by cold (0 °C) satd. aq. ammonium chloride solution (25 mL). The reaction mixture was extracted with EtOAc (4 x 50 mL). The organic phases were combined, dried over anhydrous $MgSO_4$, filtered and solvent removed in vacuo. Purification via flash chromatography using 2% EtOAc/Hexane (R_f = 0.31 in 5%EtOAC/Hex) produced pure **64** as an oil (4.82 g, 18.12 mmol, 83%) and (0.46 g, 1.93 mmol) of unreacted aldehyde. ^{1}H NMR: 5.87 (1H, ddd,J = 15.8, 5.72, 2.83 Hz), 5.67 (1H, dt, 15.3, 6.6 Hz), 5.46 (1H, dd, 15.4, 6.6 Hz), 5.24 (1H, dd, J = 15.9, 1.3 Hz), 5.13 (1H, dm, J = 10.8 Hz), 4.54 (1H, t, J = 5.9 Hz), 2.04 - 1.97 (22H, m), 0.85 (3H, t, J = 6.1 Hz). ^{13}C NMR: 140.0, 132.8, 131.0, 114.4, 73.7, 32.2, 31.9, 29.6, 29.5, 29.3, 29.1, 29.0, 22.3, 14.0. IR (neat, cm^{-1}): 3339, 2935, 2850, 1466, 1116, 1087, 988. HRMS (EI) calcd for $C_{18}H_{34}O$ = 266.2609, found 266.2613.



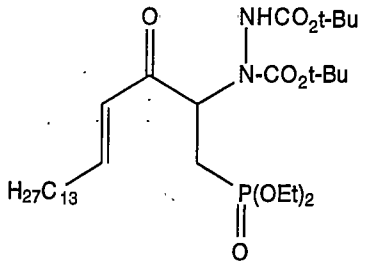
66

Diisopropyl (2-methyl-3-oxobutyl)phosphonate (66): A LDA solution (1.27 mL, 1.0 M cyclohexane) was put into a flame dried round bottom flask and dry THF (5 mL) was added. The mixture was cooled to -78 °C. The hydrolysis product of P(V) derived from isopropyl phosphite and MVK (0.149 g, 0.635 mmol), dissolved in 10 mL THF, was added drop by drop to the cold solution of LDA via cannula. The reaction mixture was allowed to stir at -78°C for 1 h. The flask was then warmed to -10 °C, and stirred at -10 °C for 1/2 h. The reaction mixture was cooled again to -78°C, and quenched by addition of MeI (500 µL, 7.7 mmol). The solution was allowed to stir for 1/2 h at -78° C, slowly warmed to rt, and then allowed to stir at rt for another hour. The reaction mixture was quenched by addition of cold saturated NH_4Cl solution (10 mL). The mixture was extracted with ethyl acetate (3 x 25 mL). The organic phases were combined, dried over $MgSO_4$, filtered, and the solvent was removed in vacuo. Purification via flash chromatography on silica gel yielded the product (0.123g, 0.49 mmol, 78%) as a clear oil. ^1H NMR: 4.61 (2H, m), 2.92 - 2.81 (1H, m), 2.24 - 2.15 (5H, m), 1.96 - 1.53 (2H, m, J_{P-H} = 6.7 Hz), 1.26 -1.24 (12H, m), 1.19 (3H, d, J = 7.2 Hz). ^{13}C NMR: 210.1, 70.2, 70.1, 41.5, 29.6, 29.3 (J_{P-C} = 143.4 Hz), 28.1, 23.9, 18.1 (J_{P-C} = 9.6 Hz). ^{31}P NMR : 28.8 ppm, HRMS (EI^+) calculated for $C_{11}H_{23}O_4P$ = 250.1347, mass found 250.1344.



