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The synthesis of phosphonate analogues of phosphatidic acid and related compounds

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The Synthesis of Phosphonate Analogues of Phosphatidic Acid and Related Compounds

by Patricia Waters Schwartz

A dissertation submitted to the Graduate Faculty in Biochemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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The City University of New York

Abstract

The Synthesis of Phosphonate Analogues of Phosphatidic Acid and Related Compounds

by Patricia Waters Schwartz

Advisor: Dr. Robert Engel

The main focus of this thesis was the development of phosphonate analogues to be used as probes of metabolic activity and as chemotherapeutic agents. Phosphonates are compounds in which a methylene group has been substituted for the esteratic oxygen in a phosphate ester. The carbon-phosphorus bond of a phosphonate is not hydrolyzed by the enzymes normally involved in cleavage of phosphate esters.

Studies with the phosphonate analogue of glycerol-3-phosphate, 3,4-dihydroxybutylphosphonate, showed profound perturbations of phospholipid metabolism in bacterial cells. In view of these and other studies with phosphonate analogues, the synthesis of phosphonolipids isosteric to phosphatidic acid and phosphatidyl choline were attempted.

For greater solubility in aqueous systems, analogues bearing ten carbon or caproyl functional groups were

synthesized. This was in addition to analogues with the naturally prevalent sixteen carbon or palmitoyl groups.

The synthesis of the following phosphonates were attempted and accomplished: 3,4-dicaproylbutyl-1-phosphonic acid, 3,4-dipalmitoylbut-1-enylphosphonate, and 3,4-dicaproylbut-1-enyl-1-phosphonate.

In studies of enzyme reactions, the loss of the electron pair of the oxygen may have a significant effect on binding of a potential substrate to the catalyst. The preparation of phosphonates containing a hydroxymethylene group or an epoxy function provide an electon rich site adjacent to the phosphorous. They also increase solubility of the analogue with respect to the natural substrate.

3,4-Dipalmitoyloxy-1-hydroxybutyl-1-phosphonate and 3,4-dicaproyloxy-1-hydroxybutyl-1-phosphonate were successfully prepared by hydroboration of the corresponding diisopropyl protected vinylphosphonate and subsequent deprotection. Several approaches for synthesis of the 3,4-dipalmitoyloxy-1,2-epoxybutylphosphonate were undertaken without complete success.

Three phosphonate analogues of phosphatidylcholine were synthesized: 3,4-dicaproylbutyl-1-phosphonylcholine, 3,4-O-isopropylidene-3,4-dihydroxybutyl-1-phosphonylcholine and 3,4-dipalmitoyloxybut-1-enyl-1-phosphonylcholine.

For Steven Fishel Schwartz,

God Only Knows.

Acknowledgements

For my parents, Gloria and Patrick Waters, who have always given me love and support in everything I have done. Thank you for always being there when I need you.

For Robert Engel, my mentor, who has been for me a model of excellence in teaching and research. I thank you for your skill, your patience and your sense of humor.

For George Axelrod. Thank you for your suggestions on everything - teaching, research, and life in general.

For all the members of the Queens College Chemistry Dept., past and present. I thank you all for your friendships, your ideas, and your help.

For Julia C Wegener, always remembered.

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INTRODUCTION

Rationale

Phosphorus is essential for metabolism in all living species. Fundamental life processes, such as reproduction and growth, rely on the important roles of derivatives of orthophosphate in various forms such as nucleotides, phospholipids, and phosphorylated carbohydrates. The search to understand the details of life processes and how these phosphorus-containing compounds function in living organisms has led to the synthesis of various analogues which may function as antimetabolites or biochemical probes.

During the past twenty years there has been increased attention focused on an interesting class of phosphate analogues, specifically, phosphonates and the related phosphonic acids. The introduction of a phosphonic acid linkage in place of a phosphate ester linkage gives the resulting compound potential as a metabolic regulator or probe. Normal phosphate esters are reasonably stable in aqueous solutions at physiological pH and temperature, but are readily cleaved by specific hydrolytic enzymes. The carbon-phosphorus bond in a phosphonate is generally not cleaved by normal enzymes although phosphorus ester linkages that may also be present may be hydrolyzed. (1)

In the design of a potential metabolic probe or regulator, it is desirable that minimal modifications be made from the original parent molecule. In the case of a phosphonate, the substitution of a methylene group for the esteric oxygen in a phosphate ester linkage could cause other perturbations in the molecule.

The first consideration in this design is the relative distances between the phosphorus and other functional groups. Crystallographic data for phosphonates and their corresponding phosphate esters indicate there are only minor overall variations in the distances between the other functional groups in the molecule and the phosphonyl or phosphoryl oxygen. This is in spite of significant differences in the C-O-P and C-C-P bond angles, the O-P-O and C-P-O angles, the C-O and C-C bond distances, and O-P and C-P bond distances. (2-5)

Using circular dichroism techniques (6) in the study of aqueous solutions of dinucleoside phosphates (7) and their phosphonic acid analogues, distinct conformational differences between the natural products and the phosphonates are noted. The esteric oxygen of the phosphate is presumed to interact with the solvent by hydrogen bonding. The methylene group of the phosphonate, in comparison, is thought to be rather hydrophobic. It is also noted that the phosphonic acids have decreased solubilities and poor dispersabilities when compared to the parent phosphates. (8-10)

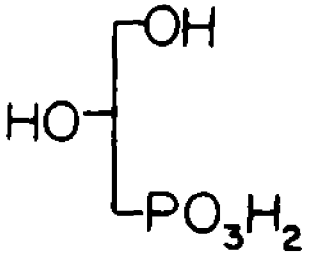
Another factor which should be considered in the use of analogues is the difference in acidities between phosphonic acids and normal phosphate esters. Both classes of compounds are diprotic acids, the first pK_a corresponding to that of a strong acid. However, the second pK_a for the phosphonic acids are generally 0.5 to 1.0 units higher, in the range 7.0 to 8.2. (11-13)

With the substitution of a methylene group for the esteric oxygen, the resulting phosphonate has lost an electron rich site adjacent to the phosphorus, i.e. the two unshared electron pairs of the oxygen. The phosphonic acids may or may not have significant reductions in binding capabilities when compared to the related phosphate esters as a result. This would depend on the the particular structural requirements for the enzymes involved in the biochemical processes under study.

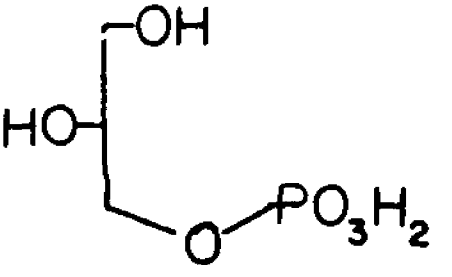
Phosphonate Analogues of Glycolysis Products:

Glycerol-3-phosphate plays an essential role in two major biochemical pathways in cellular metabolism. It is a branchpoint product of glycolysis, and it acts as a precursor of lipid synthesis. As such, it is an interesting target for investigation.

Rosenthal and Geyer (14) were the first to synthesize phosphonic acid analogues of glycerol-3-phosphate with the preparation of the non-isosteric analogue, 2,3-dihydroxypropyl-1-phosphonic acid, (I).

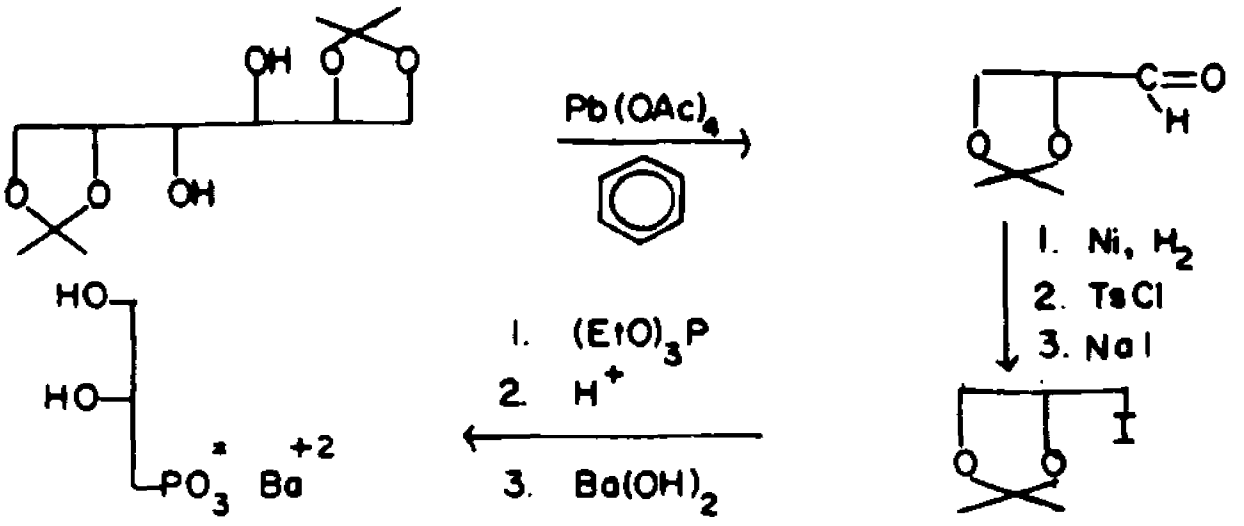


(I)



Glycerol-3-Phosphate

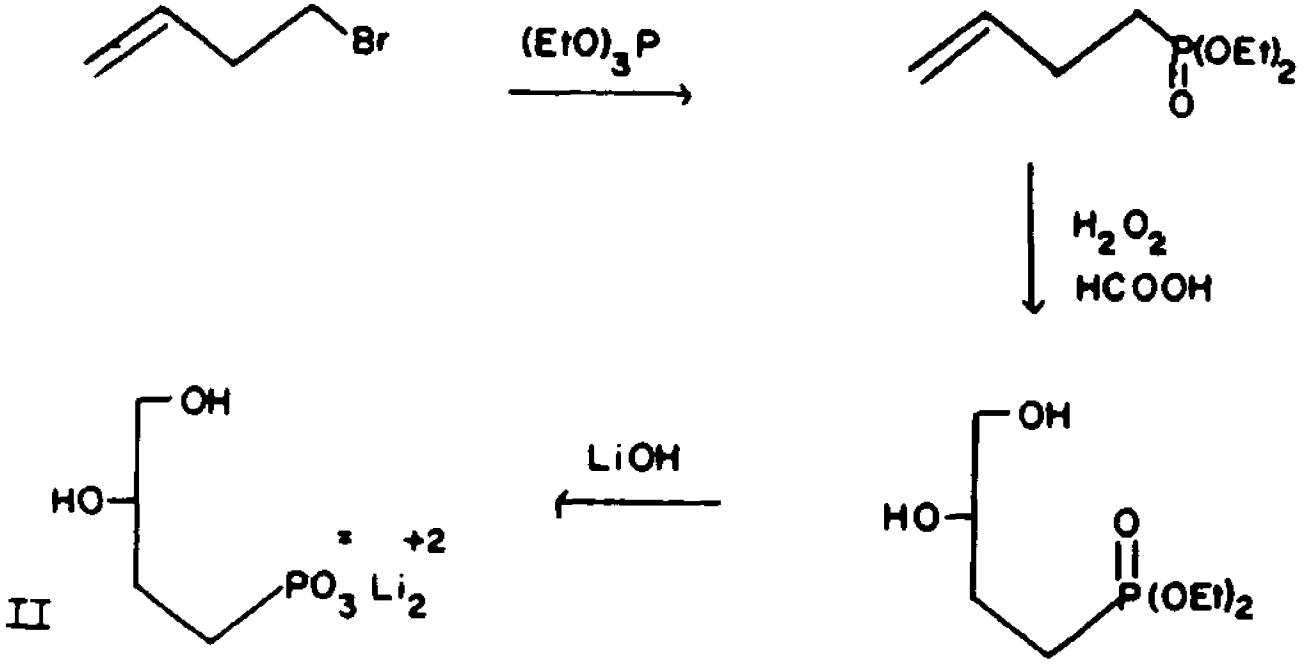
This analogue was prepared initially by an Arbuzov reaction using allyl bromide, followed by hydroxylation and ester hydrolysis. Eleven years later, Baer and Basu (15) reported the synthesis of R-(-)-2,3-dihydroxypropyl-1-phosphonic acid. This compound bears the same absolute configuration about the hydroxyl at the number two position as the natural sn-glycerol-3-phosphate. This synthesis began with 1,2:5,6-di-O-isopropylidene-D-mannitol, followed by cleavage, iodide derivitization at the 3-position, an Arbuzov reaction on the iodide, and ester hydrolysis. (Scheme 1)



I

Scheme 1

The isosteric analogue of glycerol-3-phosphate, 3,4-dihydroxybutyl-1-phosphonic acid, was then synthesized in its racemic form by Kabak, et al. (16) using a route paralleling that of Rosenthal and Geyer (14) and beginning with 4-bromo-1- butene, in an Arbuzov reaction followed by hydroxylation

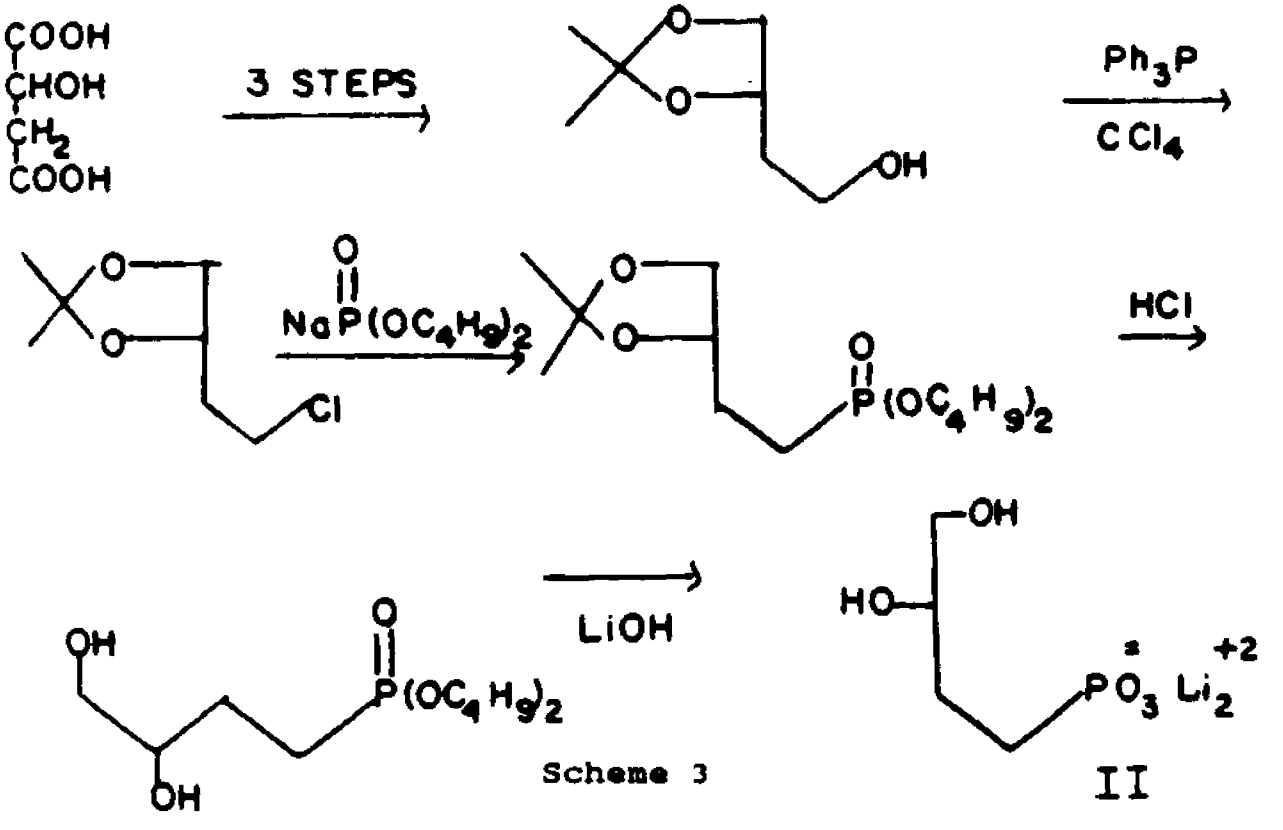


II

Scheme 2

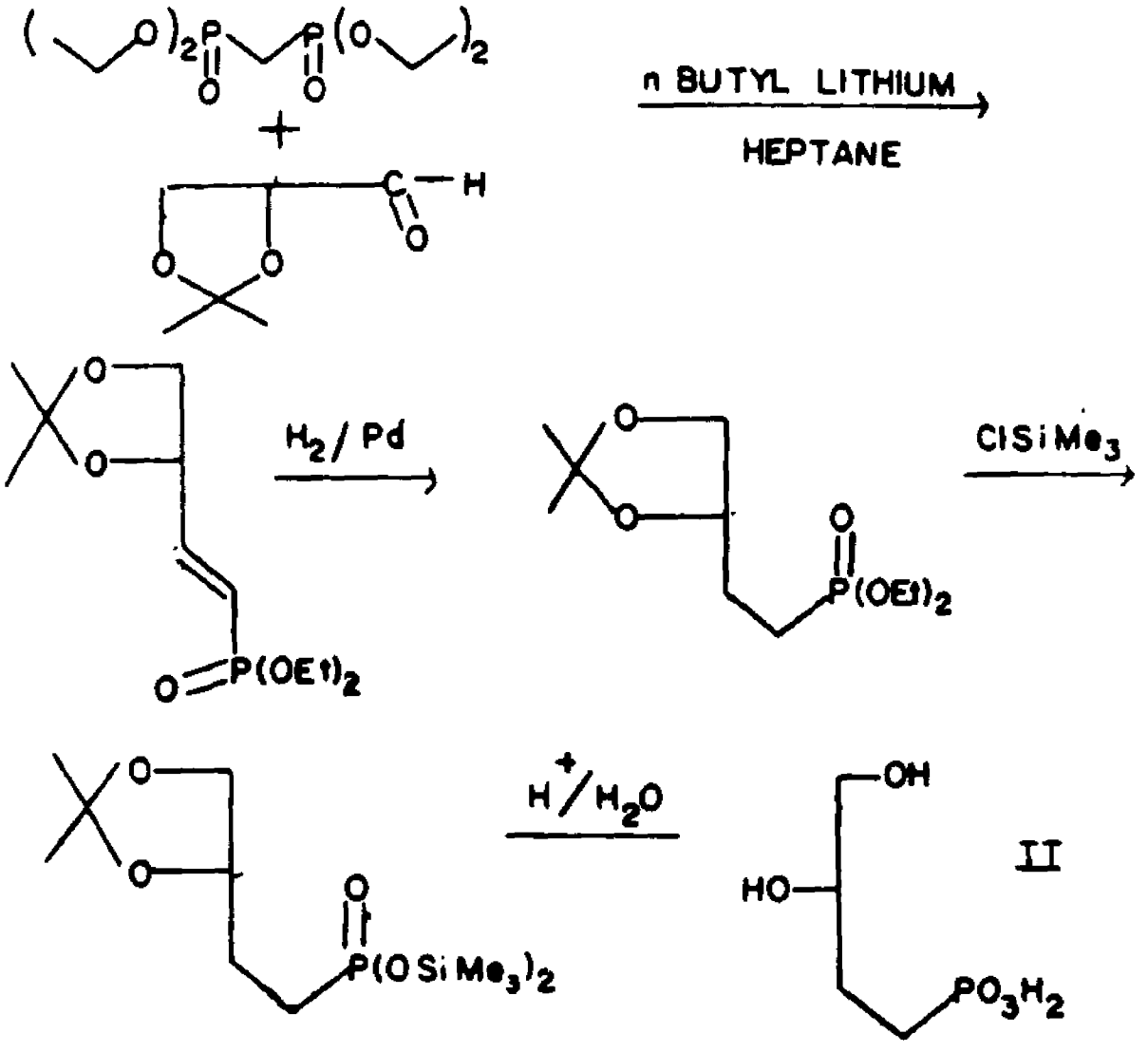
The subsequent synthesis of the optically active form of this analogue, was accomplished in 1974 (17). O-Isopropylidene-D-glyceraldehyde was prepared by cleavage of diacetone-D-mannitol. This was phosphonylated by a Wittig reaction to prepare the vinylic intermediate which was then hydrogenated.

Tang et al.(18) synthesized II starting with l-malic acid. After a three-step conversion to the acetonide, the hydroxyl was converted to a chloride by treatment with triphenylphosphine in carbon tetrachloride. This was phosphonylated <u>via</u> a Becker reaction using dibutyl phosphite and deprotected by acid treatment. The pure compound was isolated as the dilithium salt. (Scheme 3)



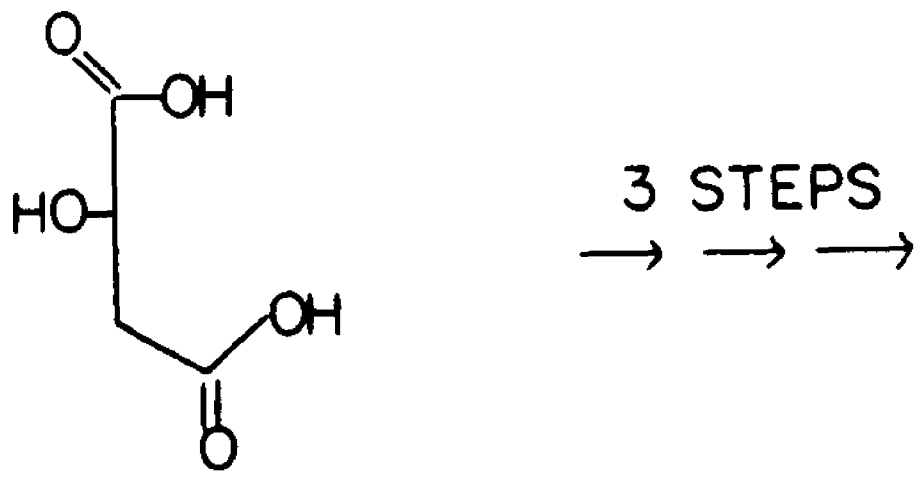
Scheme 3

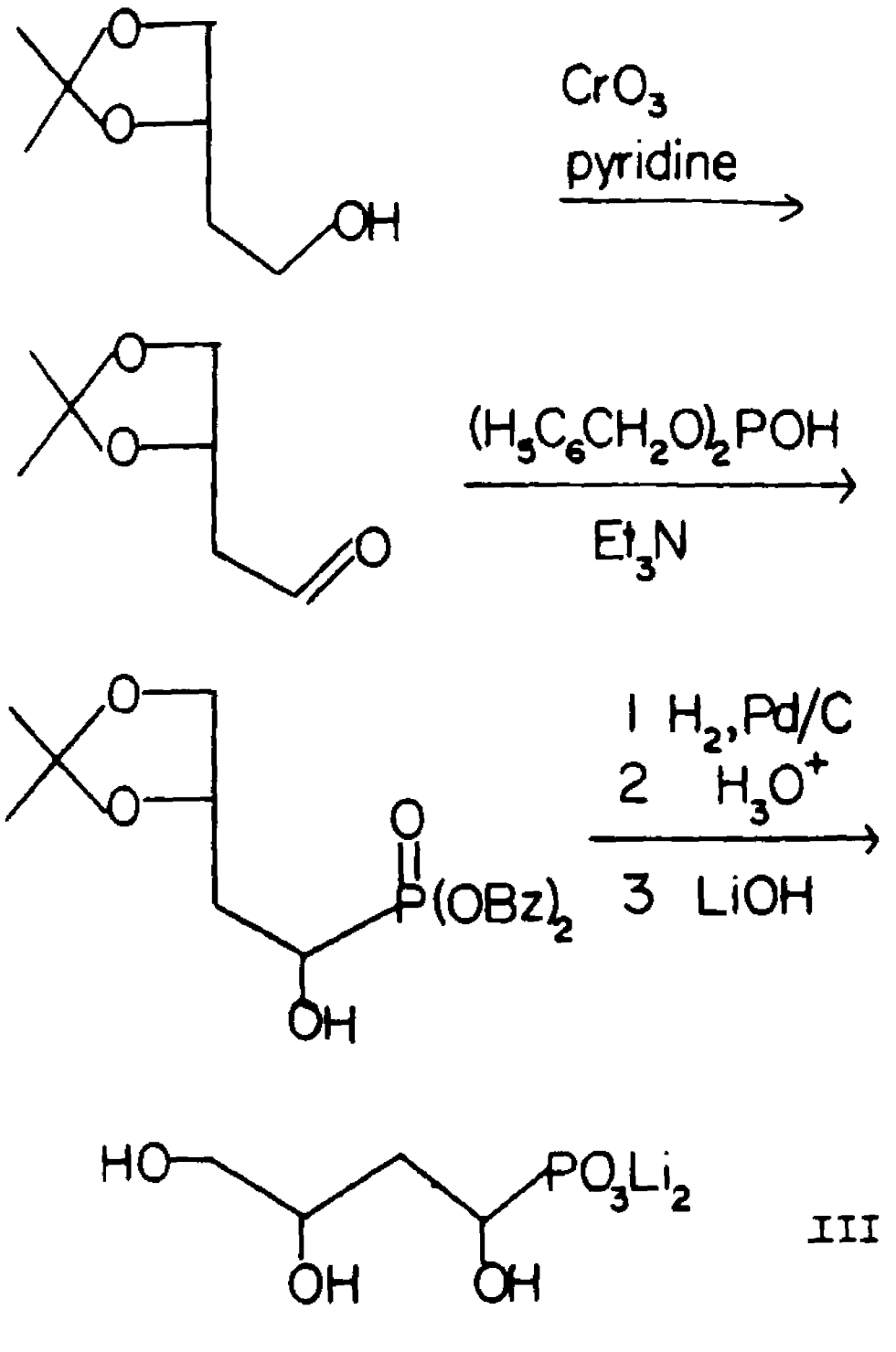
3,4-Dihydroxybutyl-1-phosphonic acid was also prepared by Paulsen and Bartsch (19) using a Horner reaction with tetraethyl methylenebisphosphonate on the D isomer of O-isopropylidene-D-glyceraldehyde followed by reduction and phosphonate ester cleavage. (Scheme 4)



