



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

© Copyright by Pranab K Mishra (1997)

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 pentavalent oxaphospholene (derived from methyl vinyl ketone and triethyl phosphite) condensed readily with bis(2,2,2-trichloroethyl) azodicarboxylate to form  $\alpha\beta$ -hydrazido- $\gamma$ -ketophosphonate in high yields. Upon reduction with  $\text{NaBH}_4$ , this  $\beta$ -hydrazido  $\gamma$ -ketophosphonate produced the desired oxazolidinone as a diastereomeric mixture of 3:1. Treatment of the oxazolidinone with  $\text{Zn}/\text{HOAc}/\text{acetone}$  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

D378

M6875

APPROVAL

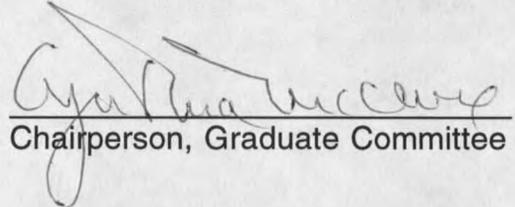
of a thesis submitted by

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.

Date

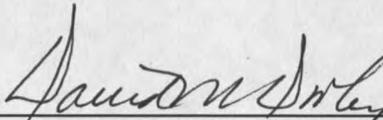
4/4/97

  
Chairperson, Graduate Committee

Approved for the Major Department

Date

April 4, 1997

  
Head, Major Department

Approved for the College of Graduate Studies

Date

4/15/97

  
Graduate Dean

## STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a doctoral degree at Montana State University-Bozeman, I agree that the Library shall make it available to borrowers under rules of the Library. I further agree that copying of this thesis is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U. S. Copyright Law. Requests for extensive copying or reproduction of this thesis should be referred to University Microfilms International, 300 North Zeeb Road, Ann Arbor, Michigan . 48106, to whom I have granted "the exclusive right to reproduce and distribute my dissertation in and from microform along with the non-exclusive right to reproduce and distribute my abstract in any format in whole or in part."

Signature ..... *Pallishma* .....

Date..... *4th April, 1997* .....

iv

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, Sonja 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!

## TABLE OF CONTENTS

INTRODUCTION	1
1.1: Organophosphates and organophosphonates.	1
1.2: The importance of analogs.	2
1.3: Phosphonates and their analogs.	3
1.4: Sphingolipids: organophosphates in animal body with unknown characteristics.	5
1.5: Historical background.	5
1.6: Why membrane sphingolipid analogs?	13
1.7: Conclusions	14
BACKGROUND	16
2.1: The phosphonate analogs of sphingolipids.	16
2.2: Organophosphorus methodology: a model study for the preparation of Sphingosine analogs.	17
2.3: Initial attempts to prepare the $\beta$ -hydrazido- $\gamma$ -keto phosphonate. A model study toward the preparation of sphingolipid analogs.	19
2.4: $\text{Sml}_2$ - Could it induce this N-N bond cleavage?	23
2.5: Conclusions.	25
RESULTS AND DISCUSSIONS PART 1	26
3.1: Search for new electrophilic nitrogen sources.	26
3.2: Attempted condensation of BTCEAD with P(V)	27
3.3: Reduction of the $\beta$ -keto hydrazide. Preparation of oxazolidinone and N-N bond cleavage.	30