67

(E)-Diethyl (3-oxooctadec-4-enyl)phosphonate (67): The dienone oxaphospholene (**54**), (129 mg, .30 mmol) was transferred into a flame dried round bottom flask via cannula, and 10 mL of pH 7 phosphate buffer was added to the flask. The reaction mixture was stirred for 4 h, and then extracted with EtOAc (3 x 10 mL). The combined organic extracts were washed with NaCl (3 x 10 mL) solution, dried over $MgSO_4$ and purified via column chromatography (silica gel) with 3.5% MeOH/CH_2Cl_2. R_f = 0.34 in 5% MeOH/CH_2Cl_2 to give a clear oily liquid, **67** (114 mg, 95 %). ^1H NMR: 6.87 (1H, dt, J = 8.95, 6.87 Hz), 6.08 (1H, d, J = 15.9 Hz), 4.08 (4H, m), 2.80 (2H, m), 2.20 (2H, m), 2.04 (2H, m), 1.44 (2H, m), 1.42 (20H, m), 1.09 (3H, t,J = 6.2 Hz). ^{13}C NMR: 197.2, 148.2, 129.5, 63.4 (d, J_{P-C} = 5.4Hz), 61.5 (d, J_{P-C} = 4.6 Hz), 32.50, 31.99 (d, J_{P-C} = 46.4 Hz), 29.4, 29.3, 29.2, 29.0, 27.8, 27.3, 27.0, 22.4, 19.4 (d, J_{P-C} = 144.4 Hz), 16.2, 15.9 (d, J_{P-C} = 6.4 Hz), 13.9. IR (neat, cm^{-1}): 3473, 2925, 2853, 1699, 1682, 1633, 1031), ^{31}P NMR: 32.4 ppm, HRMS (EI) calcd for $C_{22}H_{43}O_4P$ = 402.2894, found 402.2898.

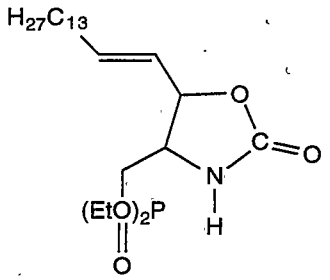


68

(±)-(4E)-Diethyl [2-(N,N'-bis(t-butoxycarbonyl)hydrazido)-3-oxo-octadec-4-enyl]phosphonate 68:

To a solution of $ZnCl_2$ (1.32 mL, 1.0 M, Et_2O) in a flame dried round bottom flask was added dry diethyl ether (4 mL) and solid di-*tert* -butyl azodicarboxylate (76 mg, 0.33 mmol) . The reaction mixture was allowed to stir at rt for 2 h. The reaction mixture was then cooled to -78 °C, and the dienone P(V) **54** (neat, 0.141 g, 0.33 mmol) the final amount in Et_2O (1 mL) rinse was added quickly dropwise via cannula over the reaction mixture at -78° C. The reaction flask was allowed to warm to rt over a period of 12 h with continuous stirring. It was further allowed to stir at rt for another 2 h. The reaction mixture was then cooled down to -40 °C and quenched by pouring into ice-cold saturated NH_4Cl solution (5 mL). This mixture was allowed to stir at -40 °C for 1 h and then slowly warming to rt over 1 h for another hour. It was then extracted with EtOAc (3 x 15 mL). The combined organic phase was dried over $MgSO_4$, filtered and solvent removed in vacuo to yield a crude oil (0.197 g). The product was purified by gradient flash chromatography (40-50 % EtOAc/Hex, R_f = 0.47 in 50 % EtOAc), to give pure product **68** (0.181 g, 87 %) as an oil. ^1H NMR: 7.01 (2H, m), 4.06 (4H, m), 2.61- 2.01 (4H, m), 1.42 (18 H, s), 1.40-1.22 (28 H, m), 0.82 (3H, t, *J* =

6.1 Hz). ^{13}C NMR: 194.2, 154.7, 153.2, 149.1, 126.3, 125.7, 82.0, 80.5, 61.9 (d, J_{P-C} = 6.6 Hz), 32.6, 31.8, 29.6, 29.5, 29.4, 29.3 (d, J_{P-C} = 143.5 Hz), 28.2, 28.0, 27.7, 22.6, 16.3, 14.1. IR (neat, cm^{-1}): 3267, 2853, 1732, 1698, 1633, 1455, 1240, 1025. ^{31}P: 30.3 ppm. HRMS (EI) calcd for $C_{32}H_{61}N_2O_8P$ = 632.4165, found 632.4187.