Scheme 4

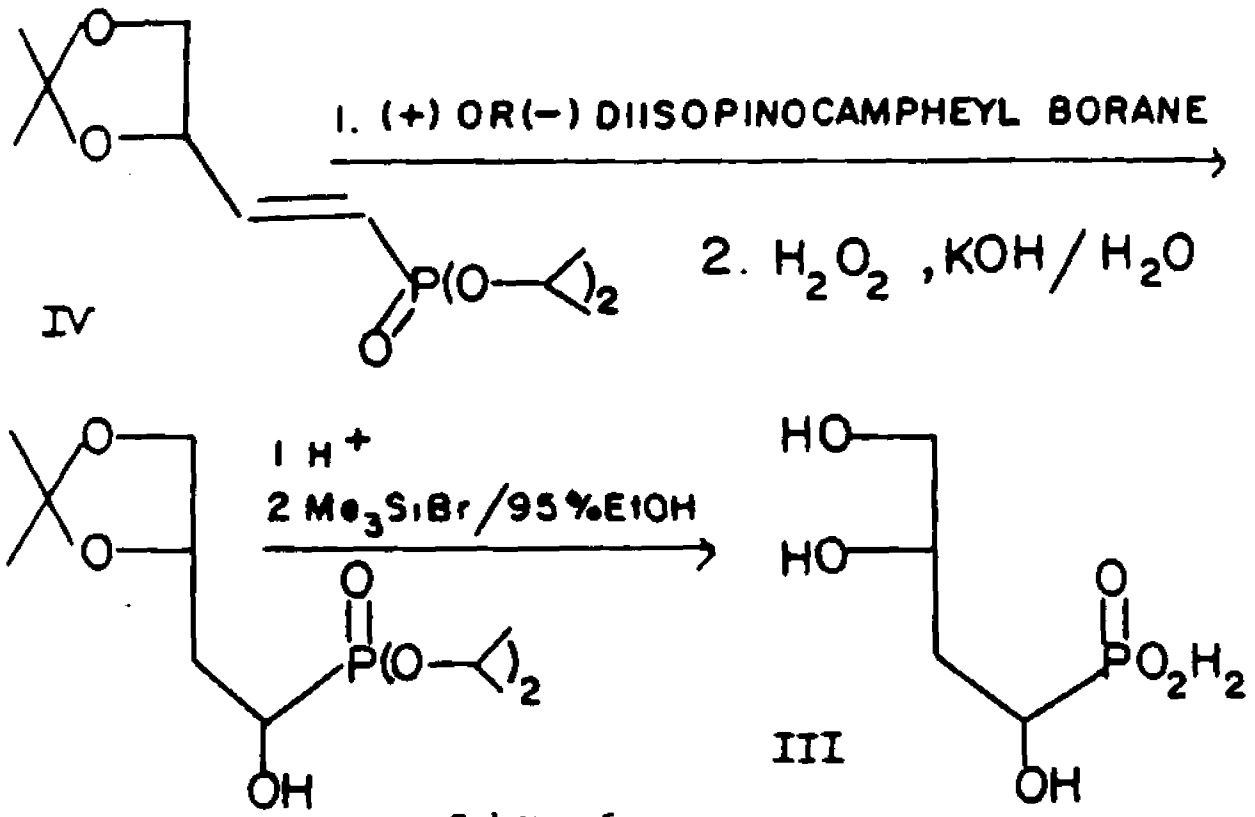
In 1978, Tang et al. (18) reported the synthesis of an hydroxymethylene analogue of glycerol-3-phosphate, 1,3,4-trihydroxybutyl-1-phosphonic acid. (III) This was prepared by a route essentially similar to that used for the preparation of chiral II using optically active malic acid as starting material. Generation of the 1,2-O-isopropylidene-1,2,4-triol was accomplished in three steps. This material was oxidized to the aldehyde with chromium trioxide in pyridine. The phosphonate was generated by treatment of the aldehyde with dibenzyl hydrogen phosphite with triethylamine in benzene. The dilithium salt of III was then prepared by hydrogenation, acid hydrolysis, and finally, treatment with LiOH. The pair of diastereomers formed in the Abramov reaction have been separated partially by column chromatography (21). The synthetic pathway is outlined in Scheme 5.




CrO3
pyridine
OH
(H5C6CH2O)2POH
Et3N
O
1 H2,Pd/C
2 H3O+
3 LiOH
P(OBz)2
HO
PO3Li2
III

Scheme 5

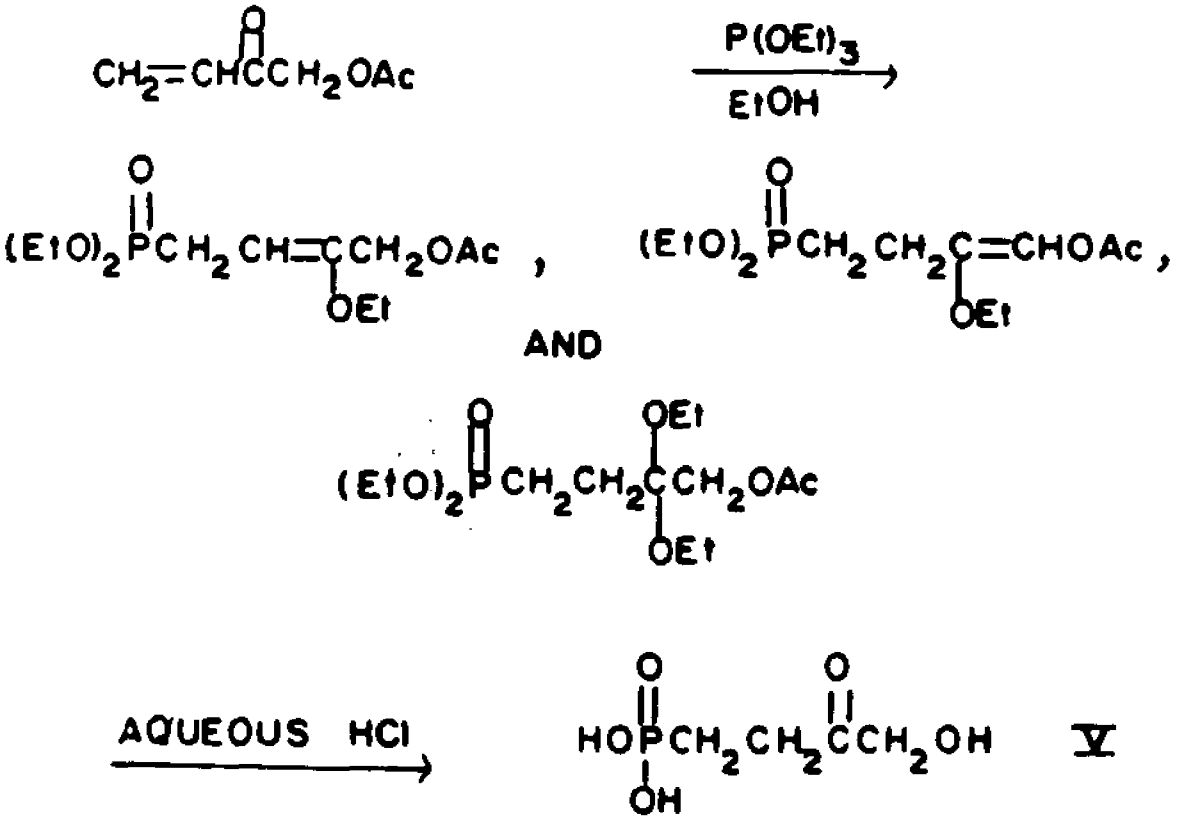
Lalinde et al. (22) synthesized the pair of diastereoisomers of III by a more efficient and stereospecific route. The vinylic phosphonate, IV, (S)-(-)-3,4-O-isopropylidene-3,4-dihydroxybut-1-enyl-phosphonate, is prepared by a Horner type reaction of O-isopropylidene-D-glyceraldehyde on tetraisopropyl methylenebisphosphonate. The phosphonate was treated with either (+) or (-) diisopinocampheylborane, followed by hydrogen peroxide and base to introduce the hydroxl stereospecifically. The acetonide was removed by acid treatment, and the isopropyl esters cleaved using trimethylbromosilane. (Scheme 6)



Scheme 6

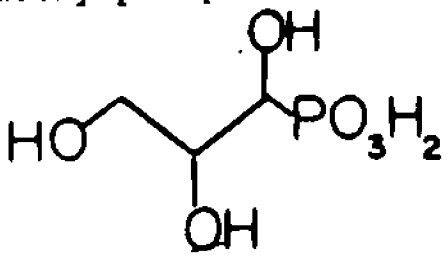
The vinylic phosphonate intermediate in this synthesis can also be reduced. When tritium gas is used, this allows 3,4-dihydroxybutyl-1-phosphonic acid (II) to be tritium labeled at a non-labile position. (vide infra) Compound II is isolated by treatment with tribromosilane followed by heating with LiOH.

In 1974 Goldstein et. al. (20) first prepared an isosteric analogue of dihydroxyacetone phosphate, 4-hydroxy-3-oxobutyl-1-phosphonic acid, V. Acetoxymethyl vinyl ketone was hydrophosphinylated (23) followed by hydrolysis. (Scheme 7) Reduction of the carbonyl with sodium boro[^{3}H]hydride generates compound II with a tritium label at the 3-carbon.

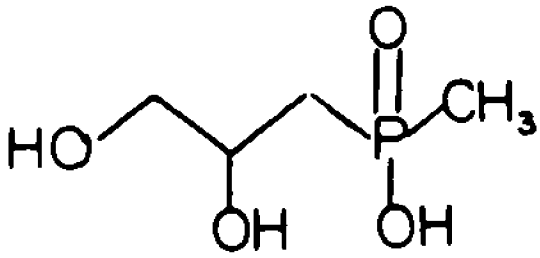


Dixon and Sparkes (24) described the synthesis of compound V in four steps starting with acrylic acid.

Two other non-isosteric analogues of glycerol-3-phosphate have been reported. (17) They are 1,2,3-trihydroxypropyl-1-phosphonic acid, VI, and the related methylphosphinate, VII. Scheme 8.



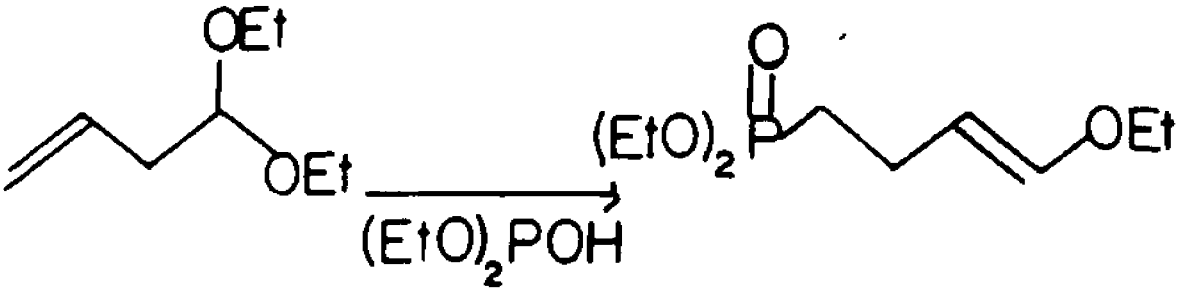
VI

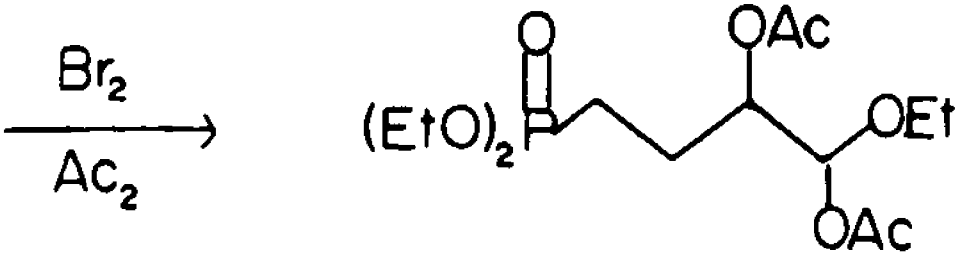


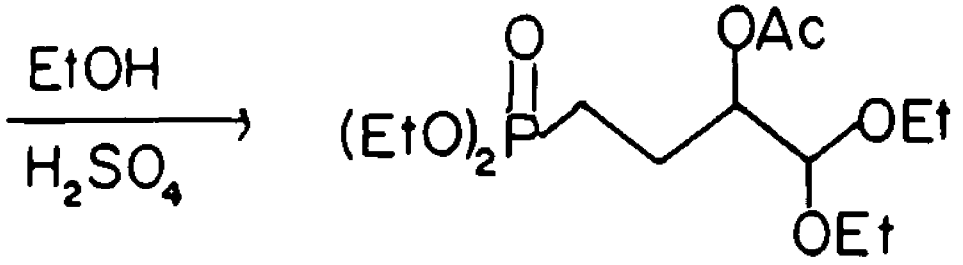
VII

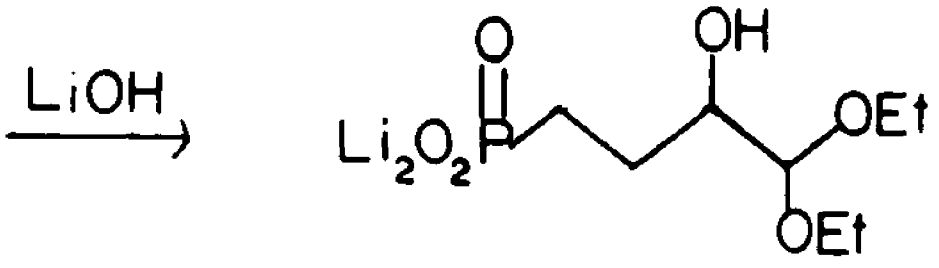
Scheme 8

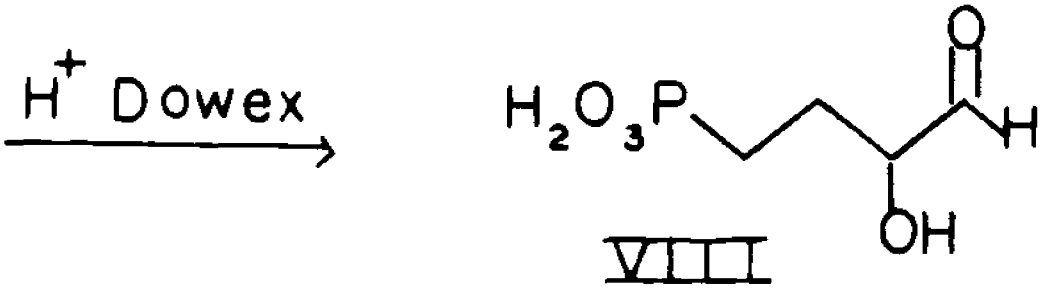
An isosteric analogue of glyceraldehyde-3-phosphate, 3-hydroxy-4-oxobutyl-1-phosphonic acid, VIII, was prepared by Goldstein et. al. (25). The synthesis was accomplished in four steps starting with a free radical addition of diethyl phosphite across the olefinic linkage of but-3-enal diethyl acetal. The diethyl 1-ethoxybut-1-enyl-4-phosphonate was converted to the diacetate by treatment with bromine in acetic anhydride. The diacetate was converted to the corresponding diethyl acetal by treatment with sulfuric acid in ethanol. Lithium hydroxide was used to cleave the phosphous ester linkages. The product was isolated and stored as the diethyl acetal of the dilithium salt. It was converted to the free aldehyde form before use by treatment with Dowex 50 in the H^+ form. (Scheme 9)







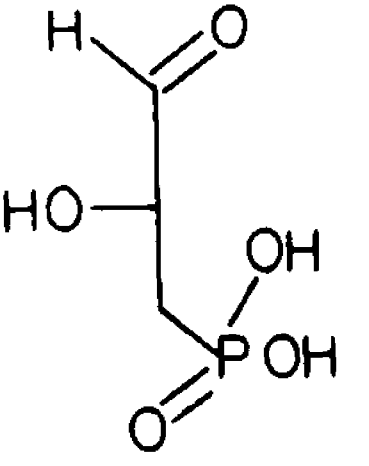




VIII

Scheme 9

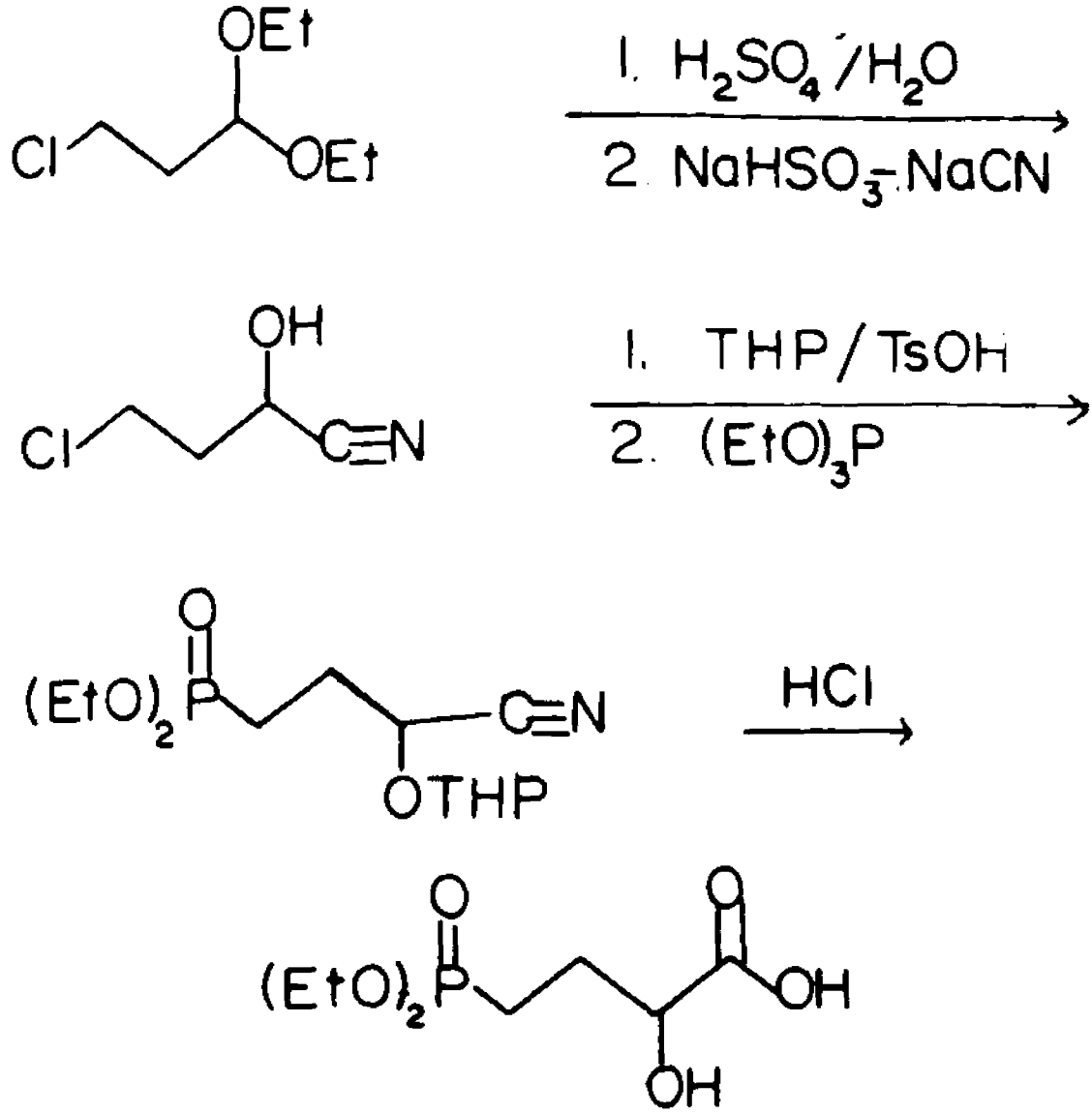
A previous paper by Baer and Robinson (26) had reported a multi-step synthesis of the non-isosteric analogue of glyceraldehyde-3-phosphate, 2-hydroxy-3-oxopropyl-1-phosphonic acid, IX. This synthesis began with DL-glyceraldehyde dimer.



IX

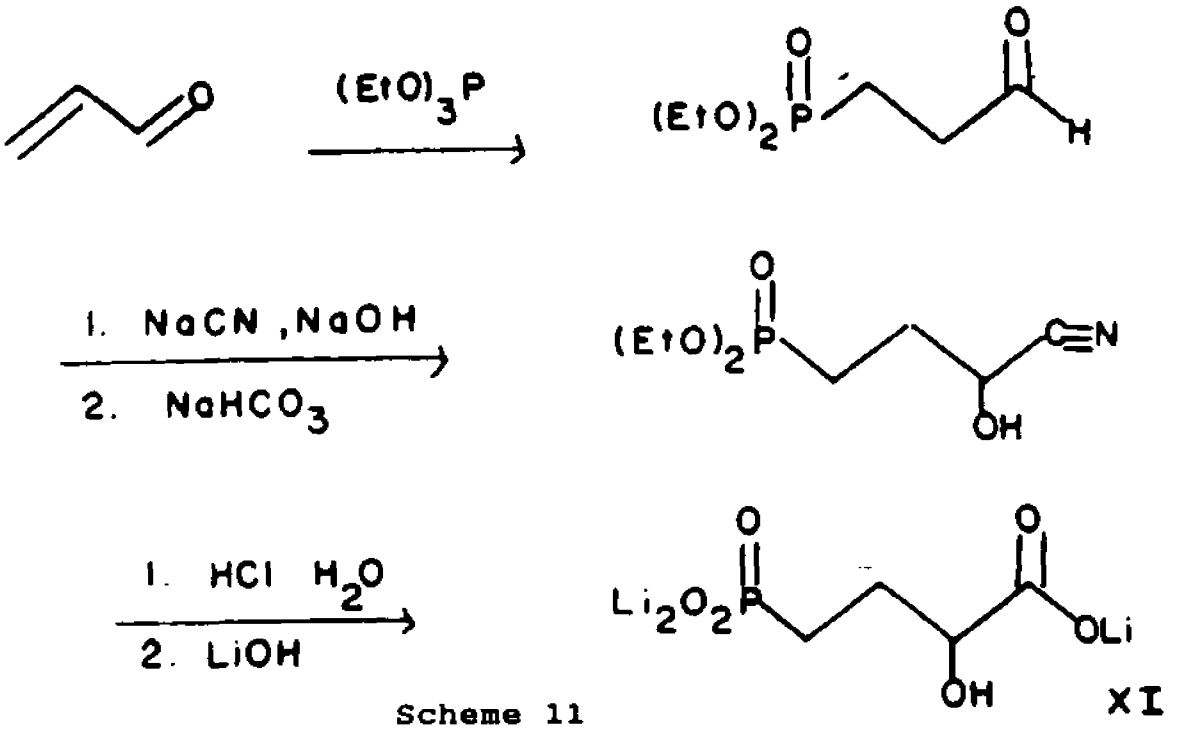
2-Hydroxy-3-Oxopropyl-1-Phosphonic Acid

Several reports of the synthesis of analogues of 3-phosphoglyceric acid have appeared in recent years. Pfeiffer et. al. (27) describe the synthesis of both the non-isosteric analogue, X, 3-carboxy-2-hydroxypropyl-1-phosphonic acid, and the isosteric analogue, XI, 4-carboxy-3-hydroxybutyl-1-phosphonic acid. The synthetic route for synthesis of the isosteric compound began with 3-chloropropionaldehyde diethyl acetal which was deprotected and cyanohydroxylated. This was followed by an Arbuzov reaction using triethyl phosphite to form the C-P bond. The product was isolated by hydrolysis of the corresponding cyanohydrin. (Scheme 10)



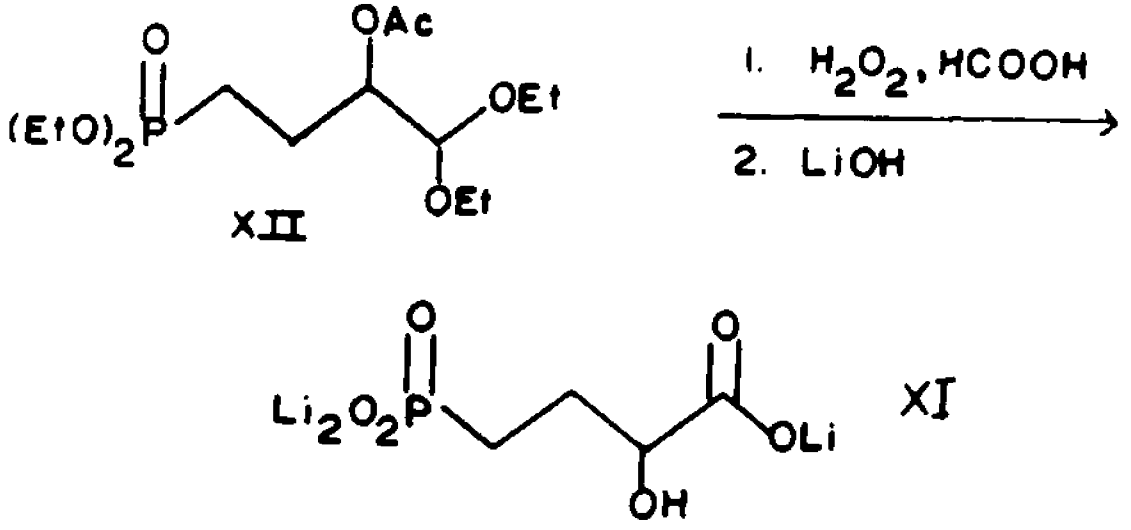
Scheme 10

K. C. Tang, et. al. (18) reported the synthesis of the trilithium salt of XI starting with the reaction of acrolein with triethyl phosphite in a Michael-type addition to form the diethyl acetal. This was a modification of the procedure of Razumov and Moskva. (28) Cyanohydration was the next step, followed by hydrolysis. (Scheme 11)



