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:
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 αβ-hydrazido-γ-ketophosphonate in high yields. Upon reduction with NaBH4, 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.

SYNTHETIC STUDIES TOWARD THE PREPARATION OF PHOSPHONATE 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
Bozeman, Montana 59717
April 1997

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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Date

Chairperson, Graduate Committee

Approved for the Major Department

April 4, 1997
Date

Head, Major Department

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To

The Two Who Gave Me Life

And

The One Who Touched My Soul

ACKNOWLEDGEMENTS

I would like to express my sincere thanks and respects to Professor Cynthia K. McClure. Without her encouragements, wisdom, patience and friendship this dissertation would have not been completed. She made my life exciting as well as challenging.

I would also like to take this opportunity to thank my advisory committee, Professor Arnold Craig, Professor Tom Livinghouse, Professor Sam Rogers, Professor Pat Callis, and Professor Warren Jones for their helpful suggestions and ideas.

My special thanks go to my group members, Larry Alegria, Jeff Link, Todd Nichols, Ross Fisher, Andrew Ross, Steve Renner, Todd Madsen, Carrie Blomquist (former group member), and our postdoctoral fellow, Dr. Baozhong Cai. My sincere respect and thanks go to the staff members of our chemistry office: Carol Thurston, Sissi Philips, Sonia Duffie, Kristin Bay, Michelle Atyeo. Dr. Joe Sears and Dr. Scott Busse deserve special thanks for their help in running Mass Spec and NMR experiments.

Dr. Debashis Ganguly deserves special thanks and gratitude for his help in discussions of many problems. My love and respect go to my parents, who are not in this world any more. They waited for this day so long. I wish they were with me today to bless me in person. My love, respect and gratitude for Mr. Bhabani Mishra, Tapan Bhattacharya, Sutapa Mishra and Papia Bhattacharya, who were always in my heart! I am thankful to all my relatives and family members for their support and encouragement throughout my studies. Thank you all!

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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:

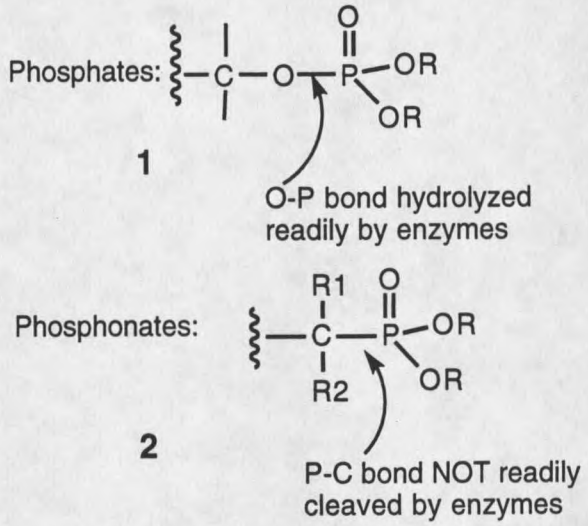
Phosphorus plays one of the most vital and life sustaining roles in all living organisms. Its amazing and diverse behavior is very well displayed by its presence in compounds such as ATP, phospholipids, coenzymes, carbohydrates, proteins and DNA.[1-4] In most of these compounds, phosphorus is present 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.

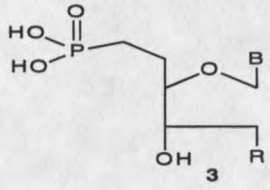
1.3: Phosphonates and their Analogs:

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

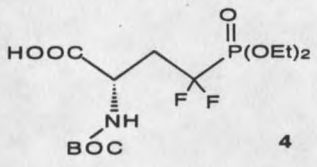


3

Phosphorylated nucleotide

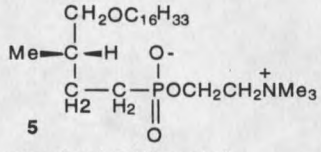
B=cytosine, guanine thymine, uracil

R= H or OH



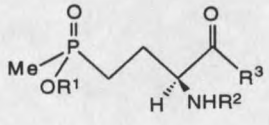
4

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:

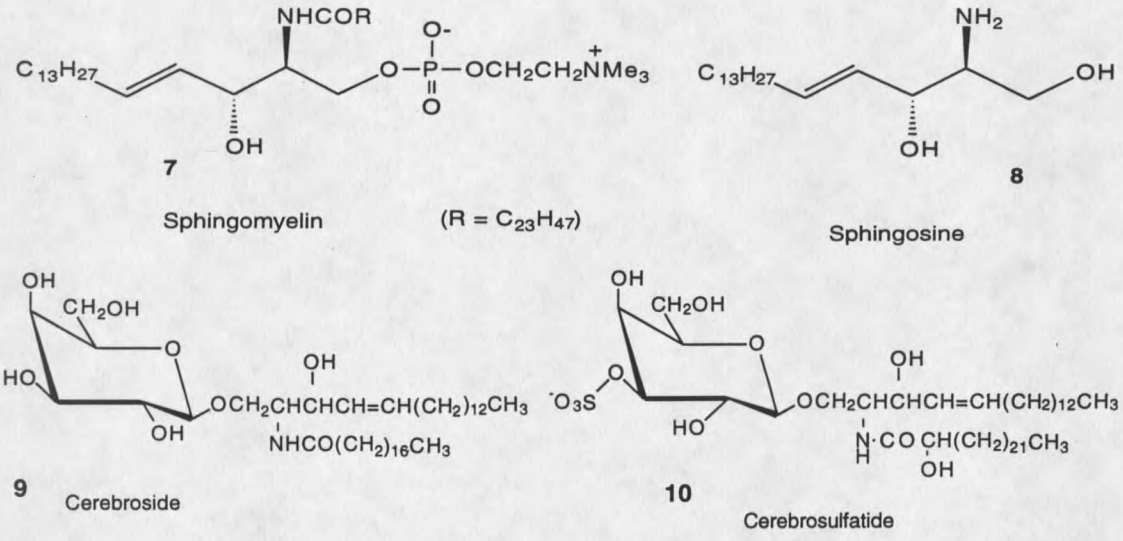
In the human body, there are many organophosphates which are responsible for signal transduction and many other functions. Most of these activities and their biological pathways are still unknown.[2,8] Phosphonate containing analogs of these compounds would be helpful in the search for better understanding 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]