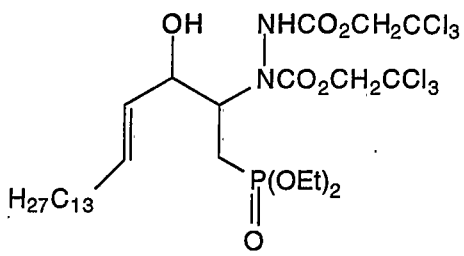


71a,b

(4*S, 5*R**) Diethyl [(5-((E)-pentadec-1-enyl)-2-oxazolidinon-4-yl)methyl]phosphonate (71a), and (4*R**, 5*R**) diethyl [(5-((E)-pentadec-1-enyl)-2-oxazolidinon-4-yl)methyl]phosphonate (71b):**

The oxazolidinones (mixture of isomers **68a** and **68b**) (426 mg, 0.67 mmol) were dissolved in dry HOAc (3 mL) in a flame dried flask, and Zn dust (439 mg, 6.71 mmol) was added to the reaction flask portion-wise over 5 minutes. The reaction mixture was stirred for 4 h and acetone (450 μL, 7.5 mmol) was added via syringe. The reaction mixture was stirred for another 17 h. CH_2Cl_2 (5 mL), was then added, the reaction mixture was filtered through celite, and then was slowly neutralized by addition of ice cold (0 °C) saturated $NaHCO_3$ solution. After reaching neutral pH the reaction mixture was extracted with CH_2Cl_2 (3 x 10mL) and EtOAc (1 x 10 mL). The organic layers were combined together and washed with water (10 mL). The organic extract was then dried over $MgSO_4$,

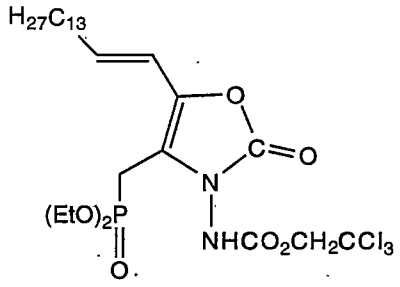
filtered, and solvent was removed in vacuo. Purification via flash chromatography (3 % MeOH/CH_2Cl_2, R_f = 0.38 in 6% MeOH/CH_2Cl_2) yielded the title compounds as a clear oil (**56a:56b** = 7:1) (mixture of **56a** and **56b**), (226 mg, 0.51 mmol, 77%) along with the acetylated product **89a,b** R_f = 0.32, (4% MeOH/CH_2Cl_2) (44 mg, 0.08 mmol, 13%). **56a**: ^{1}H NMR: 5.84 (1H, dt, *J* = 15.3, 6.9 Hz), 5.70 (1H, bs, NH), 5.47 (1H, dd, *J* = 15.5, 7.5 Hz), 4.46 (1H, dt, *J* = 7.4 , 6.8 Hz), 4.10 (4H, m), 3.77 (1H, dm, *J* = 6.8 Hz), 2.06 - 1.89 (4H, m), 1.35 - 1.22 (28H, m), 0.84 (3H, t, *J* = 6.1 Hz). Major Isomer: ^{13}C NMR: 157.7, 138.7, 124.5, 83.9 (d, $J_{P\text{-}C}$ = 20.9 Hz), 62.3, 53.8, 32.1, 31.9, 30.7 (d, $J_{P\text{-}C}$ = 147.1 Hz), 29.6, 29.5, 29.4, 29.3, 29.1, 28.6, 24.7, 22.6, 16.4, 14.1, ^{31}P NMR: 27.1 ppm. **56b**: ^{1}H NMR: 5.84 (1H, dt, *J* = 15.3, 6.9 Hz), 5.70 (1H, bs, NH), 5.47 (1H, dd, *J* = 15.5, 7.6 Hz), 5.01 (1H, dt, *J* = 7.6 , 7.5 Hz), 4.10 (4H, m), 3.26 (1H, dm, *J* = 7.5 Hz), 2.06 - 1.89 (4H, m), 1.35 - 1.22 (28H, m), 0.84 (3H, t, *J* = 6.1 Hz). Minor Isomer: ^{13}C NMR: 157.8, 139.1, 121.8, 83.9 (d, $J_{P\text{-}C}$ = 20.9 Hz), 62.3, 53.8, 32.1, 31.9, 30.7 (d, $J_{P\text{-}C}$ = 147.1 Hz), 29.6, 29.5, 29.4, 29.3, 29.1, 28.6, 24.7, 22.6, 16.4, 14.1. ^{31}P NMR: 28.7 ppm. IR (mixture of diastereomers, **56a,b**, neat, cm^{-1}): 3257, 2924, 2853, 1767, 1466, 1027, 967. HRMS (EI) calculated for (mixture of diastereomers, **56a,b**) $C_{23}H_{44}O_5PN$ = 445.2957, found = 445.2943.