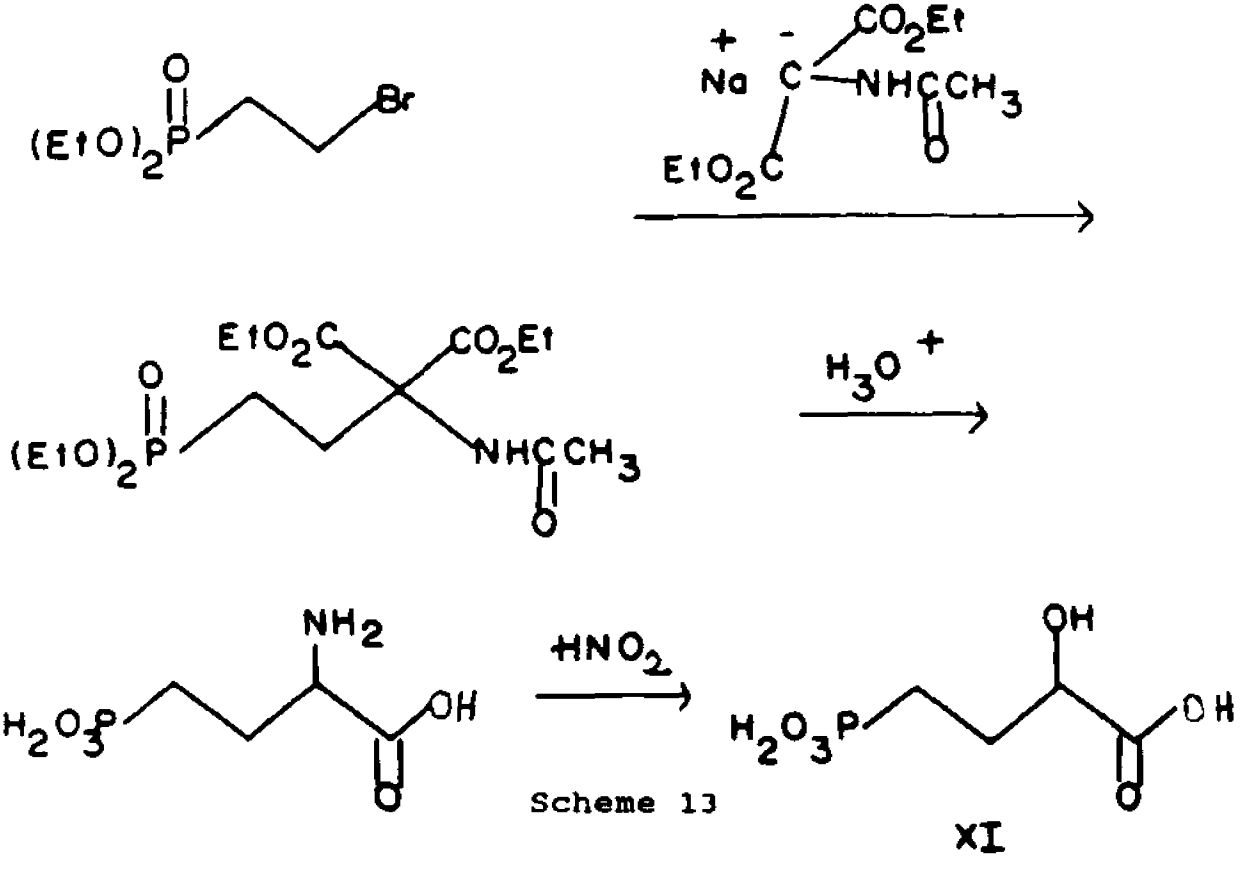
Scheme 11

The trilithium salt of XI was also prepared by Goldstein, et. al. (25) by the oxidation of XIII, an intermediate in the synthesis of 3-hydroxy-4-oxobutyl-1-phosphonic acid. VII. (Scheme 12)



Scheme 12

Dixon and Sparkes (24) reported a route for the preparation of XI starting with diethyl 2-bromoethylphosphonate. (Scheme 13)



Scheme 13

XI

Phosphonate Analogues of Phospholipids

The term phosphonolipid was introduced by Baer to describe analogues of phospholipids containing a C-P bond. Two classes of phosphonolipids will be considered here. The first class is characterized by the glycerol-derived portion possesessing a C-P bond instead of the esteric oxygen. The second type's structural variation is that the C-P bond is

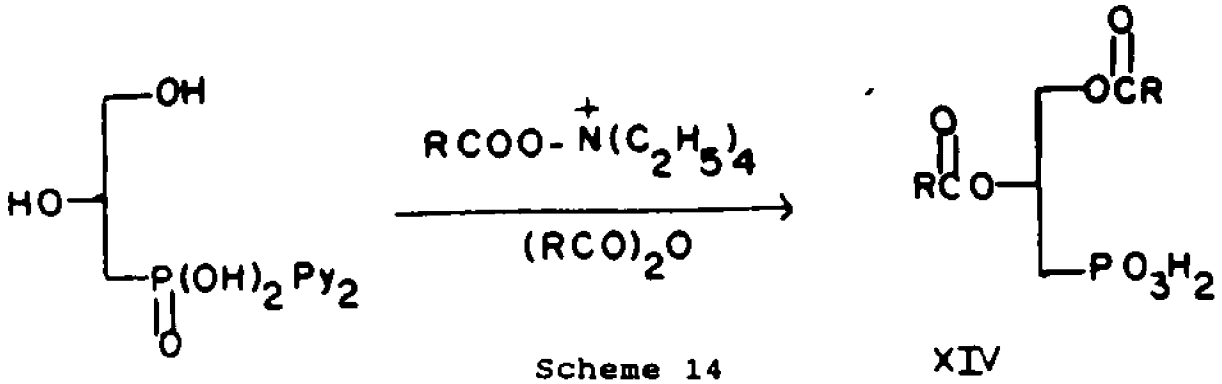
substituted for the esteric oxygen of the head group. This latter class of lipids is related to 2-aminoethylphosphonic acid, XIII, which was found in 1959 to be a naturally occurring component molecule in many species. (29-36)

XIII

2-aminoethylphosphonic acid

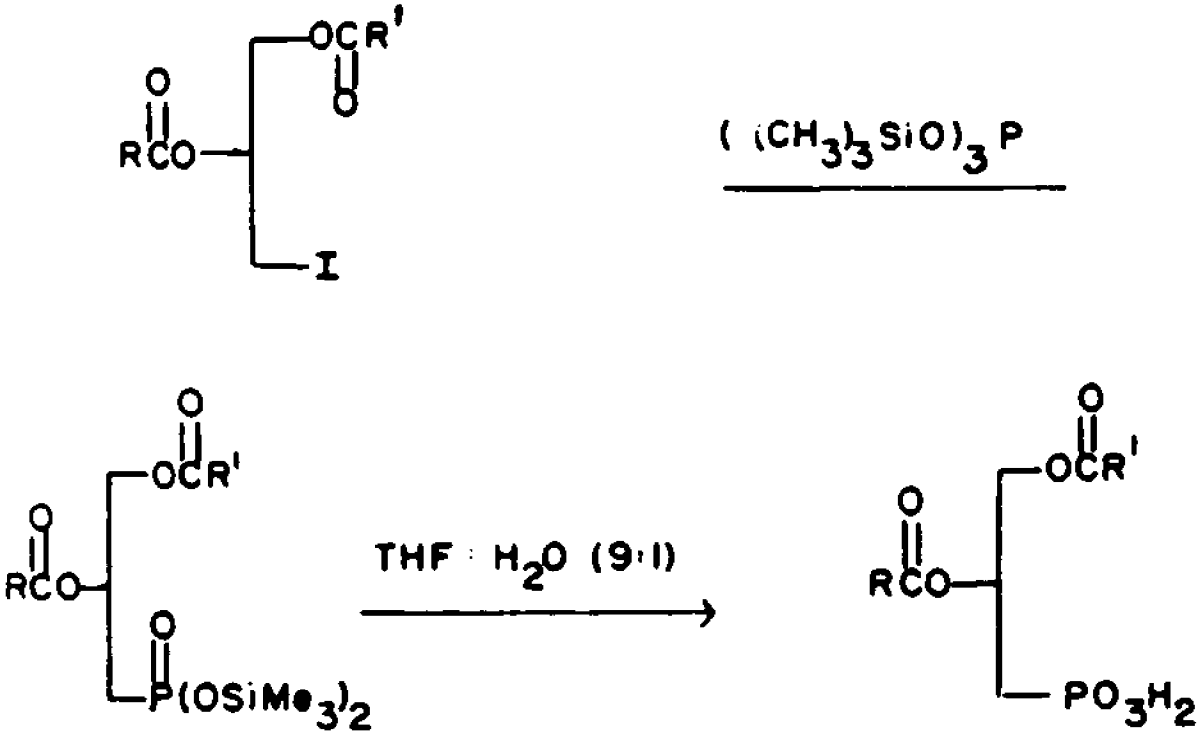
In the first category, the phosphonolipids are derived from analogues of phosphatidic acid and are referred to as phosphotidic acids. Two subcategories of this class may be considered, those which are related to the non-isosteric analogue of glycerol-3-phosphate, 2,3-dihydroxypropyl-1-phosphonic acid, I, and those which are related to the isosteric analogue, 3,4-dihydroxybutyl-1-phosphonic acid, II.

The non-isosteric analogue of phosphatidic acid, XIV, bearing saturated fatty acid functions, was first prepared by Baer and Basu (37) and later by Bonson, et. al. (38). This was synthesized by standard acylation of I. (Scheme 14) Bonson, et. al. (39) also prepared the mono-acyl derivative, XV, by acylating diethyl 2,3-dihydroxypropyl-1-phosphonate.



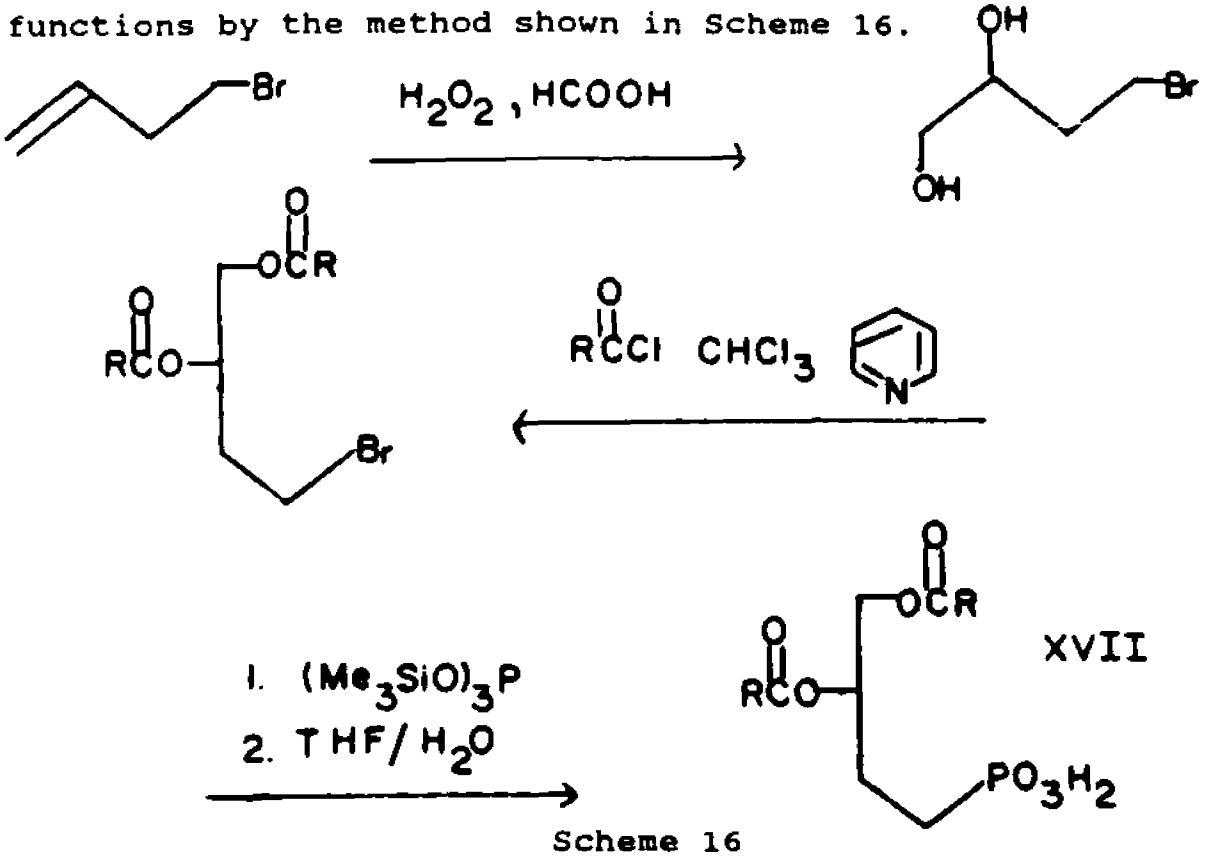
Scheme 14 XIV

Rosenthal, et. al. (40-41) also reported the synthesis of non-isosteric analogues, XVI, of phosphatidic acid of this type using a much more versatile technique. They utilized an Arbuzov reaction of the 2,3-diacyl-1-iodopropanes with tris(trimethylsilyl) phosphite (42-43) followed by mild hydrolysis. (Scheme 15).



Scheme 15 XVI

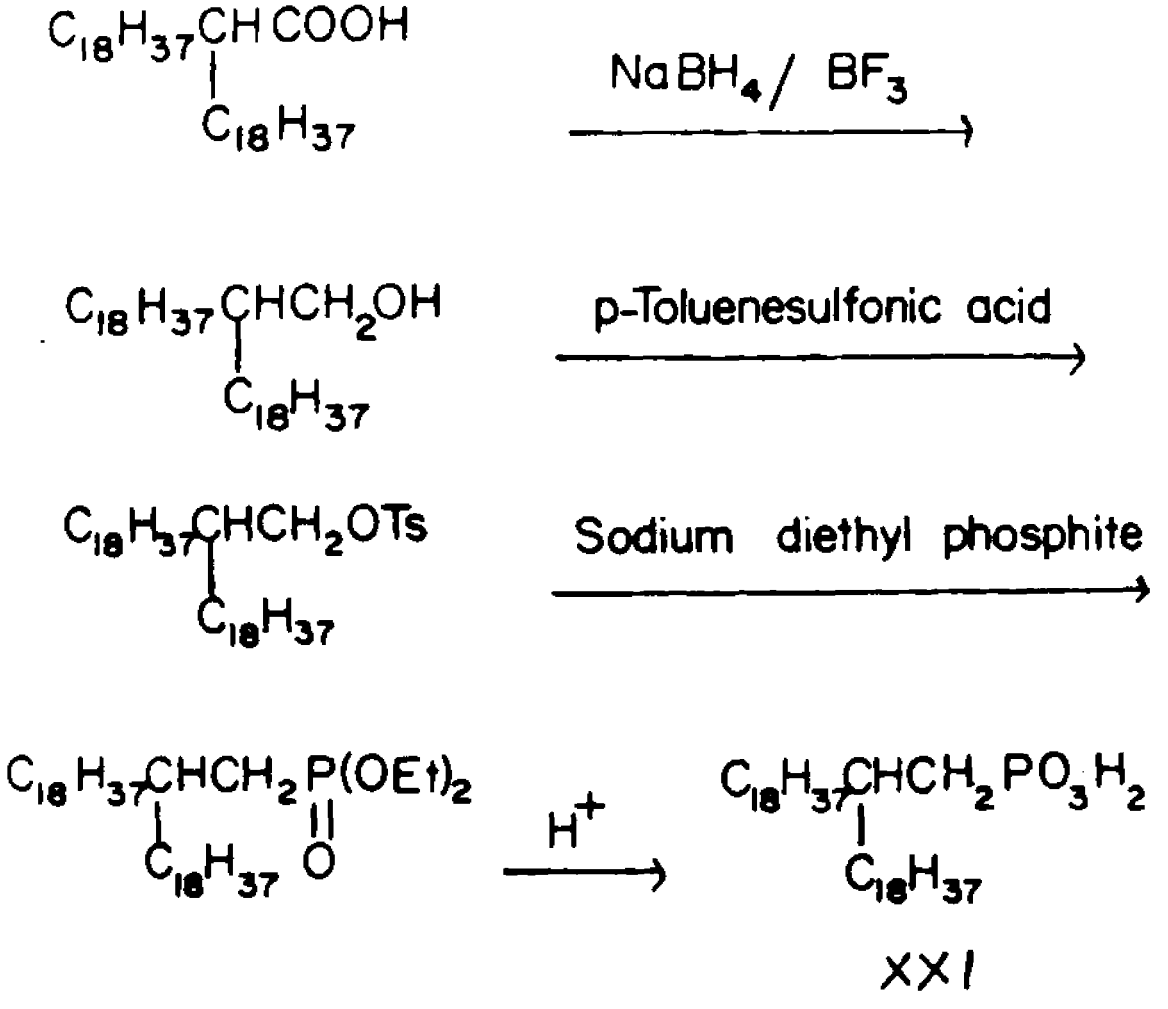
Tang, et. al. (8) prepared the isosteric phosphotidic acids, XVII, with both saturated and unsaturated acyl functions by the method shown in Scheme 16.



Scheme 16

Phosphotidic acids bearing ether linkages instead of the normal fatty acid ester linkages have been prepared by Rosenthal, et. al. (44) Non-isosteric analogues, XVIII, of phosphatidic acid were prepared from the corresponding 2,3-dialkoxy-1-iodopropanes in an Arbuzov reaction. These preparations also included compounds with different alkyl groups sustituted at positions 2 and 3, XIX. The isosteric analogues, XX, were later synthesized by phosphonylation of the corresponding 3,4-dialkoxy-1-bromobutanes. (45)

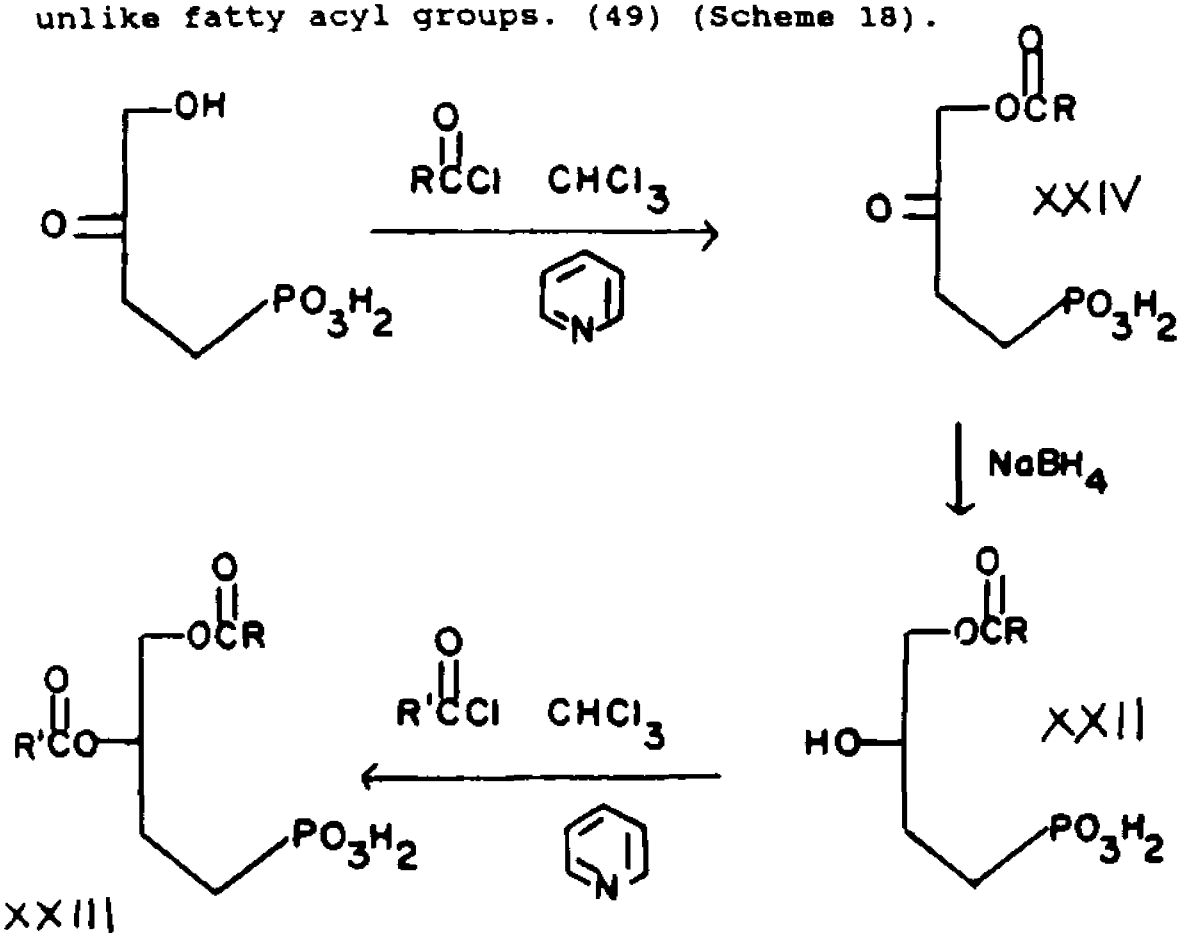
Rosenthal (46) also reported an interesting phosphonic acid, XXI, in which all the nonhydrocarbon functions of the glycerol backbone of the lipid were eliminated. This was a Becker reaction with the alkyl tosylate to give the phosphonate diester followed by acidic hydrolysis to give the free phosphonic acid. (Scheme 17)



XXI

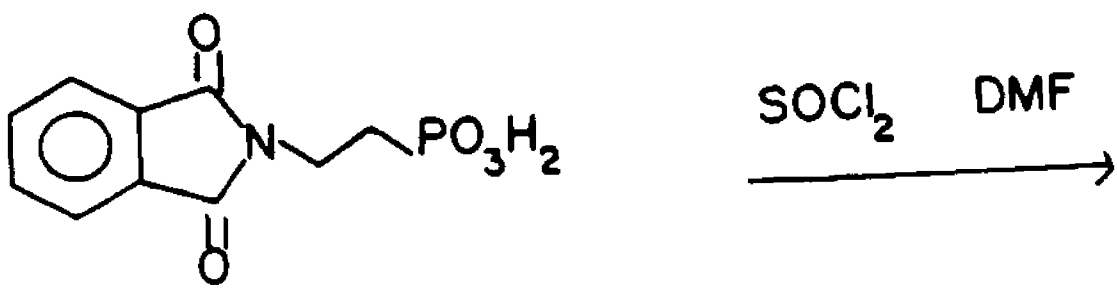
Scheme 17

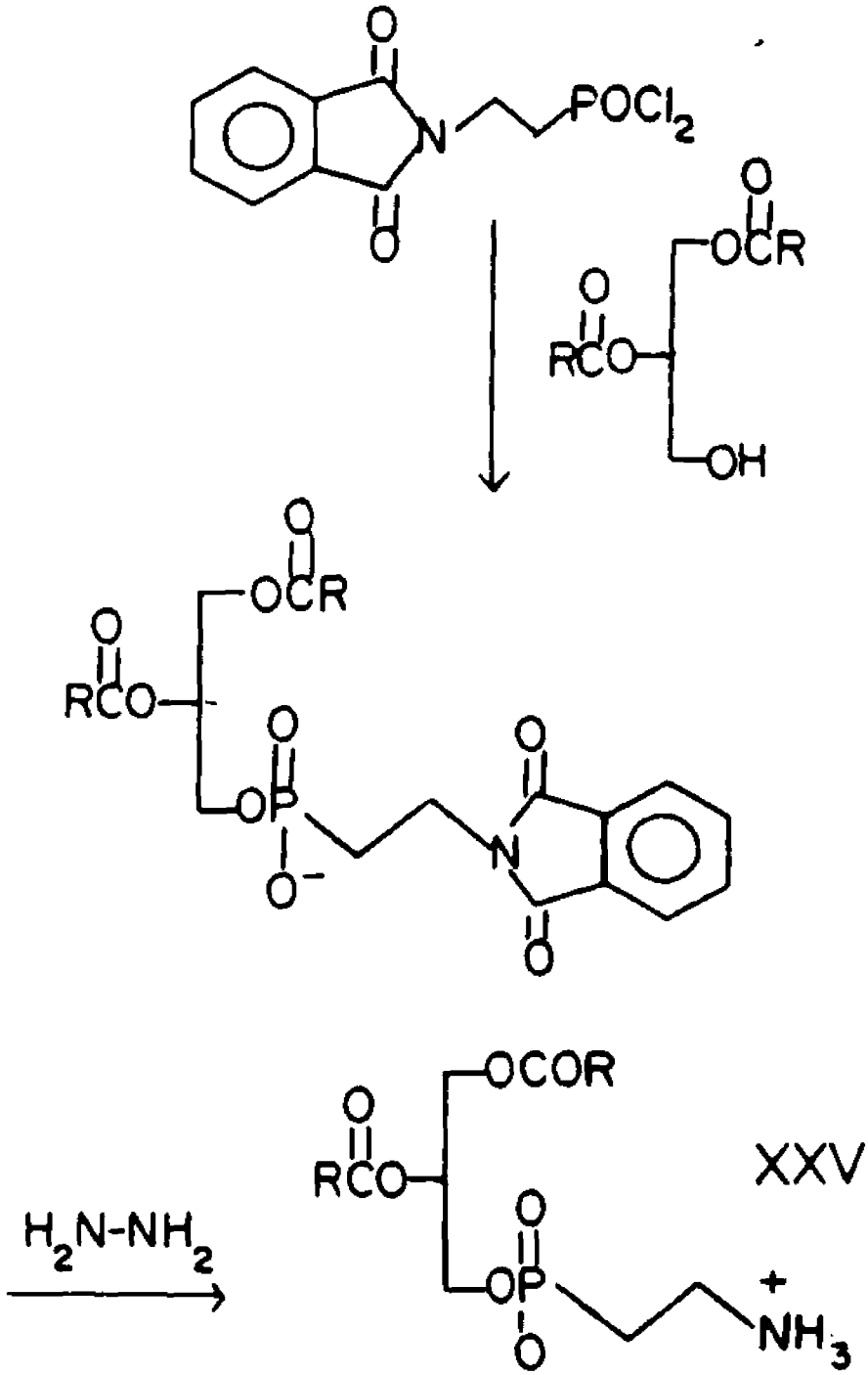
Several other classes of phosphotidic acids have been prepared by Tang, et. al. (8) Compound XXII, an analogue of acyldihydroxyacetone phosphate, was prepared by acylation of 4-hydroxy-3-oxobutyl-1-phosphonic acid, V. This phosphonolipid can be reduced either catalytically or with sodium borohydride (47,48) to generate the lyso-phosphotidic acid, XXIII. The isosteric analogue of lyso-phosphatidic acid so prepared can also be acylated in a further step to yield a phosphotidic acid of the structure, XXIV, with two unlike fatty acyl groups. (49) (Scheme 18).