3.4: Mechanistic interpretations. Isolation of different intermediates from reduction of N-N bond.	32
3.5: Stereochemical correlation of the oxazolidinone <b>44</b> .	35
3.6: Conclusions.	37
RESULTS AND DISCUSSIONS PART 2	38
4.1: Preparation of the dienone needed for the synthesis of sphingolipid analogs.	38
4.2: Preparation of the desired P(V) from the dienone.	46
4.3: Condensation of the Dienone P(V) with BTCEAD.	47
4.4: Other electrophilic amine sources that were investigated.	55
4.5: Reduction of the $\beta$ -keto hydrazide <b>55</b> .	59
4.6: Cleavage of the N-N bond in the real system; preparation of the final molecule.	67
4.7: Chiral reducing agents.	68
4.8: Future Plans: preparation of the final molecule.	71
EXPERIMENTAL	73
Preparation of ( $\pm$ )-Diethyl 2-(N,N'-bis(2,2,2-trichloroethoxycarbonyl)-hydrazido)-3-oxobutylphosphonate ( <b>41</b> )	75
Preparation of ( $4S^*$ , $5R^*$ ) Diethyl [(3-(N'-(2,2,2-trichloroethoxycarbonyl)-hydrazido)-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>42a</b> ), and ( $4R^*$ , $5R^*$ ) diethyl [(3-(N'-(2,2,2-trichloroethoxycarbonyl)-hydrazido)-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>42b</b> )	76
Preparation of ( $4S^*$ , $5R^*$ ) Diethyl [2-(N-N'-bis-(2,2,2-trichloroethoxycarbonyl)hydrazido)-3-hydroxybutyl]phosphonate ( <b>43a</b> ), and ( $4R^*$ , $5R^*$ ) diethyl [2-(N-N'-bis-(2,2,2-trichloroethoxycarbonyl)hydrazido)-3-hydroxybutyl]phosphonate ( <b>43b</b> )	77
Preparation of ( $4S^*$ , $5R^*$ ) Diethyl [(5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>44a</b> ), and ( $4R^*$ , $5R^*$ ) diethyl [(5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>44b</b> )	78

Preparation of (4 <i>S</i> *, 5 <i>R</i> *) Diethyl [(3-acetamido-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>45a</b> ), and (4 <i>R</i> *, 5 <i>R</i> *) diethyl [(3-acetamido-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>45b</b> )	80
Preparation of (4 <i>S</i> *, 5 <i>R</i> *) Diethyl [(3-amino-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>46a</b> ), and (4 <i>R</i> *, 5 <i>R</i> *) diethyl [(3-amino-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>46b</b> )	81
Preparation of (4 <i>S</i> *, 5 <i>R</i> *) Diethyl [(3-( <i>N</i> '-isopropylideneamino)-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>47a</b> ), and (4 <i>R</i> *, 5 <i>R</i> *) diethyl [(3-( <i>N</i> '-isopropylideneamino)-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>47b</b> )	82
Preparation of (2 <i>S</i> *, 3 <i>R</i> *) Diethyl [2-( <i>N</i> - <i>N</i> '-bis( <i>t</i> -butoxycarbonyl)hydrazido)-3-hydroxybutyl]phosphonate ( <b>48a</b> ), and (2 <i>R</i> *, 3 <i>R</i> *) diethyl [2-( <i>N</i> - <i>N</i> '-bis( <i>t</i> -butoxycarbonyl)hydrazido)-3-hydroxybutyl]phosphonate ( <b>48b</b> )	83
Preparation of (4 <i>S</i> *, 5 <i>R</i> *) Diethyl [(3-( <i>N</i> '-( <i>t</i> -butoxycarbonyl)hydrazido)-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>49a</b> ), and (4 <i>R</i> *, 5 <i>R</i> *) diethyl [(3-( <i>N</i> '-( <i>t</i> -butoxycarbonyl)-hydrazido)-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>49b</b> )	84
Preparation of (4 <i>S</i> *, 5 <i>R</i> *) Diethyl [(3-( <i>N</i> -butoxycarbonyl)-5-methyl-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>50a</b> )	86
Preparation of (4 <i>E</i> )-1,4-octadecen-3-one ( <b>53</b> )	87
Preparation of 2,2,2-triethoxy-2,2-dihydro-5-(( <i>E</i> )-pentadec-1-enyl)-1,2λ <sup>5</sup> -oxaphospholene ( <b>54</b> )	88
Preparation of (±) (4 <i>E</i> )-Diethyl [2-( <i>N</i> , <i>N</i> '-bis(2,2,2-trichloroethoxycarbonyl)hydrazido)-3-oxo-octadecenyl]phosphonate ( <b>55</b> )	89
Preparation of (4 <i>S</i> *, 5 <i>R</i> *) Diethyl [(3-( <i>N</i> '-(2,2,2-trichloroethoxycarbonyl)amino)-5-(( <i>E</i> )-pentadec-1-enyl)-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>56a</b> ), and (4 <i>R</i> *, 5 <i>R</i> *) diethyl [(3-( <i>N</i> '-(2,2,2-trichloroethoxycarbonyl)-amino)-5-(( <i>E</i> )-pentadec-1-enyl)-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>56b</b> )	90
Preparation of (±) (4 <i>E</i> )-3-hydroxy-octadec-1,4-dienol ( <b>64</b> )	92
Preparation of Diisopropyl (2-methyl-3-oxobutyl)phosphonate ( <b>66</b> )	93
Preparation of ( <i>E</i> )-Diethyl (3-oxooctadec-4-enyl)phosphonate ( <b>67</b> )	94