81a,b

(4E)-(2*S, 3*R**) Diethyl [2-(N-N'-bis-(2,2,2-trichloroethoxycarbonyl) hydrazido)-3-hydroxyoctadec-4-enyl]phosphonate (81a), and (2*R**, 3*R**) diethyl [2-(N-N'-bis-(2,2,2-trichloroethoxycarbonyl)hydrazido)-3-hydroxyoctadec-4-enyl]phosphonate (81b):** The dienone-P(V)-BTCEAD condensation product **55** (200 mg, 0.25 mmol) was dissolved in MeOH (10 mL) in a gflame dried rbf. Ceric chloride heptahydrate (46 mg, .125 mmol) was added in one portion. The mixture was allowed to stir for 10 minutes. The reaction mixture was then cooled to 0 °C and a solution of $NaBH_4$ in MeOH (3 mL), (11 mg, 30 mmol) was added via cannula. The icebath was removed, and the reaction mixture was allowed to stir for another 3 h at rt. The reaction mixture was cooled to 0 °C, quenched with satd. aq. ammonium chloride solution (12 mL). The reaction mixture was extracted with EtOAc (3 x15 mL) and the organic layers were combined, dried with $MgSO_4$, filtered and the solvent removed in vacuo. Purification via gradient flash chromatography using MeOH/CH_2Cl_2 (1.5 - 3 % MeOH/CH_2Cl_2) yielded the alcohol **81** (19 mg, 0.025 mmol, 10%) along with **55** (110, 0.17 mmol, 70 %) as (2 :1 ratio) as clear oils. R_f = 0.32 (5 % MeOH/CH_2Cl_2). ^1H NMR: (mixture of isomers **81a,b**): 8.01 (1H, bs, NH), 5.75 (1H, dd, J = 15.3, 7.2 Hz), 5.42 (1H, dt, J = 15.4, 7.3 Hz), 4.75 (4H, m), 4.45 (1H, m), 4.09 - 4.06 (4H, m), 3.76 (1H, m), 3.08- 2.18 (2H, m), 2.03 - 2.01 (2H, m), 1.54 - 1.22 (28H, m), 0.85 (3H, t, J = 6.2 Hz). ^{13}C NMR: (major

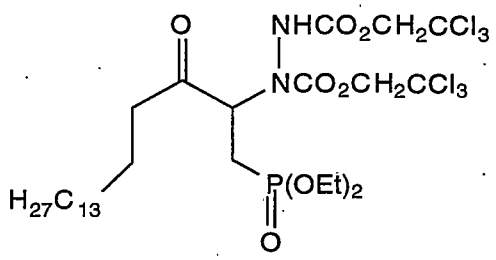
isomer, **81a**): 156.7, 153.8, 136.5, 127.2, 94.6, 75.6, 75.1, 73.5, 62.1 (d, $J_{P\text{-}C}$ = 10.54 Hz), 32.3, 31.8, 29.6, 29.4, 29.3, 29.2, 29.1, 25.1 (d, $J_{P\text{-}C}$ = 143.3 Hz), 23.5, 22.6, 21.6, 16.3, 14.0. ^{13}C NMR: (minor isomer, **81b**): 154.0, 153.6, 136.8, 133.7, 94.5, 75.8, 75.3, 73.8, 62.3 (d, $J_{P\text{-}C}$ = 18.3 Hz), 32.3, 31.8, 29.6, 29.4, 29.3, 29.2, 29.1, 25.1 (d, $J_{P\text{-}C}$ = 143.3 Hz), 23.5, 22.6, 21.6, 16.3, 14.0. ^{31}P NMR (major isomer): 28.2 ppm, minor isomer 29.3 ppm. HRMS (EI) calculated (mixture of isomers) for $C_{28}H_{49}N_2O_8PCl_6$ = 782.1357, found 782.1339.