Derivatives of phosphatidic acids would include such classes as phosphatidyl ethanolamines (cephalins), phosphatidyl cholines (lecithins), phosphatidyl serines, phosphatidyl glycerols, and cytidine diphosphate diglycerides. In these derivatives there are wider possibilities for structural variation. Again, two types of molecules are possible, the first type being those with the C-P bond in place of the glyceric oxygen in the backbone of the lipid, and the second those in which the oxygen of the head group has been eliminated. Much of the attention of research in this area has been focused on the second class of compounds, particularly phosphonolipids which are derivatives of 2-aminoethylphosphonic acid.

Rosenthal and Pousada (49) reported the preparation of dipalmitate and distearate derivatives of compound XXV. The preparation of the analogues requires 2-phthalimidoethylphosphonic acid from which an acid chloride is prepared. Reaction with a 1,2-diglyceride gives the N-phthaloyl ethylphosphonate lipid, which is treated with hydrazine to liberate the amine. (Scheme 19).





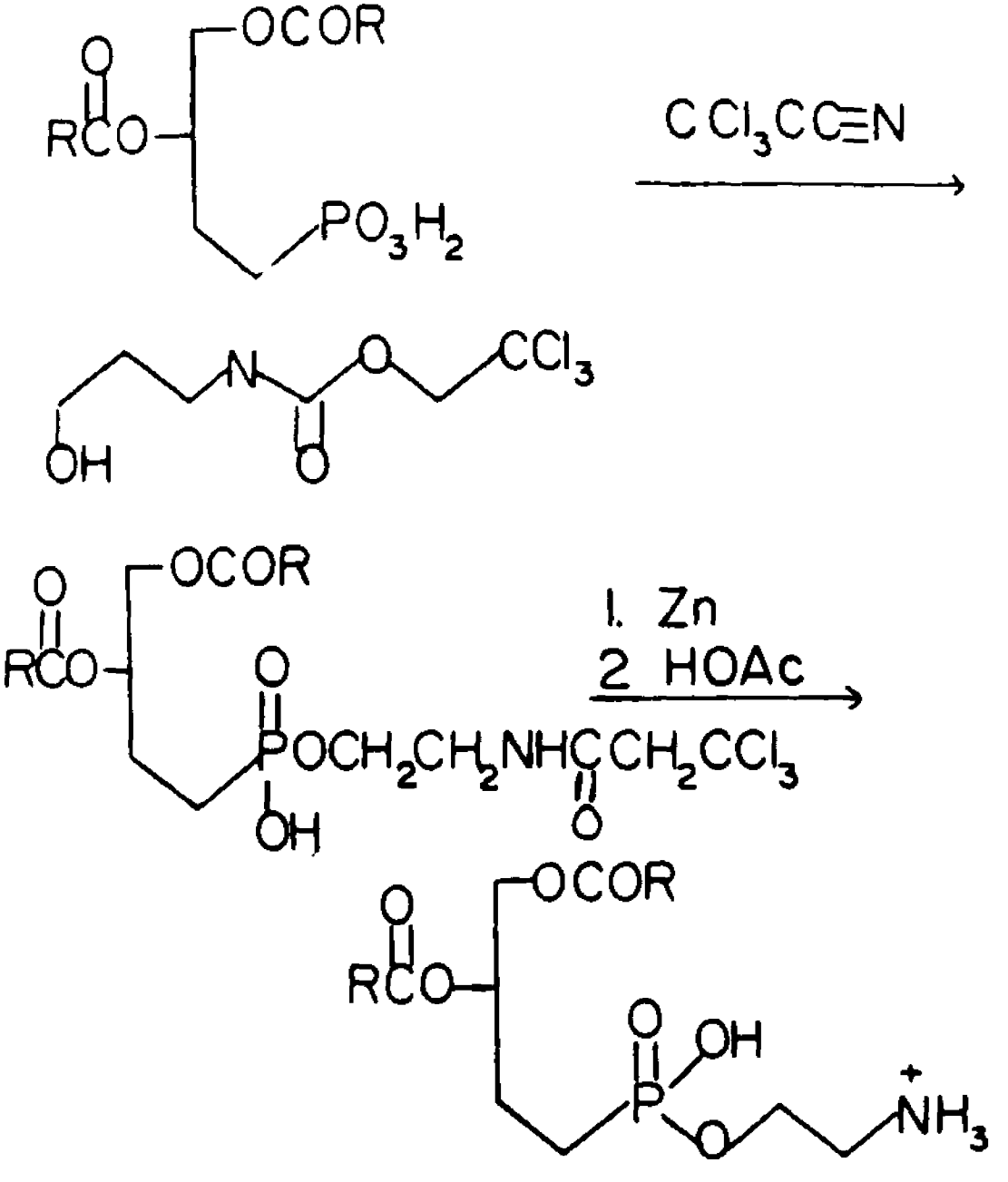
Scheme 19

Almost simultaneously, Baer and Stanacev (50) reported the synthesis of a series of compounds analogous to XXV. At first, the phosphonic monochloride, 2-phthalimidoethylphosphonic acid monochloride in triethylamine followed by hydrazinolysis was used. Subsequent reports by Baer, et. al. (51-3) prepared the analogues by 2-dibenzylaminoethylphosphonic acid with deprotection of the amine function by hydrogenolysis.

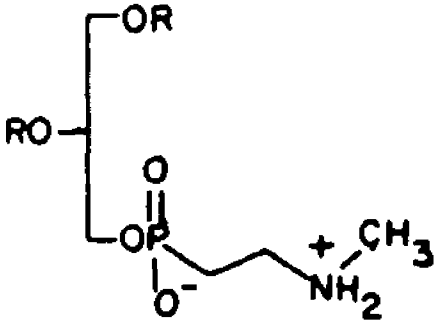
Baer (54) later reported the synthesis of a compound in the series XXV bearing a saturated alkoxy function at the 3 position and an unsaturated function at the 2 position. Pfeiffer, et. al. (55) reported a compound in which the alkyl functions were adamantyl. The diether, XXVI, (56) as well as the ether-ester system, XXVII, (57) have also been synthesized.

Compounds in which the C-P bond is present in the glyceric backbone were reported by Rosenthal (46,49). The nonisosteric diether compounds, XXVIII, were synthesized with similar and dissimilar alkoxy groups by coupling of the corresponding phosphotidic acid with the phthalimide-protected ethanolamine via trichloroacetonitrile. This was followed by deprotection to liberate the free amine.

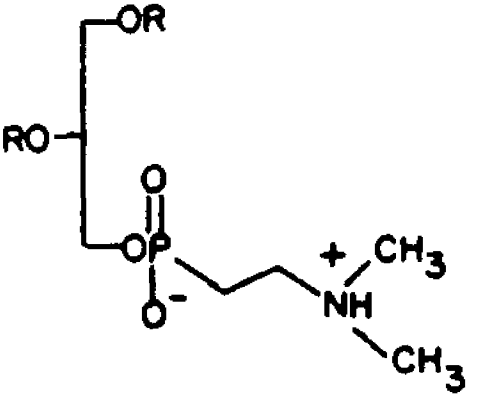
The isosteric dipalmitoyl analogue, XXIX, was prepared by Braksmayer, et. al. (58) by a similar route. (Scheme 20). Pfeiffer, et. al. (59) prepared the nonisosteric shortened acyl species, XXX, with the alkyl group being adamantyl.



Baer and Pavanaram (60) have synthesized N-methyl substituted systems, XXXI, by a route utilizing phosphonic monochlorides to accomplish ester formation. Baer and Rao (61) reported the synthesis of N,N-dimethylaminoethylphosphonate with two ether functions on the glycerol backbone, XXXII. This was synthesized by reaction of dimethylamine with the 2-bromoethyl phosphonate.

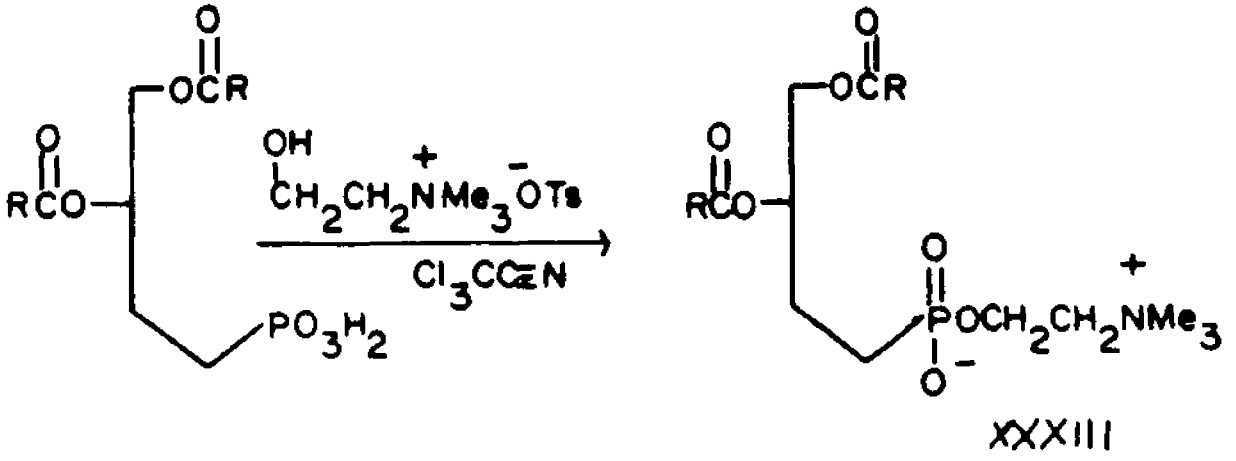


XXXI



XXXIi

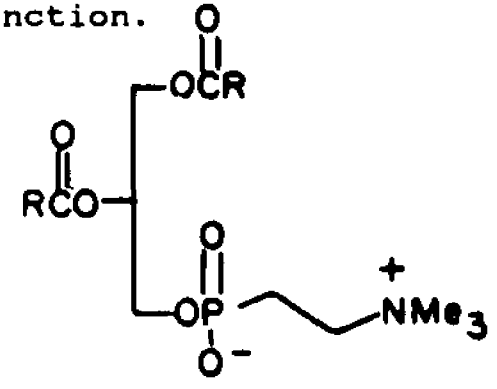
The lecithins, phosphatidyl cholines, present the same possibilities for structural variations as seen in the cephalins. The nonisosteric analogues of phosphatidyl choline with the oxygen eliminated from the glycerol backbone were synthesized with diether linkages (46, 62) or with diesters (40, 41). The phosphotidic acid is coupled with the choline moiety using trichloroacetonitrile or an acid chloride. 3,4-Dipalmitoyloxybutylphosphonylcholine, the isosteric analogue XXXIII, was reported by Braksmayer, et. al. (58). (Scheme 21).



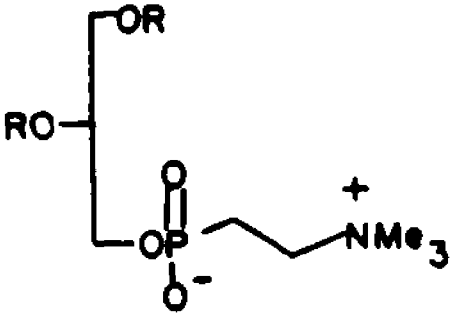
XXXIII

Scheme 21

Chiral compounds of structures XXXIV and XXXV in which the oxygen has been eliminated from the choline group have been reported by Baer, et. al. (63-67). All of these preparations proceeded through a 2-bromoethylphosphonate species followed by reaction with trimethylamine to introduce the ammonium function.

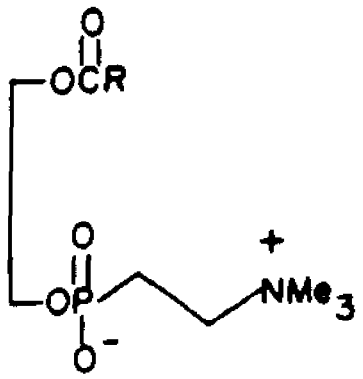


XXXIV

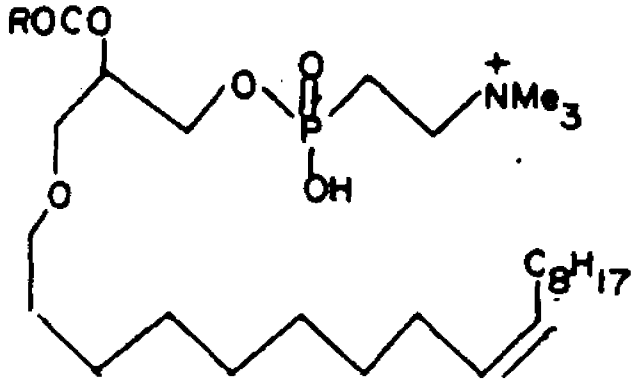


XXXV

Baer and Robinson (67) also reported the synthesis of the shortened system, XXXVI, and Chacko and Hanahan (58) the ether-ester species XXXVII.

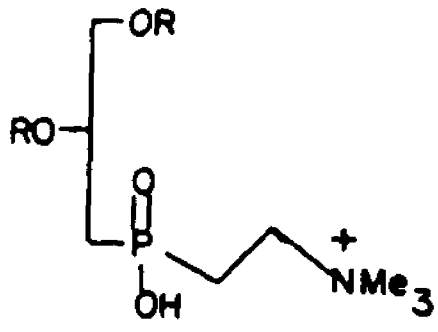


XXXVI

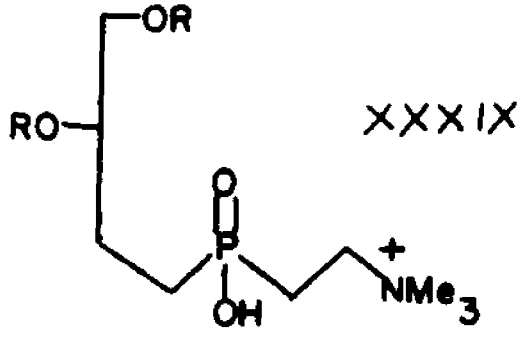


XXXVII

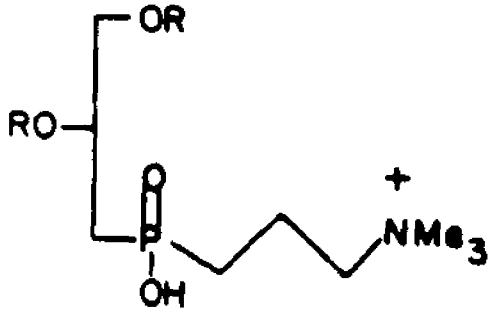
Rosenthal, et. al. (68-70) synthesized a series of phosphinates, compounds XXXVIII, XXXIX, XL, and XLI.



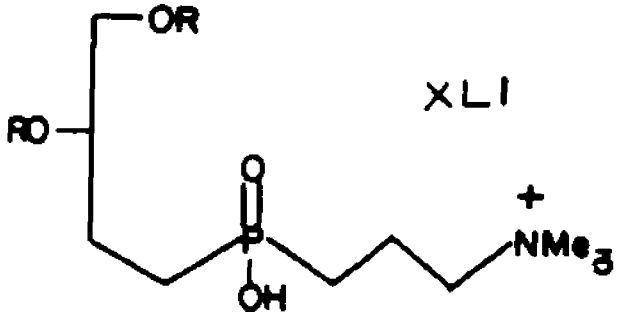
XXXVIII



XXXIX

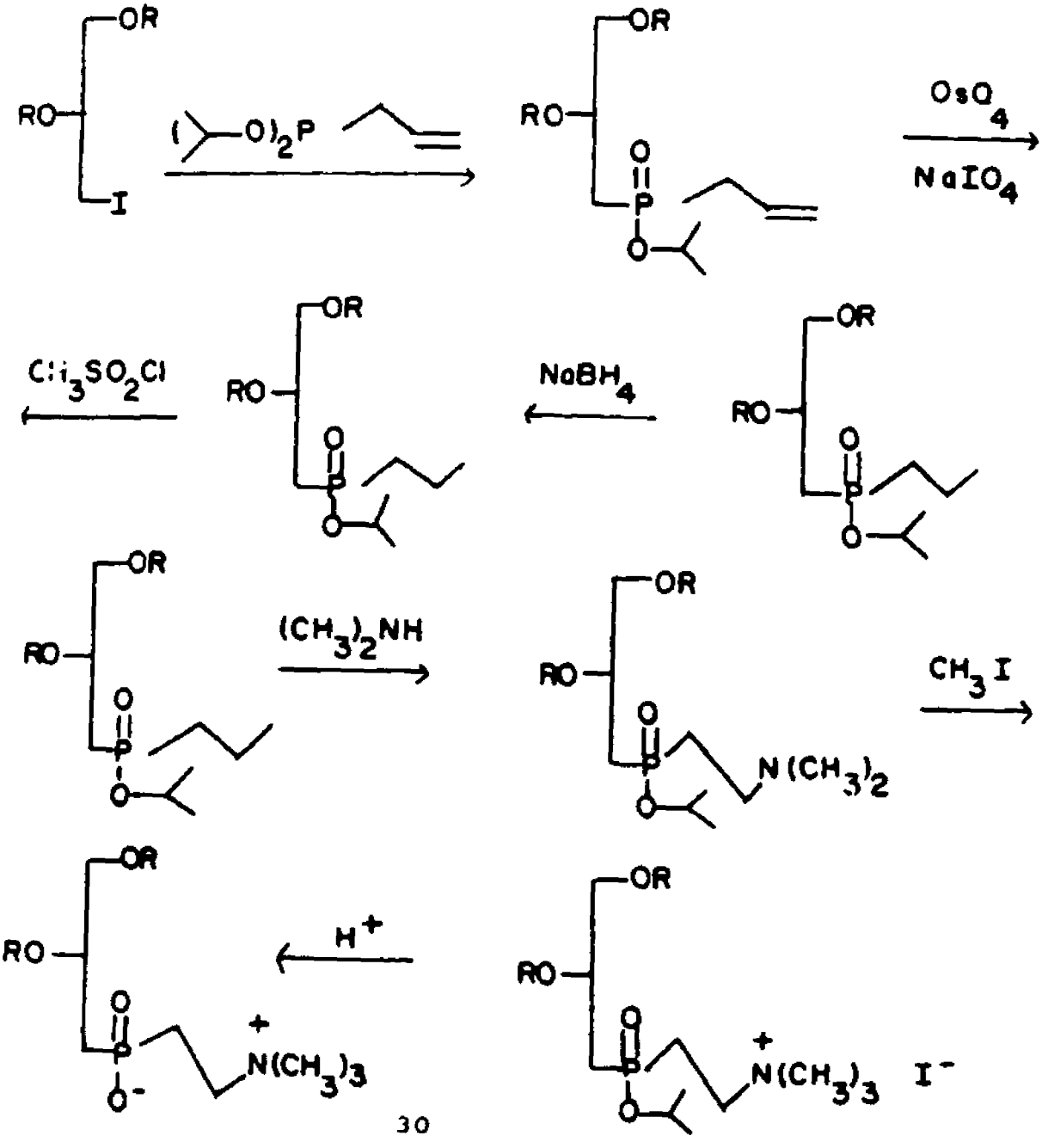


XL

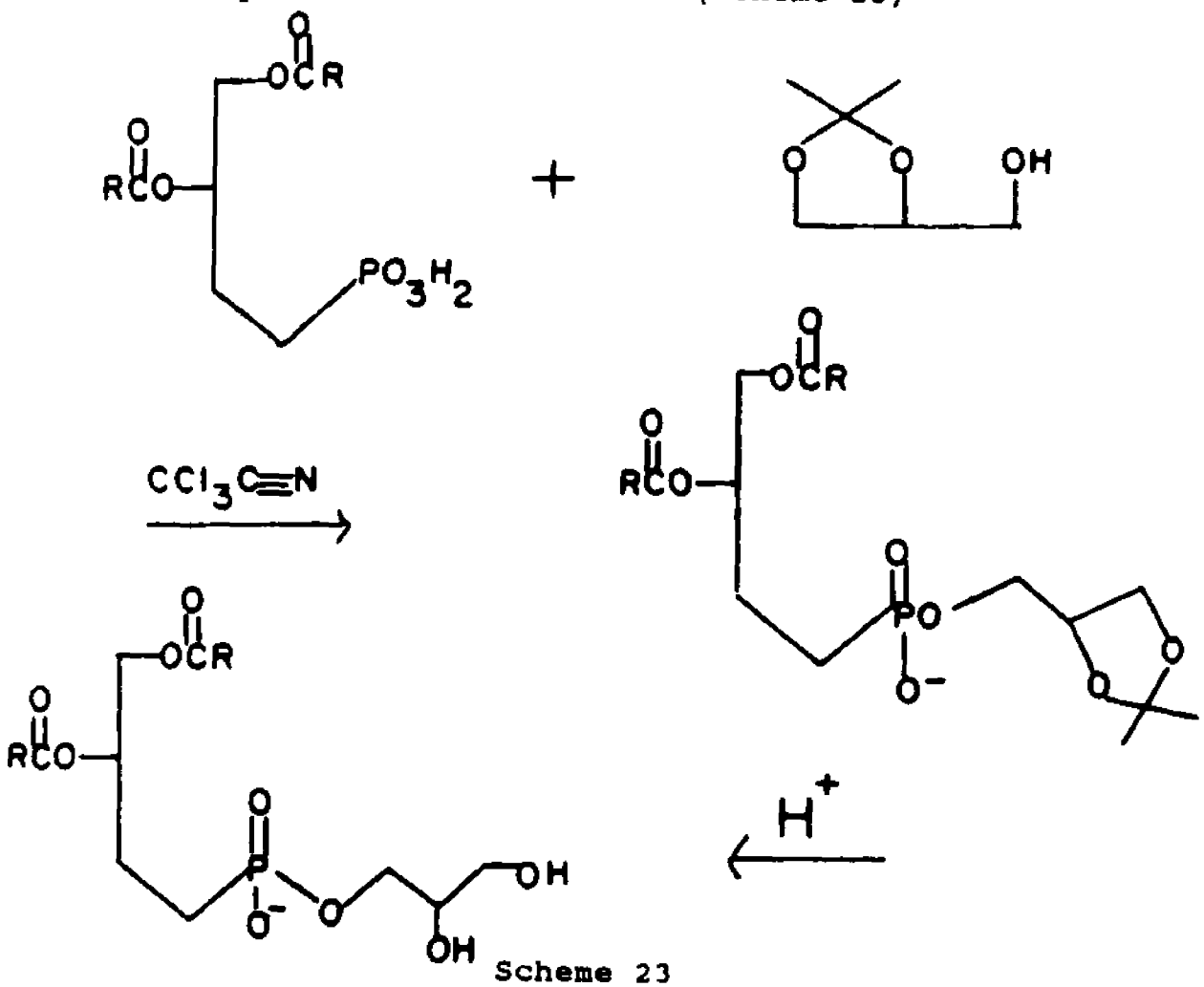


XLI

All of these lipid analogues have diether linkages. They would be expected to be completely resistant to hydrolysis. As an example of the synthetic approach, the preparation of XXXVIII is outlined in Scheme 22. (68)



Phosphatidylglycerol as a parent molecule has potential for the development of several species of analogues containing a C-P bond. Only the isosteric analogue, XLII, with a methylene group in place of the backbone glyceric oxygen has been synthesized. (59) The diacylphosphonic acid was coupled to isopropylidene-protected glycerol by trichloroacetonitrile. The protection on the glycerol was removed by treatment with acid. (Scheme 23)