Preparation of ( $\pm$ )-(4E)-Diethyl [2-(N,N'-bis(t-butoxycarbonyl)hydrazido)-3-oxo-octadec-4-enyl]phosphonate ( <b>68</b> )	95
Preparation of (4S*, 5R*) Diethyl [(5-((E)-pentadec-1-enyl)-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>71a</b> ), and (4R*, 5R*) diethyl [(5-((E)-pentadec-1-enyl)-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>71b</b> )	96
Preparation of (4E)-(2S*, 3R*) Diethyl [2-(N,N'-bis-(2,2,2-trichloroethoxycarbonyl)hydrazido)-3-hydroxyoctadec-4-enyl]phosphonate ( <b>81a</b> ), and (2R*, 3R*) diethyl [2-(N,N'-bis-(2,2,2-trichloroethoxycarbonyl)hydrazido)-3-hydroxyoctadec-4-enyl]phosphonate ( <b>81b</b> )	98
Preparation of Diethyl [((3-(N'-2,2,2-trichloroethoxy)carbonyl)-amino)-(5-(E)-pentadec-1-enyl)-2-oxazolidinon-4-en-4-yl)methyl]phosphonate ( <b>82</b> )	99
Preparation of ( $\pm$ )-Diethyl [2-(N,N'-bis(2,2,2-trichloroethoxycarbonyl)hydrazido)-3-oxo-octadecanyl]phosphonate ( <b>85</b> )	100
Preparation of (4S*, 5R*) Diethyl [(3-acetamido-5((E)-pentadec-1-enyl)-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>83a</b> ), and (4R*, 5R*) diethyl [(3-acetamido-5((E)-pentadec-1-enyl)-2-oxazolidinon-4-yl)methyl]phosphonate ( <b>83b</b> )	102
REFERENCES	103

## LIST OF TABLES

Table 3.1	Conditions for condensation of model P(V) with BTCEAD	30
Table 4.1	First trial to condense Dienone-P(V) with BTCEAD	49
Table 4.2	Second trial to condense Dienone-P(V) with BTCEAD	52

## LIST OF SCHEMES

Scheme 2.1	Organophosphorus methodology	18
Scheme 2.2	Use of organophosphorus methodology	19
Scheme 2.3	Condensation of standard oxaphospholene <b>17</b> with DEAD and DBAD	20
Scheme 2.4	Cleavage of the N-N bond in $\beta$ -amino- $\gamma$ -keto hydrazide <b>23</b> or <b>24</b>	21
Scheme 2.5	Retrosynthetic analysis for the sphingolipid analogs	22
Scheme 2.6	Initial attempts for N-N bond cleavage in dissolving metal reduction conditions	23
Scheme 2.7	Mark Burk's report of N-N bond cleavage by $\text{SmI}_2$	24
Scheme 2.8	Attempt to cleave the N-N bond by $\text{SmI}_2$ in protected keto-hydrazide of the DEAD derivative <b>33</b>	25
Scheme 3.1	Leblanc's report of N-N bond cleavage by using $\text{Zn}/\text{HOAc}/\text{acetone}$	27
Scheme 3.2	Low temperature NMR study in condensation of BTCEAD with oxaphospholene <b>17</b>	29
Scheme 3.3	Preparation of oxazolidinone <b>42</b> from condensation product <b>41</b>	31
Scheme 3.4	Cleavage of N-N bond on oxazolidinone <b>42</b>	32
Scheme 3.5	Mechanistic interpretation of the cleavage in the model system	33
Scheme 3.6	Preparation of the t-butyl derivative <b>24</b> and oxazolidinone <b>49a,b</b>	34
Scheme 3.7	Hydrolysis of Boc group in t-butyl-oxazolidinone	35
Scheme 3.8	Attempt for stereochemical correlation with threonine and allo-threonine	36
Scheme 4.1	Synthetic approach towards Sphingolipid analogs	39