82

Diethyl [((3-(N'-2,2,2-trichloroethoxy)carbonyl)-amino)-(5-(E)-pentadec-1-enyl)-2-oxazolidinon-4-en-4-yl)methyl]phosphonate (82): The α-β-unsaturated β-keto hydrazide **55** (233 mg, 0.298 mmol) was dissolved in dry THF (10 mL) in a 25 mL flame-dried round bottom flask, cooled to -78°C, and K-selectride (358 µL, 1.0 M in THF) was added drop wise via syringe. The TLC was taken every hour, with no conversion even after 6 h. The reaction mixture was slowly warmed up step by step to 0°C. There was no reaction indicated by TLC until 0 °C. The reaction was very quick at 0 °C, and there was no starting material left after 1 h at 0 °C (TLC). The reaction mixture was then quenched with 30 % H_2O_2 (5 mL) and NaOH (10%, 7 mL), and stirred for 10 h. The reaction mixture was extracted with EtOAc (3 x 15 mL). The

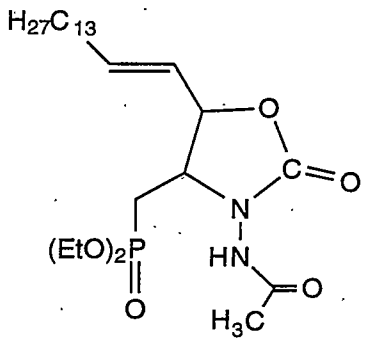
combined organic layers were washed twice with water (20 mL), twice with saturated Na_2SO_3 (2 x 10 mL) to destroy any excess peroxide, and once with saturated NaCl (1 x 10 mL). The organic phase was then dried over $MgSO_4$, filtered, and solvent was removed in vacuo. Flash column chromatography (R_f = 0.34 in 50 % EtOAc/ Hex) yielded **82** as a clear oil (171 mg, 0.27 mmol, 91 %). ^1H NMR: 8.66 (1H, bs, NH), 6.11 (1H, dt, J = 15.7, 6.7 Hz), 5.86 (1H, d, J = 15.7 Hz), 5.10- 4.52 (2H, m), 4.20- 3.89 (4H, m), 2.85 (2H, m), 2.14 (2H, m), 1.38 - 1.10 (28H, m), 0.84 (3H, t, J = 6.3 Hz). ^{13}C NMR: 153.7, 152.2, 134.6 (J_{P-C} = 13.0 Hz), 133.3, 113.2 (J_{P-C} = 14.0 Hz), 112.6 (J_{P-C} = 4.9 Hz), 94.7, 75.1, 63.2, 32.8, 31.8, 29.6, 29.5, 29.4, 29.3, 29.1, 28.9, 27.8, 25.7, 22.6, 21.0 (d, J_{P-C} = 146.1 Hz), 16.4 (J_{P-C} = 5.6 Hz), 14.1. ^{31}P NMR: 22.1 ppm, IR (neat, cm^{-1}) : 3149, 2924, 2853, 1791, 1761, 1096, 1025, 969, 795. HRMS calculated for $C_{26}H_{44}O_7N_2Cl_3P$ = 632.1951, found 632.1971.



85

(±)-Diethyl [2-(N,N'-bis(2,2,2-trichloroethoxycarbonyl)hydrazido)-3-oxo-octadecanyl]phosphonate (85): S-2-methyl CBS oxazaborolidine (1.9 mL, 0.02 M toluene) was transferred into a 25 mL round bottomed flask and $BH_3 \cdot Me_2S$ (143 µL, 1M in THF) was added into the reaction flask via syringe. The reaction mixture was stirred for half hour at rt, cooled down to -20