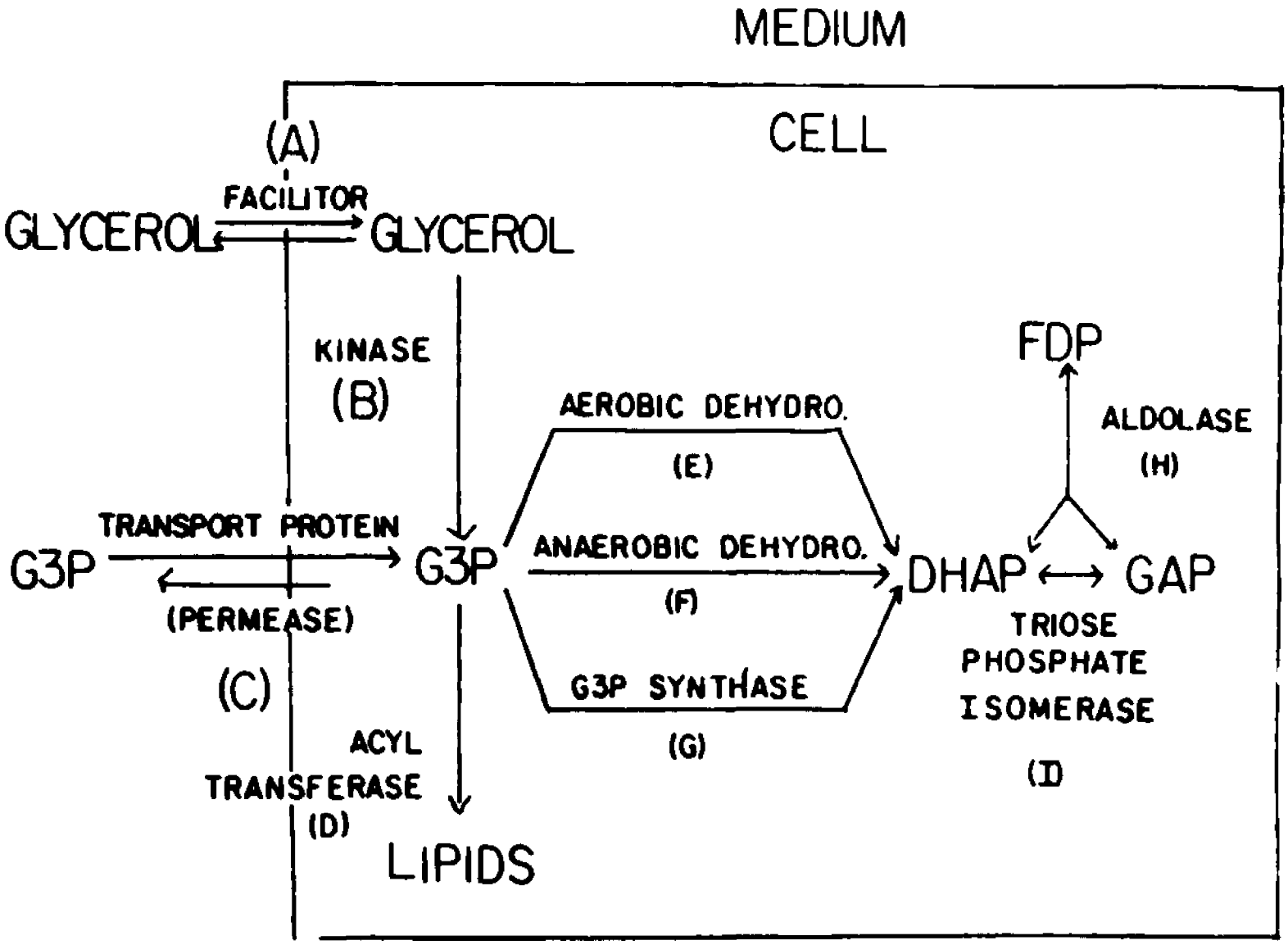
Scheme 23

Biological Investigations:

Glycerol has the biological significance of being one of the possible precursors to glycerol-3-phosphate, G-3-P, an important molecule which provides the carbon skeleton in gluconeogenesis and acts as the backbone of phosphoglycerides or glycolipids. In eukaryotic cells, G-3-P carries reducing equivalents from the cytosol to the mitochondria for oxidative-phosphorylation.(71)

In bacteria, glycerol is dissimilated by only two methods: dehydrogenation, followed by phosphorylation, or phosphorylation, followed by dehydrogenation. The terminal product in either sequence is dihydroxyacetone phosphate, DHAP. The metabolism of glycerol is extremely complex for several reasons in spite of what appears to be only two simple pathways. First, some organisms are equipped with both pathways, each functioning under specific conditions. Second, certain organisms can not only utilize external glycerol, but also the intermediates of the two pathways; glycerol-3-phosphate and/or dihydroxyacetone, DHA. As a third consideration, the actual mechanisms of dehydrogenation may vary depending on the enzyme involved and its particular protein and prosthetic group components. (72)

The special features of glycerol dissimilation in Escherichia coli will be noted here as a model system. The network for the metabolism of glycerol in this species is summarized below in Scheme 24. (71)


MEDIUM
CELL
(A)
FACILITOR
GLYCEROL
GLYCEROL
KINASE
(B)
AEROBIC DEHYDRO.
(E)
FDP
ALDOLASE
(H)
TRANSPORT PROTEIN
G3P
G3P
ANAEROBIC DEHYDRO.
(F)
DHAP
GAP
(PERMEASE)
TRIOSE
PHOSPHATE
ISOMERASE
(I)
(C)
G3P SYNTHASE
(G)
ACYL
TRANSFERASE
(D)
LIPIDS

Most of the work done on glycerol metabolism was performed on strains of E. coli descended from a branch of the K12 family that originated from strain E15. Strain E15 was isolated as a deletion mutant in the structural gene of alkaline phosphatase.(73) This enzyme in the wild-type organism furnishes the cell with phosphate by hydrolyzing organophosphate esters when inorganic phosphorus is not available. Strain E15 was chosen as the parent or wild-type strain for the development of mutants to be used in the study of glycerol and its metabolism, and was renamed strain 1.

Strain 1 will grow aerobically on glycerol or G-3-P as the sole carbon source or anaerobically with fumarate or nitrate as the exogenous hydrogen acceptor.

Glycerol enters the cell by means of a glycerol facilitator protein (A). This protein catalyzes the equilibration of glycerol across the cell membrane as opposed to active transport against a concentration gradient.

Glycerol kinase (EC 2.7.1.30: ATP:glycerol 3-phosphotransferase) catalyzes the formation of glycerol-3-phosphate by the hydrolysis of ATP. This catabolic enzyme has the unique feature of being subject to feedback inhibition by fructose-1,6-diphosphate (FDP) which acts as a non-competitive inhibitor. It will also catalyze the phosphorylation of DHA and L-glyceraldehyde.

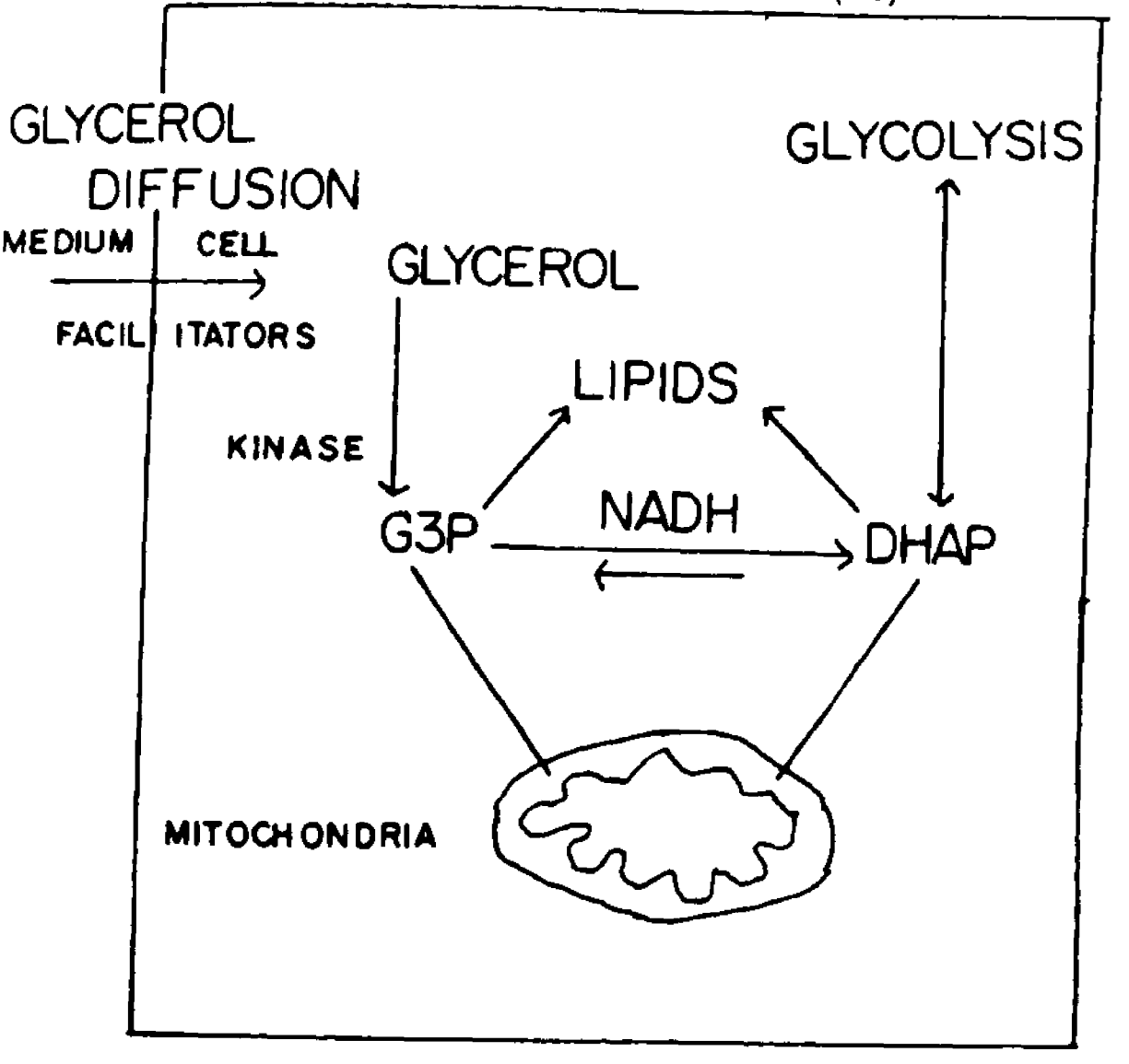
Glycerol-3-phosphate is actively transported across the cell membrane by the G-3-P transport protein (C) otherwise known as the G-3-P permease. This enzyme is competitively inhibited by glyceraldehyde-3-phosphate but is not by DHAP.

Glycerol-3-phosphate, as a precursor of lipid synthesis, can be acylated to lyso-phosphatidic acid by the action of G3P acyltransferase (EC 2.3.1.15; Acyl-CoA: G3P O-acyltransferase; D

Three enzymes catalyze the conversion of G3P to DHAP; the aerobic dehydrogenase (E), the anaerobic G3P dehydrogenase (F), and G3P synthase(G). The aerobic G3P dehydrogenase (EC 1.1.99.5) has noncovalently bound FAD as its coenzyme. Cells deficient in this enzyme will not grow on either glycerol or G3P in the presence of molecular oxygen. The anaerobic dehydrogenase (EC 1.1.99.5) contains a flavin coenzyme and catalyzes the same reaction when molecular oxygen is not present and uses fumarate or nitrate as a terminal electron acceptor. The G3P synthase (EC 1.1.1.94; G3P:$NADP^+$ 2-oxidoreductase) is also called the DHAP reductase. Equilibrium favors the reduction of DHAP to G3P via oxidation of NADPH.

Dihydroxyacetone phosphate is isomerized to glyceralde-3-phosphate by the action of triose isomerase, an enzyme of the glycolytic pathway.

In mammalian tissues, glycerol is made available from lipolysis, hydrolysis of triglycerides, and to a small extent from ingestion. Glycerol in mammalian cells is dissimilated according to the diagram outlined in Scheme 25.(74)



Scheme 25

Specific membrane components that facilitate the diffusion of glycerol across the cell membrane have been studied in both erythrocytes and rat hepatoma cells. (71) After glycerol enters the cell it is phosphorylated by glycerol kinase to form G3P. Kinetic features of the enzyme indicate that glycerol is phosphorylated when the cell is not in an energy starved state. G3P and ADP act as inhibitors. As with the bacterial systems, glycerol-3-phosphate can be acylated by acyltransferase as the first step in lipid synthesis.

The presence of a G3P shuttle was proposed by Bucher and Klingenberg (75) and Estabrook and Sacktor (76). In this process reducing equivalents in the cytosol from glycolysis are deposited in DHAP converting it to G3P by the action of a cytosolic NAD^+-linked G3P-dehydrogenase (EC G3P:cytochrome oxidoreductase). The G3P enters the mitochondrion and is reoxidized to DHAP, thereby depositing reducing equivalents from glycolysis for work in oxidative phosphorylation. The DHAP then exits the mitochondrion.

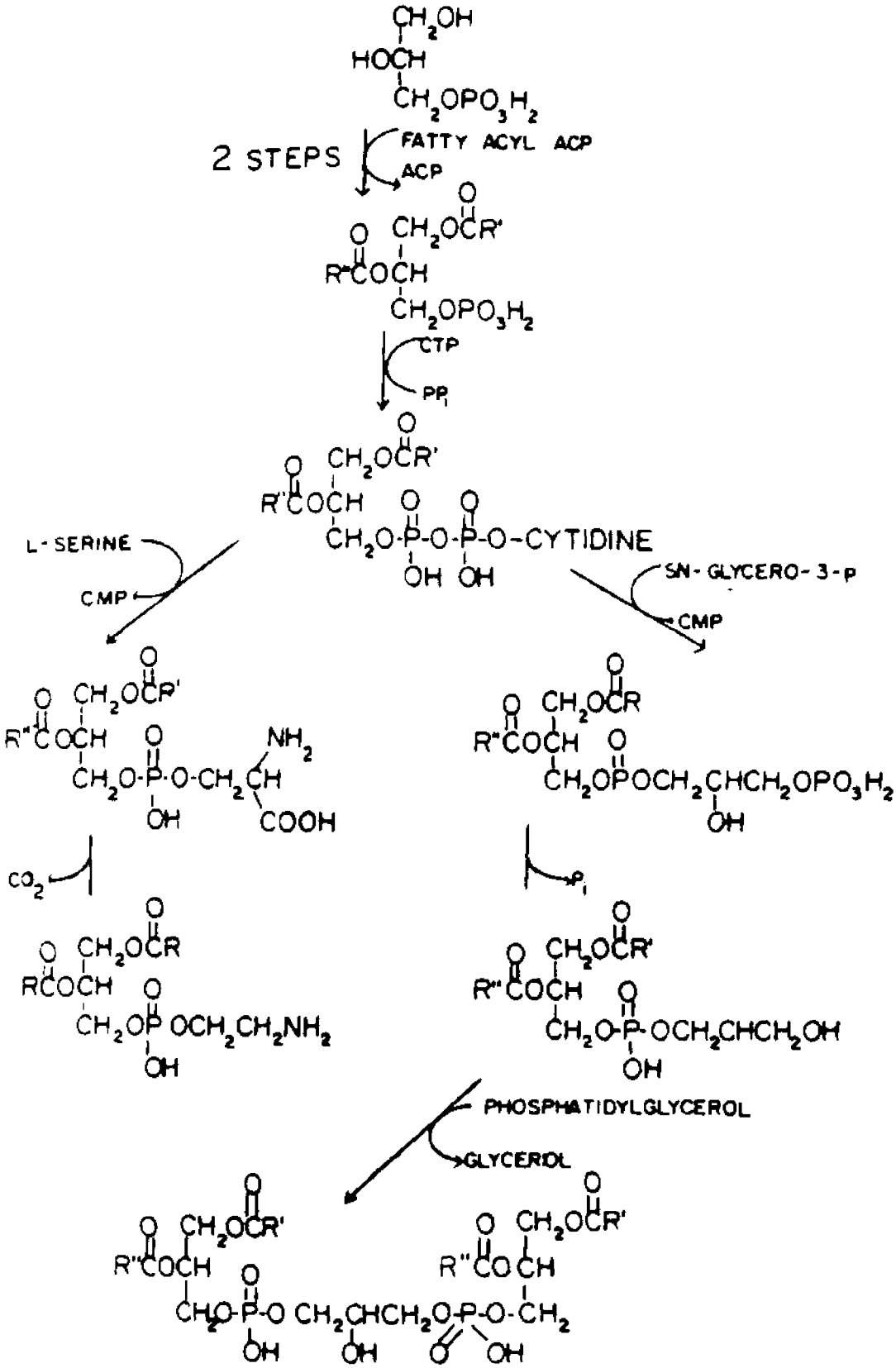
By the action of a G3P oxidoreductase (EC 1.1.1.94; G3P: NAD^+ 2-oxidoreductase) G3P can be oxidized to DHAP but the chemical equilibrium for this reaction favors the formation of G3P. DHAP from the G3P shuttle, from oxidation of G3P in the cytosol, or from glycolysis can be acylated as precursors of lipid synthesis by the action of a cytosolic DHAP

acyltransferase (EC 2.3.1.42: acyl-CoA:DHAP o-acyltransferase) associated with the peroxisomes or the endoplasmic reticulum.

The biogenesis of phospholipids in both animal and bacterial cells plays an essential role in both cell and membrane growth. The biosynthetic pathway of E. coli will be used as a model system and is illustrated in Scheme 26.

In the initial step, sn-glycerol-3-phosphate is acylated in the one position with a saturated fatty acyl group derived from a fatty acyl-ACP complex. (77) The enzyme responsible for this step is sn-Glycerol-3-phosphate acyltransferase. Very little lyso-phosphatidic acid accumulates in wild-type organisms since this compound is rapidly acylated by a second enzyme with an unsaturated fatty acid to form phosphatidic acid. (78,79) Generally, position number one is a palmitoyl group and number two a palmitoleic or cis-vaccinic functional group.

Phosphatidic acid reacts with cytidine triphosphate, CTP, or deoxy-CTP to form the corresponding liponucleotide by means of the cytoplasmic membrane-bound enzyme, cytidine triphosphate:phosphatidic acid cytidyltransferase (CDP-diglyceride synthase). (80,81) In marked contrast to the enzymes that follow in this pathway, CDP-diglyceride synthase specifically requires phosphatidic acid esterified with unsaturated fatty acyl groups and does not utilize


2 STEPS
FATTY ACYL ACP
ACP
CTP
PPi
L-SERINE
CMP
SN-GLYCERO-3-P
CMP
CO2
Pi
PHOSPHATIDYLGLYCEROL
GLYCEROL

dipalmitoylphosphatidic acid. This very unusual enzymatic feature as well as other requirements for the physical state of the substrates may represent artifacts of the in vitro assay system. (80)

There is some, but not conclusive, evidence that the formation of CDP-diglyceride is rate limiting for the pathway. (82) Certainly CDP-diglyceride is located at an important biosynthetic branch point. The polar headgroup composition of wild-type strains of E. coli is apparently constant even under changes in growth conditions. It is presumed that the biogenesis of CDP-diglyceride is the site at which the ratio of phosphatidylethanolamine to cardiolipin and phosphatidylglycerol is regulated. (83)

CDP-diglyceride can donate its phosphatidyl moiety to the hydroxyl group of L-serine. The enzyme that catalyzes this reaction, CDP-diglyceride:L-serine O-phosphatidyltransferase (phosphatidylserine synthase) was discovered by Kanfer and Kennedy. (84,85) Extensive studies have shown that phosphatidylserine synthase is the major source of phosphatidylserine and therefore phosphatidylethanolamine in gram negative bacteria.

Very little phosphatidylserine is present in wild-type strains of E. coli since it is rapidly converted to phosphatidylethanolamine by phosphatidylserine decarboxylase. This reaction prevents the reverse phosphatidylserine

synthesis *in vivo* and results in the formation of a very stable end product, phosphatidylethanolamine.

The other direction of the branching after CDP-diglyceride is the formation of phosphatidylglycerophosphate, PGP, from CDP-diglyceride and sn-glycerol-3-phosphate by the action of CDP-diglyceride:sn-glycerol-3-phosphate phosphatidyl transferase. (86,87) This reaction is analogous to the synthesis of phosphatidylserine in that it also releases CMP. The PGP does not accumulate because it is rapidly dephosphorylated to give phosphatidtylglycerol.

In procaryotic cells, cardiolipin is synthesized by the condensation of two molecules of phosphatidylglycerol and the subsequent release of glycerol.(88) This reaction does not require CDP-diglyceride and hence no metabolic energy. In stationary cells, the level of cardiolipin tends to rise at the expense of phosphatidylglycerol.

In some bacteria, specifically the genus *Agrobacterium*, phosphatidylcholine is formed by N-methylation of phosphatidylethanolamine in contrast to the alternative condensation of diglyceride with phosphorylcholine. The methyl groups are added in stepwise fashion and are derived from S-adenosyl methionine. (89).

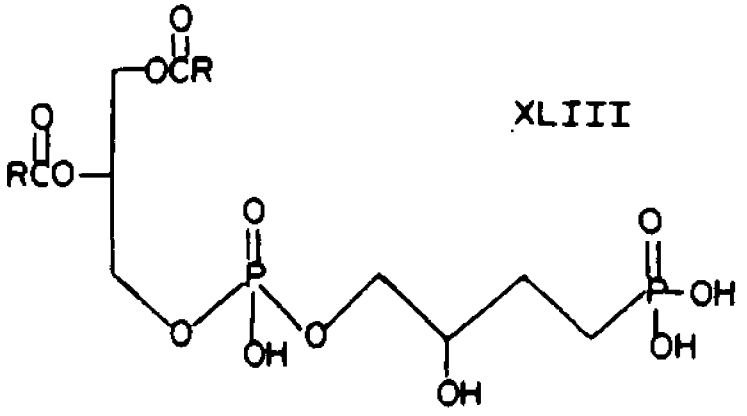
Phosphonic analogues of glycerol metabolites have been tested for biological activities with many of the bacterial and mammalian enzymes.

The preliminary work by Baer, et. al. (90) on the non-isosteric analogue 2,3-dihydroxypropyl-1-phosphonic acid, I, found it did not interact with the enzymatic systems studied. It was not a substitute for glycerol-3-phosphate in the reaction catalyzed by rabbit muscle glycerol-3-phosphate: NAD^+ oxidoreductase. They concluded the esteric oxygen was a requirement for activity.

Later work by Cheng, et. al. (91) found this compound did appear to have a slight but reproducible inhibitory effect upon the acyltransferase.

Studies by Kabak, et. al. (16) and Shopsis, et. al. (92-94) found the ester oxygen was not necessary for activity but correlation of size with the natural substrate is essential. The enantiomer of the isosteric analogue, 3,4-dihydroxybutyl-1-phosphonic acid, II, corresponding to sn-glycerol-3-phosphate is transported into a variety of bacterial species including E. coli (95-96) by means of the G3P permease or transport protein. In species in which II is transported, growth inhibition is observed. The non-isosteric analogue I probably is transported because it affects metabolism of ^{14}C acetate although the 3-carbon compound, phosphonomycin is definitely transported through this system (97-98) or it may not bind to the enzymes once it enters the cell. At low concentrations of II specific strains of E. coli show a marked perturbation of lipid synthesis and a build up of an

unusual anionic lipid, a phosphonic acid analogue of phosphatidylglycerophosphate, XLIII. (98-100)



XLIII

This is presumed to result from the inability of the bacteria to cleave the phosphonate linkage to release inorganic phosphate and synthesize normal phosphatidylglycerol (vida infra). The analogue is also active with strains of B. subtilis. 3,4-Dihydroxybutyl-1-phosphonate inhibits phosphatidylglycerol synthesis and is also incorporated into the cell wall. (101-102) With strain 168, growth is inhibited, but in strain W23 the drug is lethal.

Studies with II indicate it is a competitive substrate of the glycerol-3-phosphate:NAD(P) oxidoreductase of E. coli (91) and that of rabbit muscle G3P dehydrogenase. (103) It serves as a substrate for CDP-diglyceride:glycerol-3-phosphate phosphatidyltransferase. It does not appear to act as a substrate for the aerobic G3P dehydrogenase, CDP-

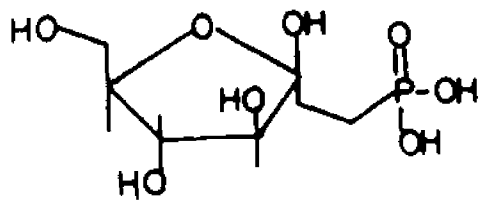
diglyceride: L-serine phosphatidyltransferase, or the acyl transferase. (91) It is presumed that binding of the substrate to these enzymes requires the presence of the esteric oxygen or at a minimum a functional group with some possible mode of interaction. The anaerobic G3P dehydrogenase was not studied.

The non-isosteric analogues of glycerol-3-phosphate, 1,2,3-trihydroxypropyl-1-phosphonic acid and its corresponding methylphosphinate analogues (Scheme 8), do not inhibit the G3P:NAD(P) oxidoreductase. (17) This is again presumably due to the size differences between the normal substrates and the analogues.

Another isosteric analogue of glycerol-3-phosphate, 1,3,4-trihydroxybutyl-1-phosphonic acid III, (18) was found to be an inhibitor of growth of E. coli (21,104) and also is a substrate for acyl coenzyme A:G3P acyltransferase. (21,104) This data supports the postulate that II does not possess activity with the acyltransferase due to loss of binding capability. The analogue III can enter the bacteria through the G3P transport system.