Scheme 4.2	Scheme for preparation of dienone <b>53</b> from the acid chloride <b>58</b>	40
Scheme 4.3	Scheme for preparation of dienone from aldehyde <b>57</b>	40
Scheme 4.4	Preparation of aldehyde <b>57</b> using Schollkopf's procedure	42
Scheme 4.5	Scheme for preparation of $\alpha,\beta$ -unsaturated acid	43
Scheme 4.6	Preparation of $\alpha,\beta$ -unsaturated acid	44
Scheme 4.7	Preparation of $\alpha,\beta$ -unsaturated ester <b>63</b>	45
Scheme 4.8	Reaction of aldehyde <b>57</b> with vinyl Grignard followed by oxidation	46
Scheme 4.9	Preparation of BTCEAD-dienone P(V) condensation product <b>55</b>	50
Scheme 4.10	Alkylation $\beta$ to the phosphonate using LDA in standard hydrolysis product <b>65</b>	54
Scheme 4.11	Alkylation $\beta$ to the phosphonate in dienone-P(V) hydrolysis product <b>67</b>	55
Scheme 4.12	Condensation of dienone P(V) with DBAD	56
Scheme 4.13	Oppolzer's methodology	57
Scheme 4.14	Attempt to condense chloro-nitroso reagent with dienone P(V) <b>54</b>	58
Scheme 4.15	Testing the efficacy of Oppolzer's chloro-nitroso reagent <b>72</b>	59
Scheme 4.16	Failure to react standard P(V) <b>17</b> with Oppolzer's reagent	59
Scheme 4.17	Reduction of condensation product <b>55</b> using Luche's conditions	62
Scheme 4.18	Reduction of <b>55</b> by $\text{Zn}(\text{BH}_4)_2$	63
Scheme 4.19	Reduction of <b>55</b> by $\text{LiNH}_3\text{B}(\text{iPr})_2$	63
Scheme 4.20	Reduction of <b>55</b> using K- and L-Selectride	64
Scheme 4.21	Effect of LDA on the condensation product	65

Scheme 4.22	Reduction of <b>55</b> by using alkoxyborohydride	65
Scheme 4.23	Reduction of <b>55</b> by using Sucrose/NaBH <sub>4</sub>	66
Scheme 4.24	Cleavage of N-N bond in the mixture of oxazolidinone <b>56a,b</b>	68
Scheme 4.25	Reduction of <b>55</b> by (S)-CBS reagent	69
Scheme 4.26	Reduction by (S)-CBS reagent to prepare the trans isomer <b>56a</b>	70
Scheme 4.27	Reduction by (R)-CBS reagent to prepare the cis isomer <b>56b</b>	71
Scheme 4.28	Preparation of chiral P(V) <b>87</b> with dienone <b>53</b>	72
Scheme 4.29	Final steps toward the sphingolipid analogs	72

## LIST OF FIGURES

Figure 1.1	Structure of Phosphates and Phosphonates	2
Figure 1.2	Some representative examples of phosphonate analogs	4
Figure 1.3	Thudichum's initial structures of sphingolipids	6
Figure 1.4	Structures of sphingosine-1-phosphate, sphingomyelins and ceramide-1-phosphate	10
Figure 3.1	NOE studies on compound <b>44a</b> and <b>44b</b>	37
Figure 4.1	Structure of Dienone-P(V)	47
Figure 4.2	Acidity of protons in compound <b>67</b>	55
Figure 4.3	Transition state for Luche reduction on compound <b>80</b>	61

## LIST OF CHARTS

Chart 1: Sphingosine-1-phosphate: major functions	11
Chart 2: Sphingomyelins: major functions	12
Chart 3: Ceramide-1-phosphate: major functions	13

## 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 pentavalent oxaphospholene (derived from methyl vinyl ketone and triethyl phosphite) condensed readily with bis(2,2,2-trichloroethyl) azodicarboxylate to form  $\alpha,\beta$ -hydrazido- $\gamma$ -ketophosphonate in high yields. Upon reduction with  $\text{NaBH}_4$ , this  $\beta$ -hydrazido  $\gamma$ -ketophosphonate produced the desired oxazolidinone as a diastereomeric mixture of 3:1. Treatment of the oxazolidinone with  $\text{Zn}/\text{HOAc}/\text{acetone}$  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:

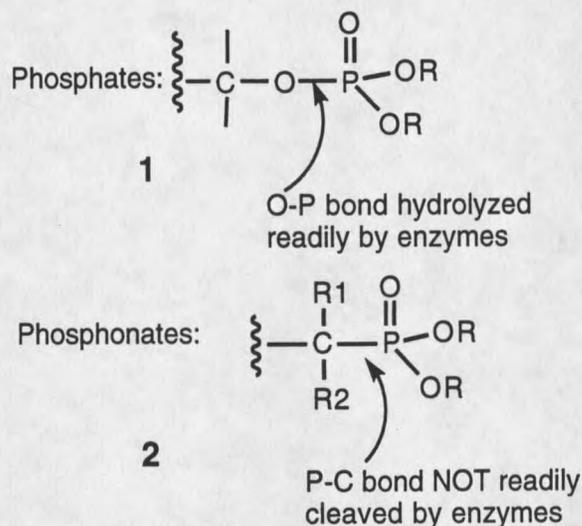
Phosphorus plays one of the most vital and life sustaining roles in all living organisms. Its amazing and diverse behavior is very well displayed by its presence in compounds such as ATP, phospholipids, coenzymes, carbohydrates, proteins and DNA.<sup>1-4</sup> In most of these compounds, phosphorus is present as a phosphate group. The phosphate functionality is known to be the active site in many metabolic processes.<sup>3a</sup> 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,<sup>1d,2a,c</sup> calcification,<sup>5d,e</sup> cell proliferation,<sup>5a-c</sup> etc. However, the exact biological functions of many of these organophosphates are not well understood and are still under critical investigation.<sup>1,2,3</sup> While organophosphates are very abundant in nature, interestingly enough naturally occurring 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.<sup>3,4,6</sup>

**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.<sup>4a,d</sup> Such studies can provide insight into the physiological processes that occur in animal bodies.

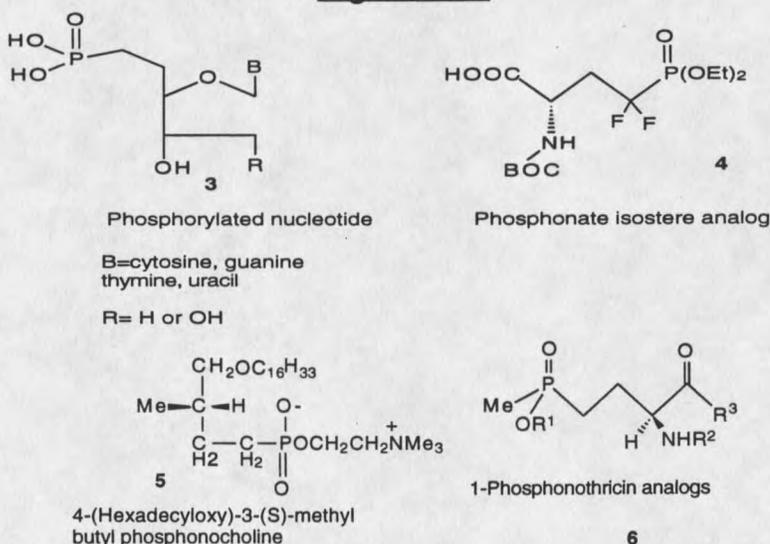
### **1.3: Phosphonates and their Analogs:**

The phosphonic acids and their esters (phosphonates) have been shown to behave as analogs for naturally occurring phosphates in regulating or perturbing metabolic processes.<sup>3,4,6</sup> 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 occurring phosphates.<sup>3a,4,6</sup> 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.<sup>1a</sup>

Phosphonates have been found to be useful for treatment of calcification diseases.<sup>5d,e</sup> They exhibit antiviral, antiHIV, antibiotic, and antiacidosis properties.<sup>6,7a</sup> Phosphonate analogs are also being used as important tools for deducing the mechanism of signal transduction across biomembranes.<sup>2a,c</sup> 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.<sup>7b</sup> 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,<sup>7d</sup> 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.<sup>7d</sup> Compound **4** is a phosphonate isostere which is being used as a substrate for host-cell phosphorylating enzymes.<sup>7c</sup> 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.<sup>7e</sup>

**Figure 1.2**



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.<sup>2,8</sup> 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.<sup>9,10</sup> 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.<sup>3,4,8</sup>

#### **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.<sup>9,10</sup> 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).



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.<sup>9c</sup> 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.<sup>10</sup> 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.<sup>11</sup> 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.<sup>12-17</sup>













































































































































































