°C. Into another 25 mL round bottom flask was transferred the dienone-P(V) BTCEAD condensation product **55** (93 mg, 0.119mmol), and dissolved in THF (5 mL). This keto-hydrazide solution was then added via cannula to the cooled solution of CBS-BH_3 complex at -20 °C. The reaction mixture was allowed to stir at -20 °C for 7 h, and then allowed to warm to 0 °C over 1 h. The reaction mixture was then quenched with satd. ammonium chloride solution (10 mL) and extracted with EtOAc (3 x15mL) The organic layers were combined, dried with $MgSO_4$, filtered and the solvent was removed in vacuo. Purification via gradient flash chromatography using 2-5 %MeOH/CH_2Cl_2 yielded **81** (47 mg, 0.07 mmol) and **85** (11 mg, 0.014 mmol) R_f = .45 in 5% MeOH/CH_2Cl_2. ^1H NMR: 5.27 - 4.65 (4H, m), 4.11 - 4.07 (4H, m), 2.96 - 2.85 (1H, m), 2.35 - 2.14 (1H, m), 2.12 - 2.01 (1H, m), 1.55 (2H, m), 1.34 - 1.23 (28H, m), 0.87 - 0.83 (3H, m). ^{13}C NMR: 204.6, 153.9, 129.1, 94.5, 75.8, 75.1, 62.3 (d, J_{P-C} = 18.3 Hz), 39.3, 32.3, 31.9, 29.6, 29.4, 29.3, 29.1, 24.3 (d, J_{P-C} = 141.5 Hz) 22.6, 21.6, 16.3, 14.1. ^{31}P NMR: 29.5 ppm. HRMS (EI) calculated for $C_{28}H_{49}N_2O_8PCl_6$ = 782.1357, found 782.1354.



83a,b

(4*S, 5*R**) Diethyl [(3-acetamido-5((E)-pentadec-1-enyl)-2-oxazolidinon-4-yl)methyl]phosphonate (83a), and (4*R**, 5*R**) diethyl [(3-acetamido-5((E)-pentadec-1-enyl)-2-oxazolidinon-4-yl)methyl]-phosphonate (83b):** During the preparation of **71a,b**, this compound, **83a,b** was isolated as a minor intermediate due to the contamination of HOAc with acetic anhydride. ^{1}H NMR (mixture of isomers, **83a,b**): 8.31 (1H, bs, NH), 5.89 (1H, dt, J = 15.2 Hz, J = 6.62 Hz,), 5.57 (1H, dd, J = 15.2, 7.9 Hz), 4.60 (1H, dt, J = 7.8, 7.2 Hz), 4.48 (dt, J = 7.6, 7.1 Hz), 4.10 (4H, m), 2.07 - 2.02 (4H, m), 2.02 (3H, s), 1.33 - 1.22 (32H, m), 0.84 (3H, t, J = 6.0 Hz). ^{13}C NMR (major isomer, **83a**): 169.1, 155.9, 139.5, 124.5, 80.4 (d, $J_{P\text{-}C}$ = 10.2 Hz), 62.5 (d, $J_{P\text{-}C}$ = 6.2 Hz), 57.7 (d $J_{P\text{-}C}$ = 4.5 Hz), 32,1, 31.8, 29.6, 29.5, 29.4, 29.3, 29.1, 28.5, 27.4, 23.8 (d, $J_{P\text{-}C}$ = 148.2 Hz), 22.6, 20.8, 16.4, 14.1. ^{13}C NMR (minor isomer, **83b**): 169.2, 156.1, 140.1, 121.5, 80.4 (d, $J_{P\text{-}C}$ = 10.2 Hz), 62.1 (d, $J_{P\text{-}C}$ = 6.43 Hz), 57.7 (d, $J_{P\text{-}C}$ = 4.5Hz), 32,1, 31.8, 29.6, 29.5, 29.4, 29.3, 29.1, 28.5, 27.4, 23.8 ($J_{P\text{-}C}$ = 148.2 Hz), 22.6, 20.8, 16.4, 14.1. ^{31}P: major isomer **83a**: 26.8 ppm, minor isomer **83b** 28.1 ppm. IR (neat, cm^{-1}): 3209, 2925, 1785, 1703, 1524, 1368, 1236, 1026, 967. HRMS (mixture of isomers, **83a,b**) calculated for $C_{25}H_{47}N_2O_6P$ = 502.3171, found 502.3177.

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