The phosphonate analogue of dihydroxyacetone phosphate, 4-hydroxy-3-oxobutyl-1-phosphonic acid V, (20) was tested in in vitro studies for activity. It does serve as a substrate for G3P:NAD(P) oxidoreductase from E. coli. (20, 105) Stribling (106) found that the analogue would interact with

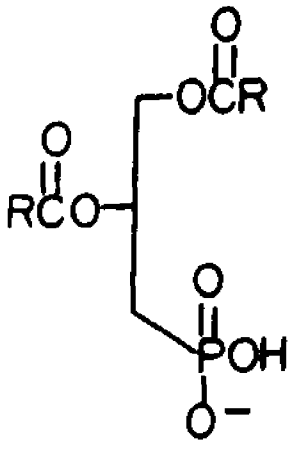
glyceraldehyde-3-phosphate and aldolase to produce a phosphonate analogue of fructose-1,6-diphosphate, XLIV.



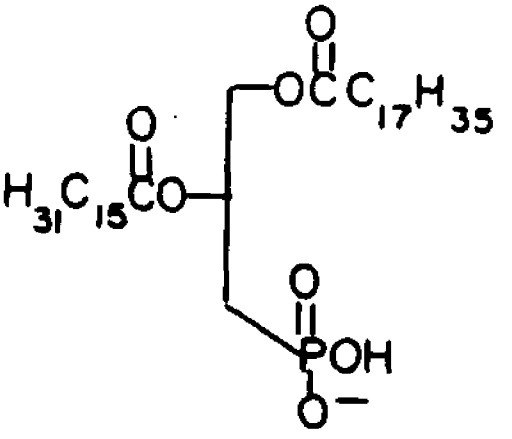
XLIV

Dixon and Sparkes (24) found this compound did not interact with triose phosphoisomerase from either chicken muscle or B. stearothermophilus. In vivo studies of V in E. coli have not been sucessful because the compound is not transported by the G3P system.

Analogues of phosphatidic acid serve as antimetabolites in several systems. In studies with phosphonates of the series XVIII, specifically, 2-hexadecyloxy-3-octadecyloxypropylphosphonic acid, XLV, the analogues show considerable activity as inhibitors of pig kidney phosphatidate phosphohydrolase (L-a-phosphatidate phosphohydrolase, E.C. 3.1.3.4.) when measured with phosphatide derived from egg lecithin.(9)



XVIII



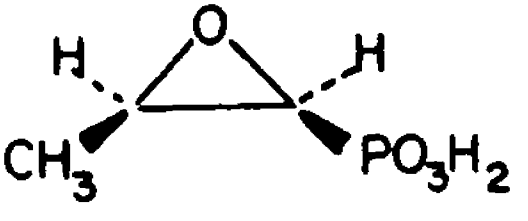
XLV

The isosteric compounds of series XX, specifically 3,4-dioctadecyloxybutylphosphonate, XLVII, also show activity in this system, but to a lower extent. The analogues have been shown to be specific inhibitors of the enzyme. The degree of inhibition was found to increase with the amount of dispersion of the inhibitor. The observation of decreased solubility with phosphonic acids is probably due to the loss of the hydrophilic esteric oxygen as possessed by the natural substrate. The analogues also completely inhibited growth of Staphylococcus epidermidis and Streptococcus salivarius and caused partial growth inhibition of several other species. (9).

The antibiotic phosphomycin, is a naturally occurring phosphonate that was first isolated from a fermentation broth in which Streptomyces fradiae was grown. (98) The antibiotic

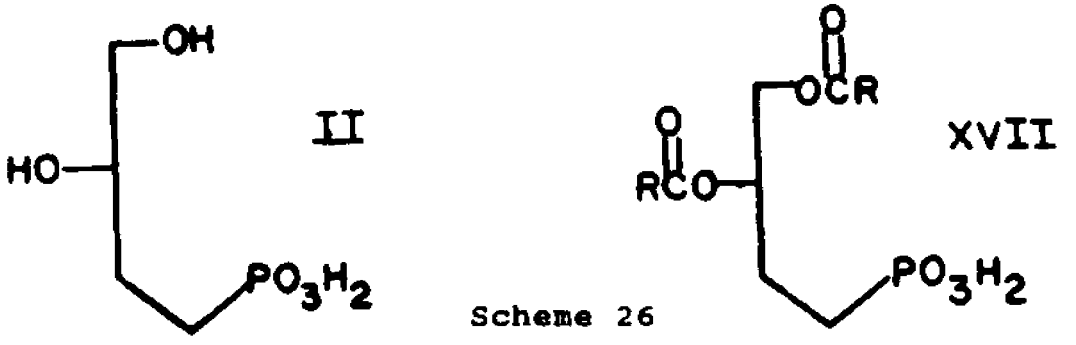
is bactericidal. In a growth media of high osmolarity, several species of bacteria are, in the presence of phosphonomycin, converted to spheroblasts. (107) These observations indicated the target activity of the antimetabolite is with cell wall formation. Later experiments with extracts from both gram negative and gram positive bacteria have shown that phosphonomycin is an irreversable inhibitor of phosphoenolpyruvate:uridine diphospho-N-acylglucosamine transferase. This enzyme is the first step in the synthesis of nucleotide muramyl peptides which serve as cell wall precursors in all bacteria. (108)

Phosphonomycin has been shown to be (-)-(1R,2S)-1,2-epoxypropylphosphonic acid. Proof of the absolute structure was obtained by synthetic chemical determination of the configuration. The phosphonomycin molecule has been synthesized from (-)-cis-propenylphosphonic acid by epoxidation with hydrogen peroxide in the presence of sodium tungstate and has been isolated as both the ammonium salt (98) and the phenethylammonium salt (110). The structure of phosphonomycin is given below.



STATEMENT OF PROBLEM

The current project has been concerned with the preparation of analogues of phosphatidic acid. The initial synthesis and biological studies on 3,4-dihydroxybutyl-1-phosphonate, DHBP, lead to the synthesis of the phosphonate analogues of structure XVII, by Tang, et. al. (8) Scheme 26



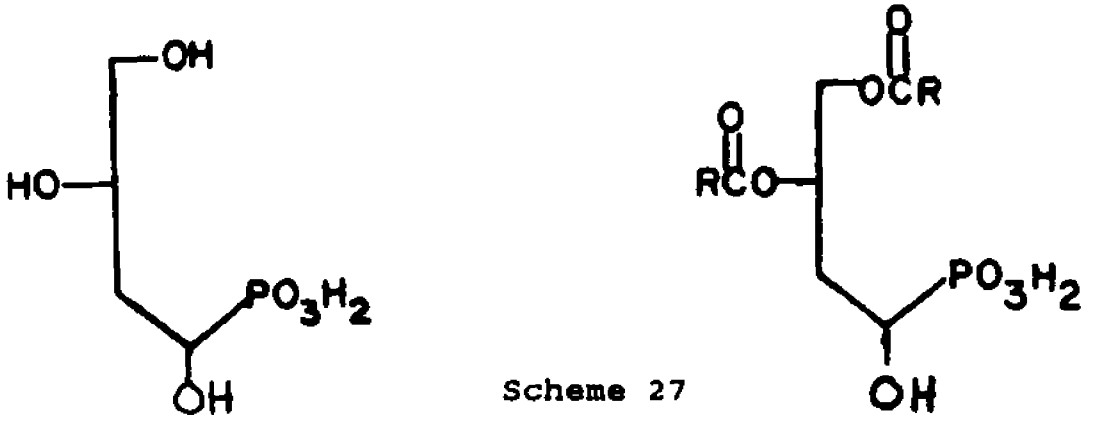
Scheme 26

In these compounds, the glycerol backbone of the phosphatidic acid is replaced by a four carbon phosphonate skeleton. The molecules are isosteric analogues of the natural compounds and can be said to be of the DHBP family. The shared features are outlined above in Scheme 26.

The C_{16} and C_{18} butyl phosphonates exhibited a marked decrease in solubility compared to the phosphatidic acids. This made *in vitro* enzymatic studies difficult and unreliable. The initial part of this project was the synthesis of the dicaproyl, C_{10}, analogue. This was prepared

by the method of Tang, et. al. (8). The phosphonic acid had a considerably lower molecular weight, increased solubility in aqueous systems and was an oily semi-solid at room temperature.

The synthesis of 1,3,4-trihydroxybutyl-1-phosphonate III, THBP, gave rise to another prospective "family" of phosphonate analogues having a THBP backbone instead of the glycerol of the natural compound. These structures are outlined in Scheme 27.



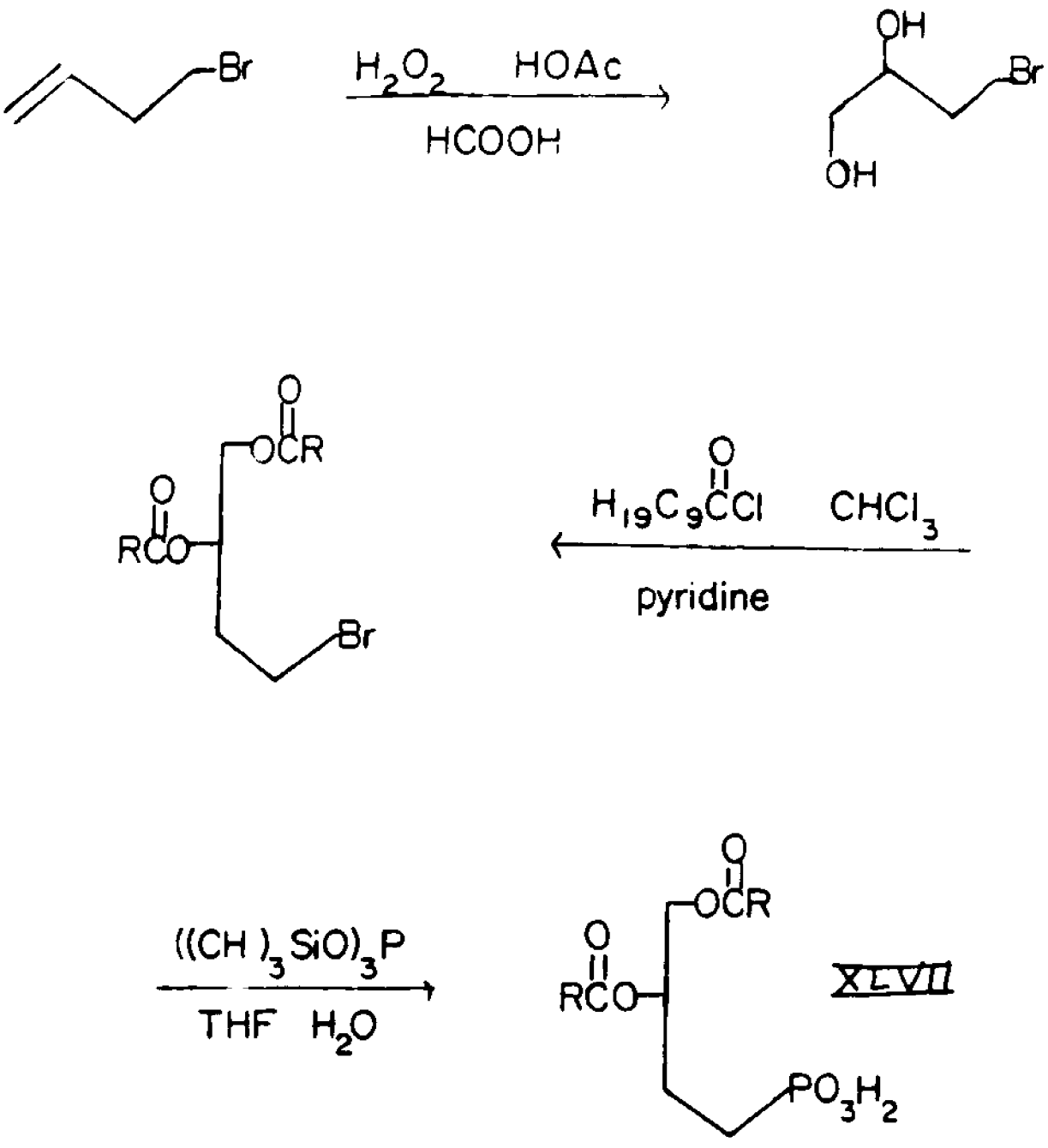
Scheme 27

The presence of a hydroxymethylene group supplies an electon-rich site adjacent to the phosphorus. To synthesize these compounds, 3,4-dihydroxybut-1-enylphosphonate diisopropyl ester was generated as an intermediate. This would be acylated and the 1-hydroxy function introduced. The diacyl vinylphosphonates themselves are analogues of phosphatidic aci and may be of biochemical interest. The vinylic system also would allow the synthesis in theory of the corresponding 3,4-diacyl-1,2-epoxy-butylphosphonates.

RESULTS AND DISCUSSION

I. Synthesis of 3,4-Didecanoylbutyl-1-phosphonate:

The synthetic approach for the preparation of XLVII was that developed by Rosenthal (40) and later modified by Tang, et. al. (8). It is outlined in Scheme 29. 4-Bromo-1-butene was oxidized to form the corresponding diol by treatment with hydrogen peroxide and formic acid. The 3,4-dihydroxybutyl-bromide was acylated by the standard treatment with the acid chloride in pyridine. The diacylated halide, 3,4-dicaprylbutyl-1-bromide, was allowed to undergo an Arbuzov reaction with tris(trimethylsilyl)phosphite. The resulting bis(trimethylsilyl)ester was hydrolyzed under relatively mild conditions by refluxing with 9:1 THF:water. The crude product was purified initially by column chromatography to remove traces of caproic acid. The resulting product had NMR spectra as predicted and migrated as a single spot with thin layer chromatography. Microanalysis revealed the percent carbon and percent hydrogen were low although the ratio of C:H was as expected. This indicated the product had to have significant contamination by inorganics. This problem was resolved by washing the material with chloroform followed by microfiltration with a series of millipore filters.


Br
H2O2 HOAc
HCOOH
OH
Br
OH
O
OCR
RCO
Br
O
H19C9CCl CHCl3
pyridine
((CH)3SiO)3P
THF H2O
O
OCR
RCO
XLVII
PO3H2

SCHEME 29

The purified 3,4-dicaprylbutyl-1-phosphonate product is a yellow orange semi-solid. The most prevalent naturally occuring phosphatidic acid, dipalmitoyl phosphatidic acid, as well as the analogue previously synthesized in our laboratory, 3,4-dipalmitoylbutyl-1-phosphonate, are both solids with significantly higher molecular weights. As previously discussed, the phosphonates of interest are generally less soluble than the natural phosphates of which they are analogues. In vitro enzyme assays using the dipalmitoyl analogue, DPBP, are extremely difficult to perform and have led to inconclusive results (110) because of decreased solubility and poor dispersability. The replacement of the sixteen carbon acyl chains with ten carbon chains provides an isosteric analogue with improved solubility and dispersability.

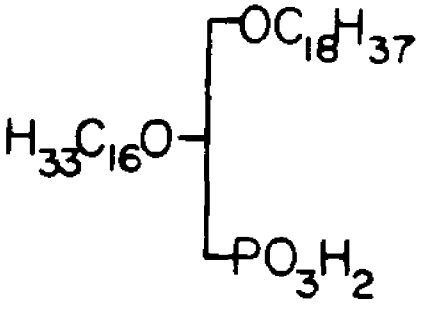
Studies by Carter and Kennedy (80,81) on the CDP-diglyceride synthase indicated the enzyme would only utilize phosphatidic acid with unsaturated acyl chains. This is in contrast to in vitro assays with the other enzymes in the phospholipid biosynthetic pathway which show a marked preference for dipalmitoyl substrates. As stated in the introduction, this may be an artifact of the assay system. The present synthesis of 3,4-dicaprylbutyl-1-phosphonic acid is a valuable tool to test the actual properties of this particular enzyme. The compound's increased solubility may

all . it to function as a substrate or inhibitor for the synthase. If not, important insights may be gained regarding specific interactions of the enzymatic system.

Oftentimes a critical factor allowing a molecule to bind to an enzyme is the presence of the non-bonded electons at the site adjacent to the phosphorous. If this were the situation, for example, by hydrogen bonding to a key amino acid, the isosteric phosphonate would not be able to function well as a substrate or inhibitor.

\nother point of consideration is a difference in the acidity of the phosphorus acid site. The phosphonate is less acidic with a difference of 0.5-1.5 pK_a units. This factor may also play an important role in determining whether the analogue has activity in an enzymne system.

Phosphatidic acid phosphatase (L- -phosphatidate phosphohydrolase, EC 3.1.3.4) occupies a key position in the synthesis of both triglycerides and lecithin. Rosenthal and Pousada (9) demonstrated the phosphonate analogue, 2-hexadecoxy-3-octadecoxypropylphosphonic acid is an inhibitor

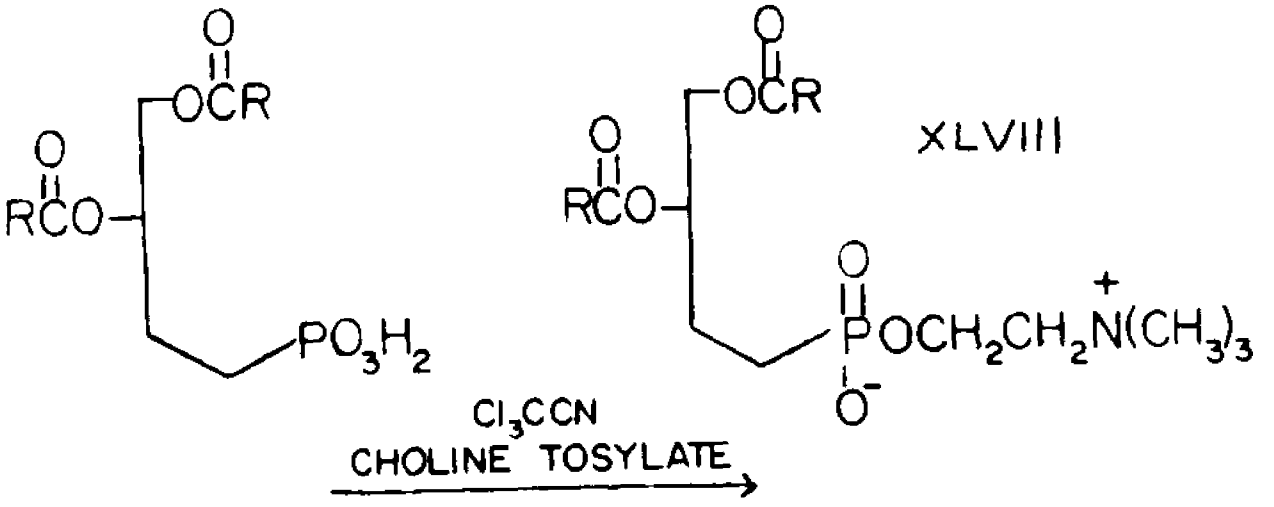


pig kidney phosphatidate phosphohydrolase. From their work, it appears that the general molecular geometry, in long chain species, is more important than specific functional groups.

The analogue 3,4-dicaprylbutyl-1-phosphonate would seem to have the following advantages compared to the longer chain analogues. The butyl backbone chain allows the molecule to be isosteric with the natural substrate and therefore, the number and spatial arrangements of functional groups should be much closer to the natural substrate. Pre-incubation of the propyl (non-isosteric) phosphonate with the enzyme led to a striking increase in inhibition.(9) This indicated an alteration in the enzyme with time suggesting the enzyme flexible active site binds the analogue loosely at first, and then readjusts to a tighter fit with time. The butyl analogue may not show this effect because it should fit better initially. The authors also reported solubility problems using the non-isosteric analogue. The shorter acyl chains should help with this problem. However, the ether linkages of the propyl analogue are relatively inert when compared to the ester linkages of the butyl species.

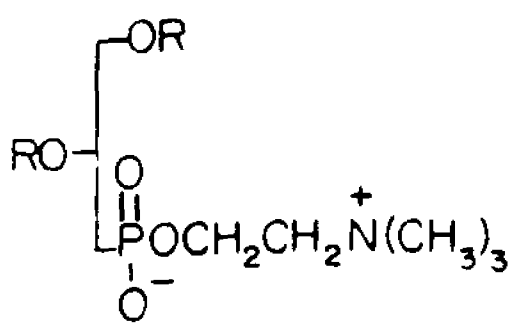
11. Synthesis of 3,4-Dicaprylbutylphosphonylcholine, XLVIII:

The synthesis of the isosteric phosphonate analogue of phosphatidylcholine was accomplished using a procedure modified by Braksmayer (58), Scheme 30, from work of

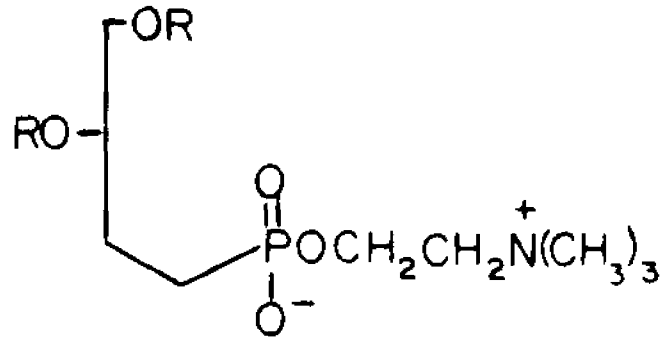


Rosenthal (46) and Cramer (111). Choline tosylate was prepared from N,N-dimethyl-2-aminoethanol and methyl tosylate according to the procedure of Rosenthal et. al. (12) The esterification of 3,4-dicaprylbutyl-1-phosphonate with choline tosylate was accomplished with trichloroacetonitrile. Purification techniques included passage through Amberlite MB-3 column. Partial purification was also facilitated by low temperature recrystalization. Silica gel chromatography involving methanol solvent systems contaminated the eluent with soluble silica materials. These impurities were removed by dissolution of the sample in chloroform followed by microfiltration.

Compounds in which a C-P bond is present in the glycerol backbone of a lecithin analogue would have the potential to act as inhibitors of enzymes that hydrolyze the C-O-P bond of the natural compound. The species XLIX and L have been



XLIX



L

found to be significant inhibitors of phospholipase C from C. perfringens. The isosteric analogue L is the better inhibitorof the two. Dicaproylbutylphosphonylcholine has the isosteric butyl backbone which should make for a better analogue. The use of the ten carbon acyl chains should improve solubility while maintaining the original carboxylate ester functional group.

Phosphonate analogues of structure L have been used to study the features of phospholipid-cholesterol interactions in model membrane systems. It was observed that methylene group substitution for the esteratic oxygen of the glycerol moiety does not alter the extent of these interactions as measured by chloride and rubidium ion efflux rate constants. Studies with dicaprylbutylphosphonylcholine would be anticipated to show similar findings since there should be no significant differences in the average conformation of the

glycerol molecule when comparing ether and ester linked chains. The shorted acyl chains may however, be anticipated to affect how cholesterol increases the order of the hydrocarbon matrix. (112)

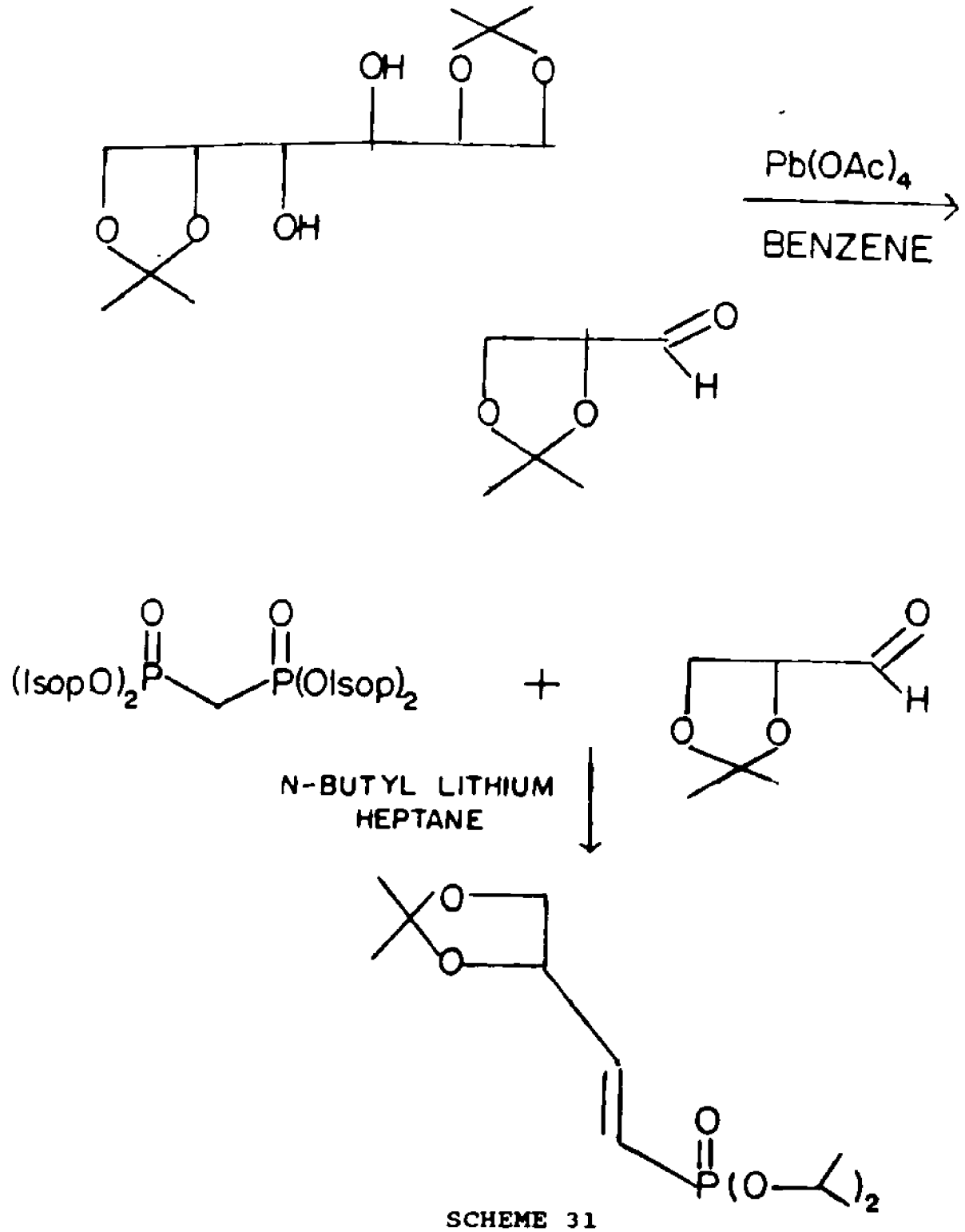
De Haas et. al. (113) reported the use of short chain lecithins as substrates for porcine pancreatic phospholipase A. A chain length of 6-8 carbon atoms was found to have the main advantage that use of a detergent was not required for dispersal. The use in such a study of dicaprylbutylphosphonylcholine introduce two structural modifications; a slightly longer chain length, and the C-P bond. Since species with acyl chains in length from 6 through 18 carbons have been incorporated in substrate molecules, the ten carbon chain length should have no significant effect in binding to the enzyme. Although it lacks the esteric oxygen of the C-O-P bond, the analogue still possesses a negative charge on the phosphorous which has been determined essential for hydrolysis.

Lipids participate in some chemical reactions by serving as enzymatic cofactors. As an example, D- -hydroxybutyrate dehydrogenase has an absolute requirement for lecithin. (114) The use of dicaprylbutylphosphonylcholine as a probe to study the requirements for activation of the apoenzyme is a particularly interesting application. The distances in the analogue between the major functional

groups, hydrophobic region, phosphorous, and quaternary ammonium function, are conserved. Studies have shown that long chain diether phosphonocholines activate the enzyme. Therefore, the presence of the esteric oxygen is not a requirement for activity. The shortened acyl chains of the dicaproylphosphonylcholine would be anticipated to demonstrate how modifications in the hydrophobic region of the lecithin molecule affect enzyme activation. (114)

III. Synthesis of Diisopropyl (S)-(E)-3,4-O-Isopropylidene-3,4-dihydroxybut-1-enyl-1-phosphonate. IV

Tetraisopropyl methylenebisphosphonate, TIMBP, was synthesized by the method of Roy.(115) The reaction of dibromomethane with triisopropyl phosphite at 185 C for several hours with concurrent removal of the isopropyl bromide side product through a column maintained at 65 C gave tetraisopropyl methylenebisphosphonate in good yield. The 1,2:5,6-di-O-isopropylidene-D-mannitol was prepared by the method of Baer and Fischer. (116) Zinc chloride catalyzed acetonization of D-mannitol at room temperature for 16 hours provided 1,2-5,6-diacetone-D-mannitol. This material on treatment with lead acetate in benzene provided the O-isopropylidene-D-glyceraldehyde. The subsequent vinylic phosphonate product, Scheme 31, is formed in a Horner


OH
O
O
OH
O
O
Pb(OAc)4
BENZENE
O
H
O
O
(IsopO)2P
P(OIsop)2
O
O
+
O
H
O
O
N-BUTYL LITHIUM
HEPTANE
O
O
O
P(O
)2

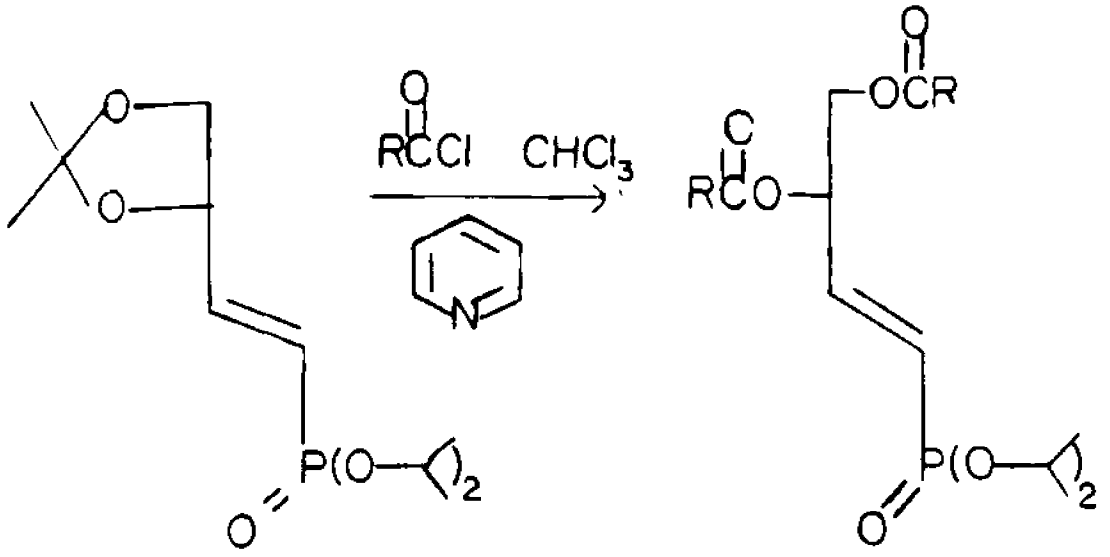
SCHEME 31

reaction in which the anion of TIMBP is generated by reaction with n-butyllithium in heptane. This intermediate then condenses with the glyceraldehyde acetonide. Reaction conditions involve stirring at room temperature for two hours followed by quenching with water and washing to remove any water soluble side products. Spectral data and optical rotation measurements indicated the correct product was formed. The vinylic phosphonate can be distilled to yield a pure, colorless product, but significant decomposition occurrs when attempting to remove excess TIMBP which distills at a temperature very close to the product.

An alternative attempt to synthesize the vinyl phosphonate by treatment of TIMBP with sodium hydride to generate the anion failed. Only starting material, TIMBP, was recovered and the aldehyde was destroyed in the process.

Removal of the isopropylidene function to deprotect the hydroxyls at position three and four is easily accomplished by stirring with 0.1 N HCl for several hours. Excess water is removed by evaporation under reduced pressure. An alternative scheme for deprotection by treatment with Dowex 50W in the acid form gave a residue with an orange color whose NMR and TLC data matched the above method.

IV. Synthesis of Diisopropyl (S)-(E)-3,4-Diacylbut-1-enyl-phosphonate. LII, LIII



The vinylic phosphonates, LII and LIII, are intermediates of great versatility. Acylation of the hydroxyls at positions three and four is accomplished by standard methods. The diol is treated with the respective acid chloride in chloroform with a small amount of pyridine as catalyst. Treatment of the vinyl phosphonate with palmitoyl chloride by stirring in the dark for 48 hours followed by washing to remove water soluble residue yielded the product 3,4-dipalmitoylbut-1-enylphosphonate, LII. The white solid was purified by recrystalization to give a product whose spectra were as expected.

Treatment of the diol with caproyl chloride under the same conditions resulted in a yellow-orange semi-solid. This product, LIII, was purified by column chromatography.

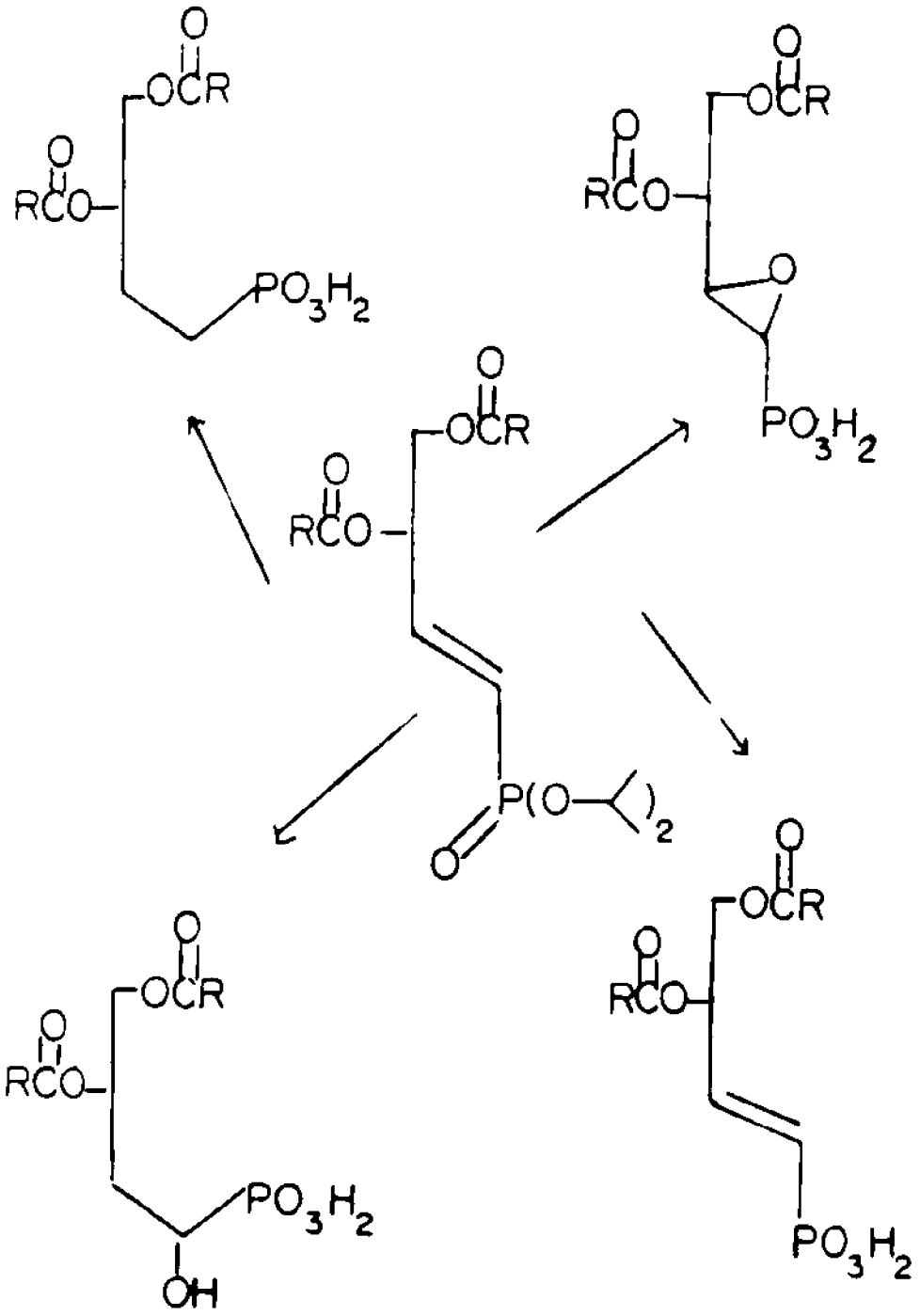
The formation of the diisopropyl-3,4-diacylbut-1-enyl-phosphonate is extemely important. These compounds, whether having ten or sixteen carbon chains, are the central compounds in the formation of a series of analogues of phosphatidic acid. This chemistry is outlined in Scheme 32.

V. Synthesis of (S)-(E)-3,4-Diacylbut-1-enylphosphonic acid. LIV, LV

3,4-Dipalmitoylbut-1-enylphosphonic acid was generated by deprotection of the phosphorous. The diisopropyl ester of the phosphonate was stirred with a six-fold molar excess of tribromomethylsilane overnight in the absence of solvent. The trimethylsilyl esters thus formed on the phosphorous were hydrolyzed by treatment with 9:1 THF:water. The product was isolated as a white solid by recrystallization from chloroform.

The use of the bromotrimethylsilane compound is far superior to use of either the chloro- or iodo- compounds. The iodo compound provides an extremely rapid reaction, but generates significant inorganic contaminants. The chloro compound is as clean as the bromo, but requires considerably longer reaction times.

The diacylbut-1-enylphosphonic acid themselves are analogues of phosphatidic acid. The presence of the double bond in the trans or (E) configuration results in a molecule with an increased rigidity of structure in the glycerol backbone with no freedom of rotation around the 1-2 bond.



SCHEME 32

Difficulties may be encountered in the use of this "kinked" analogue for some of the enzymatic work as mentioned for the 3,4-dicaproylbutyl-1-phosphonate as it is condiderably less soluble in aqueous solutions than the saturated compounds. However, it would be particulary interesting for use in model membrane investigations because of its locked structural feature.

The 3,4-dicaprylbut-1-enylphosphonic acid was prepared as above to yield a yellow semi-solid. The final product was purified by column chromatography.

VI. The Synthesis of 3,4-diacylbutyl-1-phosphonic acid. XVII, LVI

The intermediate diisopropyl 3,4-diacylbut-1-enylphosphonate can be converted into the saturated butyl phosphonic acids in a two step synthesis.

Diisopropyl 3,4-dipalmitoylbut-1-enylphosphonate was suspended in absolute ethanol and hydrogenated over a platinum oxide catalyst at atmospheric pressure until no more hydrogen uptake was observed. The solution was filtered through Celite and evaporated to give a white residue. Spectral data indicated the absence of vinylic protons and the presence only of the saturated linkages.

The saturated product was deprotected with tribromosilane by stirring at room temperature. The esters

so formed were hydrolyzed to give a white solid whose spectra, TLC data, and melting point matched that published by Tang, et. al. (8)

Subsequent treatment in a likewise fashion of the dicaproyl analogue yielded a yellow semi-solid. Spectral data, melting point, and TLC data matched that of the 3,4-dicaproylbutyl-1-phosphonic acid, XLVII, synthesized in part I of this paper.

VI. Synthesis of Diisopropyl 3,4-dipalmitoyl-1-hydroxybutyl-phosphonate. LVII

Studies by Tang, et. al. (18) and Tang, et. al. (47) on the activity of compound III, 1,3,4-trihydroxybutyl-1-phosphonate, provided an interesting insight. Although the species II, DHBP, is not an inhibitor of the acyl coenzyme A: sn-glycerol-3-phosphate acyltransferase of E. coli, the trihydroxy species is an inhibitor. Two points should be noted at this time. Blackburn (117) has noted that the second pK_a of a phosphonic acid may be lowered by the introduction of an electronegative group such as a hydroxyl function at the site adjacent to the phosphorous. Cooperman and Chiu (118) demonstrated that modification of a methylene group by addition of a hydroxyl function changed the biological activity of an analogue of pyrophosphate. The hydroxy bearing analogue was recognized by a variety of pyrophosphatases but the methylene compound was inactive.

The presence of a double bond adjacent to the phosphorous in the diisopropyl 3,4-diacyloxybut-1-enylphosphonates allows for hydration at this site. Work by Hampton et. al. (119) and Lalinde et. al. (22) have demonstrated that hydroboration-oxidation of substituted vinyl phosphonic acid esters places the hydroxyl at the position adjacent to the phosphorous. This specific orientation can be confirmed by reversion studies.

The synthesis of 3,4-dipalmitoyl-1-hydroxybutyl-1-phosphonic acid was accomplished in two steps. Hydroboration-oxidation was accomplished by treatment of the vinylic phosphonate, LI (22) with borane-dimethyl sulfide complex in ether. Excess reagent was destroyed by ethanol addition. This was followed by addition of 3M NaOH and 30% hydrogen peroxide. After washing to remove water soluble by products and evaporation under reduced presssure, the white solid was purified by column chromatography and subsequent recrystallization.

This procedure forms both the (1S,3S) and (1R,3S) pair of isomers which were not separated.

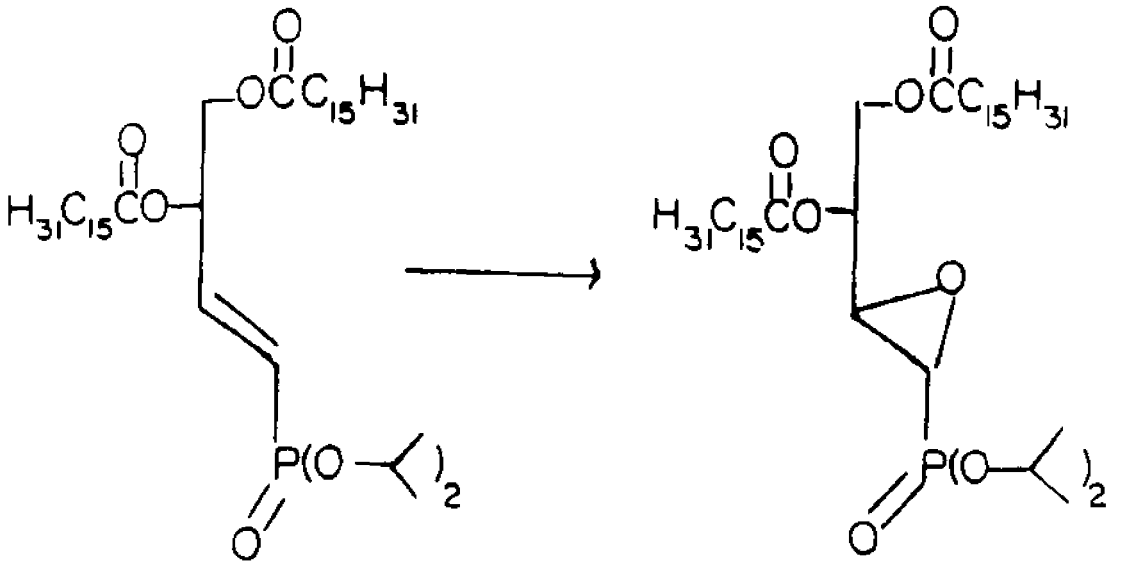
The free acid is generated by the standard treatment with tribromosilane followed by hydrolysis to give a product consisting of both isomers. Purification of the final product, LVIII, was accomplished by recrystalization of the solid.

The 3,4-dicapryl-1-hydroxybutyl-1-phosphonic acid, LIX, was synthesized as above. Purification was more difficult and consisted of a series of separations by column chromatography.

VII. The Synthesis of 3,4-diacyl-1,2-epoxybutyl-1-phosphonate. LX

The naturally occurring phosphonic acid antibiotic phosphonomycin, irreversably inhibits cell wall formation in extracts from both gram negative and gram positive bacteria. (107) The unique feature of this antimetabolite is a structure in which an epoxide function is present adjacent to a phosphonic acid linkage. As with a hydroxymethylene group, the presence of en epoxy function adjacent to a phosphorous would be anticipated to improve the binding ability and reactivity of an analogue molecule. It is presumed that while the stereochemistry of the epoxide might be important use of a diasteroisomeric mixture would be considered acceptable in the initial stages of investigation.

Thus a new target for synthesis was an analogue of phosphatidic acid bearing a 1,2-epoxy function. The intermediate diisopropyl 3,4-dipalmitoylbut-1-enylphosphonate was chosen as a candidate for direct epoxidation according to Scheme 33.



This particular species was chosen because of its availability and ease of purification. The epoxide would be expected to be difficult to purify by standard methods and a solid would aid in purification by recrystalization.

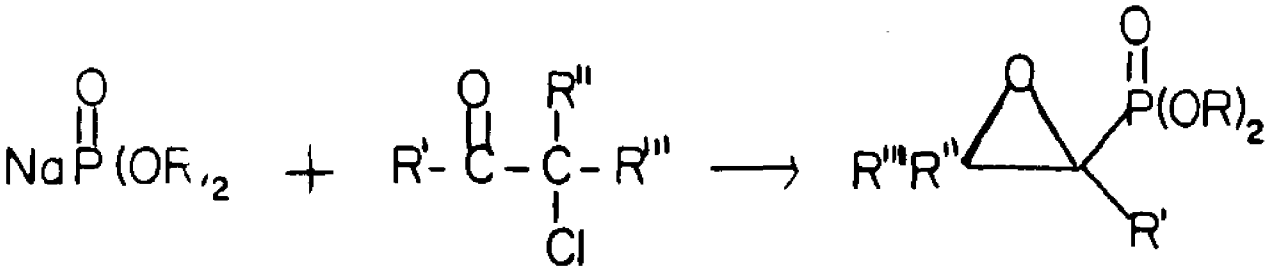
The initial synthesis attempted was direct epoxidation of the double bond by treatment with m-chloroperbenzoic acid. (120) The vinylic phosphonate was treated with a slight excess of the m-chloroperbenzoic acid with stirring in methylene chloride at room temperature. Excess peracid was destroyed by the addition of sodium sulfite. The m-chlorobenzoic acid byproduct was removed by washing with sodium bicarbonate. The residue that was isolated clearly showed the presence of vinylic protons in its NMR, indicating that no epoxidation had occurred.

The experiment was repeated with an increase in temperature to 50 C and allowed to run overnight with no significant change in results.

Another method of direct epoxidation of the double bond was attempted. This method, as utilized by Marmor (121), had several advantages. Previous work in this laboratory (122) during attempts to synthesize 1,2-epoxy-3,4-dihydroxybutylphosphonate had met with failure. The use of 30% hydrogen peroxide and very basic aqueous conditions led to opening of the epoxide and cleavage of the C-P bond during the reaction. The use of sodium hypochlorite in pyridine with organic solvents should not cause this cleavage. The reagents are also less hazardous and less expensive.

A solution of the vinyl phosphonate in pyridine was treated with an excess of sodium hypochlorite at 0 C. The solution was allowed to come to room temperature and then stirred for one hour. After workup with chloroform and water washes, only the starting vinylic phosphonate was recovered.

Since direct epoxidation of the vinyl phosphonate no longer seemed feasible, other approaches for synthesis of epoxides were considered. The reaction of a dialkyl sodioalkane phosphonate and an alpha-halocarbonyl compound, as diagramed below was attempted.

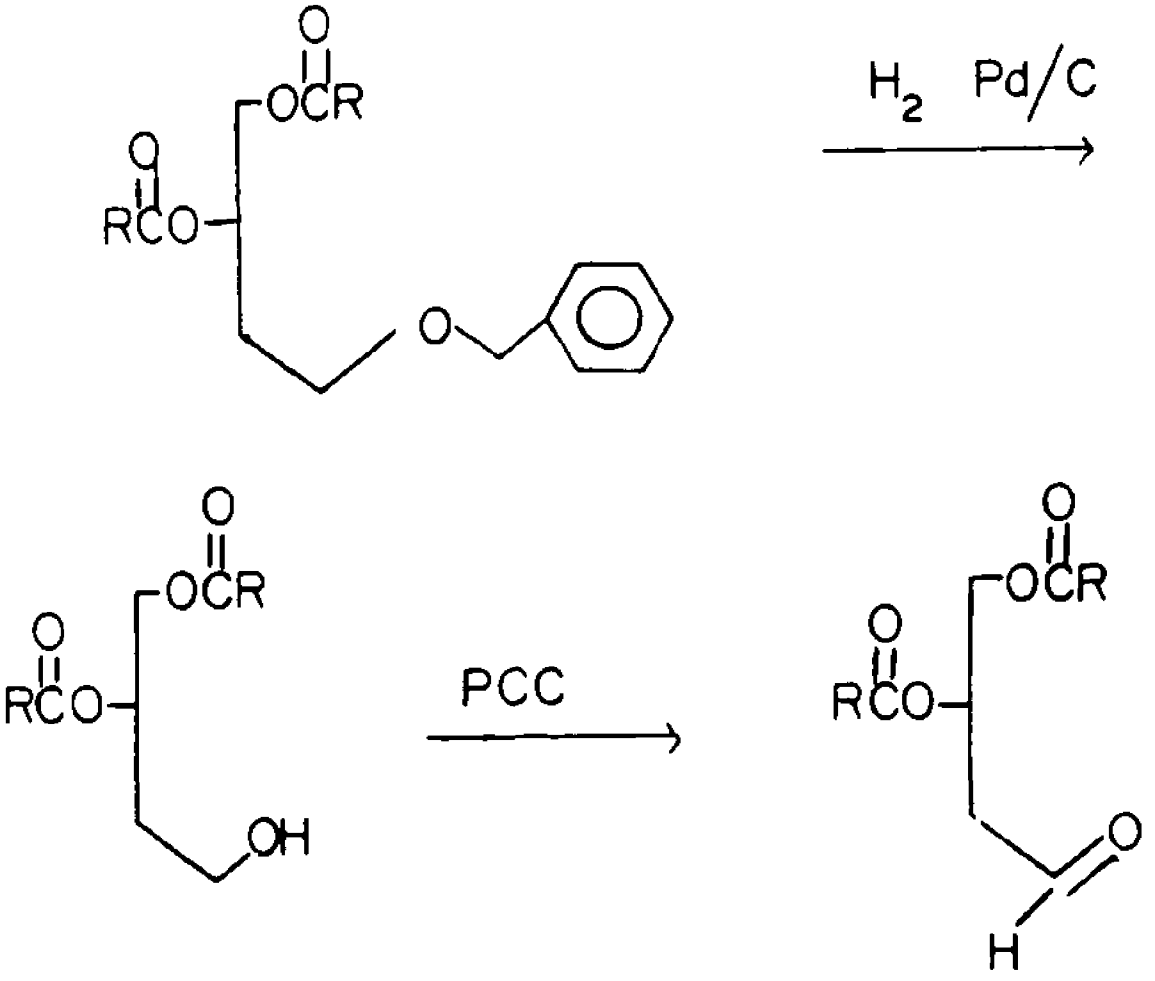


Trialkyl phosphites react with alpha-halocarbonyl compounds to give enol-phosphates in the Perkow reaction and/or phosphonates in the Arbuzov reaction, depending upon the reactants and reaction conditions. Sekine, et. al. (123) reported that a carbonyl adduct intermediate in these reactions could be trapped and converted to bis(trimethylsilyl) esters of 1,2-epoxyalkylphosphonic acids. This involved treatment with sodium methoxide in methanol followed by retrimethylsilation in dry THF. The epoxide is reported to be isolated as a monoanilinium salt.

Synthesis of the key alpha-halocarbonyl compound was performed as outlined in Scheme 34.

(S)-4-Benzyloxy-1,2-dipalmitoyloxybutane was synthesized by the method of Tang, et.al. (18) The benzyl group protecting the hydroxyl in the four position was removed by hydrogenolysis with 10 % Pd on carbon in absolute ethanol. The solution was hydrogenated at 56 psig of hydrogen until no more hydrogen was taken up. The solution was filtered

through Celite and evaporated under reduced pressure. The product was recrystalized from methanol. The melting point and spectra of the product were in agreement with published data.



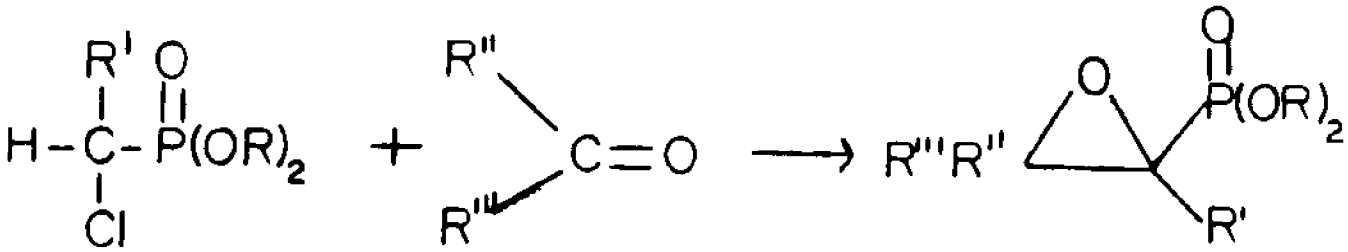
Scheme 34

The alcohol was then oxidized to the corresponding aldehyde by treatment with pyridiniumchlorochromate, PCC. (124) To a solution of PCC in methylene chloride was added sodium acetate and celite, followed by the 1-hydroxy-3,4-dipalmitoyloxybutane. The reaction was monitored by the change in color of the solution. The solution was filtered through Florisil and then evaporated under reduced pressure. The white crystalline product was recrystallized from methanol. The product exhibited spectra that were expected, showing a clear aldehyde peak in the NMR.

The aldehyde was dissolved in methylene chloride and chilled to - 50 C in a dry ice-isopropanyl alcohol bath. The halo-carbonyl compound was then generated by the addition of sulfuryl chloride. The solution was warmed to room temperature and allowed to react for one hour. Although thin layer chromatography indicated reaction to be proceeding, attempts to isolate the halocarbonyl compound failed. The product rapidly decomposes even when chilled and in an inert atmosphere.

Since the formation of the epoxide depended upon isolation and purification of the 2-chloro-3,4-dipalmitoylbutanal, another method of synthesis was necessary.

The next approach of choice was the use of a Darzens type reaction of a dialkyl chloromethylphosphonate with a carbonyl compound:



The first step in this synthetic approach is the oxidation of dipalmitin to the corresponding aldehyde. (125) To a solution of dipalmitin in methylene chloride was added anhydrous sodium acetate and pyridiniumchlorochromate. The oxidation is complete after two hours of stirring at room temperature. After filtration and solvent evaporation under reduced pressure, the aldehyde product can be isolated by simple recrystallization. The product exhibited spectra in accord with its proposed structure.

The reaction to form the epoxide consisted of initial treatment of diethyl chloromethylphosphonate with n-butyl lithium in an inert atmosphere at -70 C. Tetrahydrofuran was added, followed by the addition of the aldehyde. The reaction mixture was stirred overnight. The system was quenched by the addition of water. Isolation of product was achieved by extraction with methylene chloride , drying, and evaporation of solvent.

The white crystalline product exhibited a pair of doublets in the NMR spectra at a position consistent with the successful formation of the epoxide moiety.

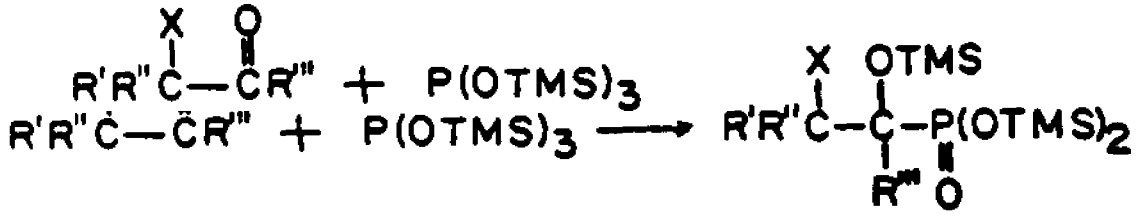
VIII. Deprotection of Diethyl 1,2-epoxy-3,4-dipalmitoylbutylphosphonate.LXI

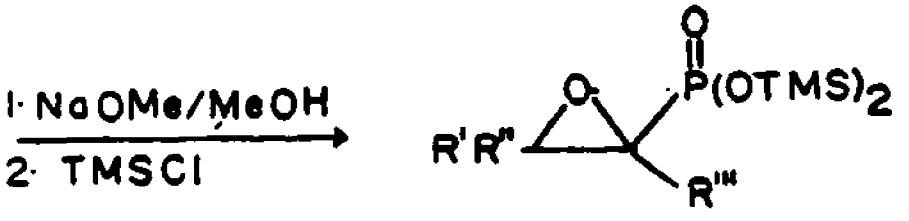
Once the epoxy function had been introduced into the phosphonate molecule successfully, the next synthetic obstacle was the removal of the protecting groups on the phosphorous.

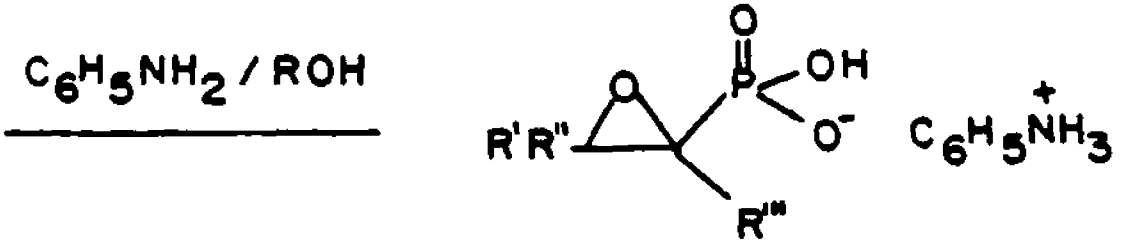
Morita, et. al. (125) have demonstrated that trimethylbromosilane is an effective agent for cleavage of alkyl esters of phosphonic acids. With an epoxide functional group present in addition to the esters on the phosphorus, it would be expected that the bromide of the reagent might also attack the beta-carbon of the epoxide. It was hoped that there would be a significant difference between the rates of ester cleavage and nucleophilic attack. Treatment of the diethyl 3,4-dipalmitoyloxy-1,2-epoxybutyl-1-phosphonate with two equivalents of trimethylbromosilane at room temperature for one hour in ether showed loss of the epoxide signals in the NMR.

Work by Sekine, et. al. (126) has demonstrated that monoanilinium salts of epoxyphosphonates can be synthesized and isolated by recrystalization. Trimethylsilyl halohydrins

are formed from the reaction of alpha-carbonyl compounds with tris(trimethylsilyl) phosphite. These are converted to the corresponding bis(trimethylsilyl)1,2-epoxyphosphonates by reaction with sodium methoxide in methanol. This is followed by resilylation with trimethylchlorosilane and isolation of the salt after treatment with aniline in alcohol. This method is outlined below:







The diisopropyl 3,4-dipalmitoyloxy-1,2-epoxybutyl-1-phosphonate was treated with 3 equivalents of trimethylbromosilane to form the bis(trimethylsilyl) halohydrin. To convert to the bis(trimethylsilyl) 1,2-epoxyphosphonate, the material was treated with three equivalents of sodium methoxide in methanol. At this point the material decomposed.

Future Research

The first priorities for further research in this area should be the biochemical studies detailed in the Results and Discussion section. Studies with the 3,4-dicaproylbutyl-1-phosphonic acid should determine whether the use of a shortened acyl group has countered the solubility problems. If this alone is not successful, studies with the 3,4-diacyl-1-hydroxybutylphosphonates should be informative as far as elucidating what effect the lack of the electron pair of the oxygen has on binding to various enzymes.

As far as the synthetic organic work to be done, the 3,4-dipalmitoyloxy-1,2-epoxybutyl-1-phosphonate was not synthesized. That the epoxide functional group can be synthesized adjacent to the phosphorus, has been demonstrated. I believe the next approach should be the reaction of dibenzyl chloromethylphosphonate (127,128) with 2,3-dipalmitoylglyceraldehyde in a Darzens reaction to form the dibenzyl 3,4-dipalmitoyloxy-1,2-epoxybutylphosphonate. The dibenzyl esters could be cleaved by hydrogenolysis.

EXPERIMENTAL

Routine NMR proton spectra were recorded at 60 MHz on a Varien EM360 instrument and the high field proton spectra were obtained on a Brucker-IBM WP200SY instrument. TMS was was used as an internal standard. Infrared spectra were recorded on a Perkin-Elmer IR598 spectrophotometer.

Column chromatography was performed in glass columns packed with Baker silica gel (60-200 mesh). Thin layer chromatography was performed on silica gel GF (250 microns) coated glass plates from Analtech.

Hydrogen peroxide (30 %) was obtained from Fisher Scientific Co. D-Mannitol and dipalmitin were obtained from Sigma. Chloromethylphosphonic dichloride was obtained from Alfa. All other materials were of reagent grade and obtained from Aldrich. They were used without further purification with the following exceptions: THF was dried and distilled from LAH and used immediately. DMSO, pyridine, hexanes, heptane, and ligroin were distilled from calcium hydride just before use. Chloroform was distilled from phosphorous pentoxide. Methanol was distillled and stored over 4 A molecular sieves. Triethylamine, triisopropyl phosphite, dibromomethane, acetonitrile, 4-bromo-1-butene, and trichloroacetonitrile were distilled just before use.

Caproyl chloride and palmitoyl chloride were distilled under vacuum. Methanolic HBr was prepared by bubbling HBr through methanol until saturation. Lead tetraacetate was always opened fresh, only white material used.

Microanalysis were performed by Galbraith Lab., Knoxville Tenn. and MicAnal, Tucson, Arizona.

Synthesis

1. Preparation of 3,4-dihydroxybutyl-1-bromide.

To 16.85 g (0.125 m) of redistilled 4-bromo-1-butene was added 81 mL of 88 % formic acid and 27.5 g of 30% hydrogen peroxide. The mixture was maintained below 50 C with stirring until a clear solution resulted, generally about one hour. The formic acid was removed by evaporation under reduced pressure. To the residue was added 81 mL methanolic HBr and the resulting solution refluxed for one hour. Volatiles were removed under reduced pressure. The 18.45 g of crude product was obtained. NMR and IR were as expected and the material showed a positive diol test.

2. Preparation of 3,4-dicaprylbutyl-1-bromide.

To a 1.94 g (0.014m) sample of 3,4-dihydroxybutyl-1-bromide was added 25 mL chloroform and 2.21 g (0.028 m) of pyridine. The solution was chilled to 0 C and freshly distilled decanoyl chloride (13.75 g, 0.072 m) was added dropwise. The solution was stirred in the dark at room temperature for 48 hours. The solution was then heated at 41-42 C for 2 hours. The reaction mixture was added to 250 mL chloroform and washed three times with 100 mL 0.25 M sulfuric acid, water 0 C, 10 % sodium thiosulfate, and

water. The organic layer was dried over sodium sulfate and then filtered. Volatiles were removed under reduced pressure. The residue was passed through a column of silica gel (5 x 32.5 cm) in chloroform. The fractions with a spot at R_f = 0.78 were combined and evaporated under reduced pressure. The material exhibited NMR and IR as expected. The material was used without further purification.

3. Synthesis of Tris(trimethylsilyl) phosphite.

To a 3 neck 3 L round bottom flask equippped with addition funnels and stirrer is added 20.4 g (0.25m) phosphorous acid. The flask is chilled to 0 C over ice and 100 mL 1,2-dimethoxyethane is added. The solution is stirred for 30 minutes until clearing is evident. Trimethylchlorosilane (119 mL, 102 g, 0.94 m) is quickly added dropwise. The solution is stirred for 30 minutes. Triethylamine (105 mL, 0.75 m) is slowly added dropwise to the reaction mixture. The solution was stirred for fortyeight hours then refluxed for 1-2 hours. After cooling, 250 mL of ligroin was added and the solution filtered. The white precipitate was washed with an additional 750 mL of ligroin. Solvent was evaporated under reduced pressure and the residue vacuum distilled to give 61.07 g of clear product. bp 46-48 C (0.5 mm). 91.3 %.

4. Synthesis of 3,4-dicaprylbutyl-1-phosphonic acid.

To 1.37 g of the 3,4-dicaprylbutyl-1-bromide (0.00287 m) was added a ten-fold excess of tris(trimethylsilyl)phosphite (7.68 g, 0.0287 m). The solution was heated to 165-170 C for sixteen hours. Excess tris phosphite was distilled under vacuum (50 C, 0.5 mm). A 25 mL solution of THF:water 9:1 was added to the residue and the solution allowed to reflux at 68 C for 2 hours. The solution was evaporated under reduced pressure to give a yellow semi solid crude product, weight 3.5 g.

Chloroform, 100 mL, was added to the crude residue and the suspension stirred with mild heating overnight. The chloroform soluble fraction was isolated. The insoluble fraction of the residue was treated in a similar fashion with 100 mL methanol. No significant amount of material was soluble in this fraction. The insoluble residue was discarded as inorganic material. The chloroform solution was evaporated under reduced pressure.

The residue was layered on a silica gel column (5.5 x 54 cm) in chloroform and eluted first with 1 L of chloroform then followed by chloroform:methanol 3:1, chloroform:methanol 1:1, chloroform:methanol 1:3. The fractions with a single spot R_f = 0.48 in chloroform were combined and evaporated under reduced pressure. Thin layer chromayography in chloroform: ethanol: formic acid:water 100:10:8:0.5 gave R_f =

0.67. The material exhibited NMR and IR spectra in accord with the proposed structure. Analysis of $C_{24}H_{47}O_7P$ requires C, 60.23 % and H, 9.90 %. The values found were C, 54.61 % and 9.00 %. Although these values were very low, the C to H ratio was almost perfect to the calculated value, 6.07 as compared to 6.08. This indicated the possiblity of inorganic contamination, probably silica gel from the chromatography. The sample was dissolved in a minimum of chloroform, washed with distilled water and evaporated under reduced pressure. The residue was redissolved in a minimum of chloroform and filtered through a series of Gelman metricel filters, alpha type 0.2 to 10 um. After filtration and isolation, the residue gave analysis values of 60.27 % C and 9.94 % H. Yield 240 mg pure product, 17.5 %.

5. Synthesis of Choline Tosylate

To a 1 L round-bottom flask was added 44.6 g (0.50 m) of redistilled N,N-dimethylethanolamine in 500 mL acetone. Methyl p-toluenesulfonate (93.1 g, 0.50 m) was added slowly. Formation of a white solid product was evident. The reaction mixture was stirred overnight at room temperature. The product was isolated by cold filtration followed by recrystalization from acetone. Yield 128 gm, 93 %.

6. Synthesis of 3,4-dicaprylbutyl-1-phosphonyl choline

To a mixture of 2.0 g (0.0042 m) of 3,4-dicaprylbutyl-1-phosphonate in 60 mL pyridine was added 6.9 g (0.025 m) of choline tosylate. Trichloroacetonitrile, 21.5 g (0.15 m) was added dropwise. The reaction mixture developed a pinkish-yellow color which upon stirring for 48 hours became dark red. The solution was concentrated to one third of its volume by evaporation under reduced pressure. The crude product was precipitated by the addition of 100 mL of acetonitrile and then isolated by filtration. The solid was dissolved in THF:water 9:1 and passed through an Amberlite MB-3 column (3 x 18 cm) by elution with the same solvent. The product was collected in the first 225 mL of eluent. The solvent was removed under reduced pressure and the product isolated by recrystallization from acetone. Yield 0.84 gm, (0.0015 m), 35.5 %. The material chromatographed as a single spot R_f =0.48 in 65:25:4 chloroform:methanol:water. Melting point of the pure material was 126-130 C. Spectral data was in accord with the proposed structure. Analysis: $C_{29}H_{58}O_7PN$ requires 61.79 % C, 10.40 % H, found 61.73 % C, 10.60 % H.

7. Synthesis of 1,2:5,6-Di-O-isopropylidene-D-mannitol.

To 900 mL of acetone in a 1 L erlenmeyer flask is added 120 g (0.88 m) anhydrous zinc chloride. The solution is vigorously shaken for 20 minutes, then stirred magnetically

for 3 hours. The solution is allowed to settle overnight and then filtered to remove excess zinc salts. To the filtrate is added 30.0 g (0.165 m) D-mannitol. After stirring at room temperature for 3 hours, the reaction mixture is filtered to remove unreacted mannitol. The solution is added to 420 g 50 % (w/w) aqueous potassium carbonate. This is covered with 900 mL diethyl ether and stirred vigorously by hand for 1.5 hours. The zinc carbonate pellets are removed by filtration and washed three times with 250 mL acetone-ether 1:1. The combined filtrates are evaporated under reduced pressure. The white residue obtained is suspended in 250 mL of petroleum ether and refluxed for 0.5 hours. The solution is chilled briefly, and the resulting crystals filtered, crushed and resuspended in solvent. This is repeated three times. The solution is then chilled to 0 C over ice and the crude crystaline product isolated by filtration. Immediately before use, the crystals are heated at 50 C for 1-2 hours in 250 mL n-butyl ether and filtered. Yield 18.0 g (0.069 m) 49.0 %. Spectral data in accord with proposed structure.

8. Synthesis of O-Isopropylidene-D-glyceraldehyde.

In a 1.0 L erlenmeyer flask, 15.6 g (0.0595 m) 1,2:5,6-di-O-isopropylidene-D-mannitol is dissolved in 550 mL benzene. To this mixture is added 26.4 g (0.0812 m) of fresh

lead tetraacetate. The gummy precipitate formed is triturated to a fine colorless powder over a period of one hour. The solution is filtered and the filtrate evaporated under reduced pressure with the water bath temperature maintained below 40 C. The yellow oil product is used immediately without further purification to prevent polymerization. Yield crude product 15 g, 97 %. NMR spectra shows the presence of aldehyde functional group.

9. Synthesis of Tetraisopropyl methylenebisphosphonate.

To 625 g (3.00 m) triisopropyl phosphite was added 174 g (1.00 m) dibromomethane. The solution was slowly heated to 140 C. Condenser temperature was maintained at 65 C by a steam-water combination. At this temperature, reluxing was apparent and isopropyl bromide condensed in the receiver vessel. The temperature of the reaction mixture was then slowly raised to 185 C over a period of 3 hours at which point it was maintained for two hours. The solution was allowed to cool overnight. The crude product was distilled first at low vacuum to remove side products and reagents, 36-42 C, 0.3 mm), then at high vacuum to remove product. The product distilled as a colorless liquid (90-100 C, 0.005 mm Hg). Yield 210 g 67 %. NMR spectra in accord with proposed structure.

10. Synthesis of Diisopropyl (S)-(E)-3,4-O-Isopropylidene-3,4-dihydroxybut-1-enyl-1-phosphonate.

To a three-necked round bottom flask equipped with addition funnel, condenser, nitrogen inlet tube and magnetic stirrer is added 32.0 g (0.093 m) tetraisopropyl methylenebis phosphonate and 500 mL dry heptane. The system is flushed with dry nitrogen for 30 minutes. Seventy milliliters of n-butyl lithium (1.6 M in hexanes) is added dropwise to the solution. The mixture is stirred at room temperature for two hours during which it develops a golden yellow color.

The reaction mixture is cooled to 0 C with an ice bath. O-Isopropylidene-D-glyceraldehyde (15 g, 0.115 m) in 50 mL hexane is added to the solution, followed by a rinse of 50 mL hexane. The ice bath is removed and the system allowed to warm to room temperature. The solution is stirred under nitrogen overnight and then heated to reflux for two hours. The solution is washed twice with 600 mL distilled water. The aqueous layers were washed with 600 mL heptane. The organic layers were combined and dried over magnesium sulfate. The solution was filtered to remove drying agent then evaporated under reduced pressure to give a dark yellow residue. The residue is distilled (125-130 C, 0.01 mm Hg) under vacuum to give a colorless liquid. Yield 13.6 g, 0.047 m) 89 %. NMR spectra in accord with expected structure.

Note: thin layer chromatography in ethyl acetate: hexanes (9:1) and chloroform on silica gel revealed the presence of trace amounts of starting material (TIMBP) in both the crude material and the distilled product. The relative points at which TIMBP and the vinylic product distill are so close, total separation of large batches, even when the distillation is very carefully performed is almost impossible. Since the material tends to decompose significantly upon prolonged heating, some further synthetic work was done using the crude product with no significant change in results.

11. Attempted synthesis of Diisopropyl (S)-(E)-3,4-O-Isopropylidene-3,4-dihydroxybut-1-enyl-1-phosphonate with sodium hydride.

In a 500 mL round bottom flask equipped with addition funnel, condenser, nitrogen inlet tube and magnetic stirrer is placed 230 mL dimethylsulfoxide. The system is flushed with dry nitrogen for 30 minutes. A 50 % oil suspension of NaH (6.0 g, 0.125 m) is added to the solvent. The solution is heated to 75 C for 45 minutes to dissolve the hydride then cooled to 0 C with an ice bath. TIMBP, 34.4 g (0.100 m) is added dropwise to the reaction mixture. The ice bath is removed and the solution stirred at room temperature for

two hours. O-Isopropylidene-D-glyceraldehyde (16.3 g, 0.125 m) in 100 mL DMSO is added dropwise to the solution. The mixture is stirred for one hour at room temperature followed by one hour at 60 C. After cooling and quenching with water, the solution is poured into 1.4 L of cold water (0 C) and stirred for several minutes. The DMSO-water mixture is washed five times with 225 mL of diethyl ether. The ethereal layers were washed twice with 85 mL water. The organic layers were combined and dried over magnesium sulfate. The solution was filtered and then evaporated under reduced pressure. The NMR spectra revealed vinylic protons were absent. A large percent of the starting material TIMBP could be recovered.

12. Synthesis of Diisopropyl (S)-(E)-3,4-dihydroxybut-1-enyl-1-phosphonate.

To 8.0 g (0.027 m) of diisopropyl isopropylidene-3,4-dihydroxybut-1-enyl-1-phosphonate was added 100 mL of 1 % aqueous HCl. The solution was stirred overnight at room temperature. The solvent was removed by evaporation under reduced pressure to give a red oil as product. NMR spectra revealed the isopropylidene group had been cleaved to give free hydroxyl functions. Yield 6.7 g, 0.026 m, 97 %.

13. Alternate Deprotection of Diisopropyl (S)-(E)-3,4-O-Isopropylidene-3,4-dihydroxybut-1-enyl-1-phosphonate.

Biorad Dowex 50W-X8 was stirred in water at 100 C for 48 hours. The material was washed with 0.1 N NaOH, followed by water, 0.1 N HCl, and again by water. To 6.0 g of the diisopropyl vinyl phosphonate (0.021 m) was added 50.0 g (dry weight) of the prepared Dowex (0.255 meq) and 50 mL water. This mixture was stirred at room temperature for 48 hours and then filtered. The Dowex residue was washed three times with water and then with absolute ethanol. The filtrates were evaporated under reduced pressure. The residue was treated several times with small amounts of isopropanol to remove traces of water. Yield 4.2 g, 0.017 m, 81 % of a deep red product. Although the NMR spectra matched that of the HCl treated material (method 12) a deep red colored persisted. This is believed to come from trace amounts of organic dyes leached from the ion exchange resin.

14. Synthesis of Diisopropyl (S)-(E)-3,4-Dipalmitoylbut-1-enyl-1-phosphonate.

To 2.0 g (0.0080 m) of the diisopropyl 3,4-dihydroxyvinyl phosphonate is added 95 mL chloroform and 1.9 g (0.024 m) pyridine. The solution is chilled to 0 C with

an ice bath. Freshly distilled palmitoyl chloride (6.6 g, 0.024 m) in 20 mL chloroform is added dropwise. The solution is warmed to room temperature and allowed to stir in the dark for 48 hours. The solution was heated to 41-2 C for two hours, cooled, and then added to 300 mL of diethyl ether. The ethereal solution was washed three times each with 100 mL of 0.1 N sulfuric acid, water (0 C), 10 % sodium thiosulfate, and water. The combined aqueous layers were washed with 100 mL ether. The organics were combined and dried over sodium sulfate. The solution was filtered and evaporated under reduced pressure to give a yellow residue. The material was purified on a silica gel column (30 x 4.5 cm) packed and eluted with chloroform. Fractions with a single spot R_f=0.44 in chloroform were combined. The solvent was evaporated. The residue was recrystallized from hot methanol. Yield 4.4 g, 0.0060 m, 75 %. NMR spectra in accord with proposed structure. Analysis: $C_{42}H_{81}O_7P$ requires 69.19 % C and 11.20 % H, actual values 69.37 % C and 11.31 % H.

15. Synthesis of Diisopropyl (S)-(E)-3,4-Dicaproylbut-1-enyl-1-phosphonate

To a solution of 3.5 g (0.014 m) of the diisopropyl 3,4-dihydroxyvinylphosphonate in 100 mL of chloroform was added 2.2 g (0.028 m) of pyridine. The solution was chilled to 0 C

with an ice bath. To the cold mixture, was added dropwise 6.6 g (0.035 m) of freshly distilled capryl chloride. The mixture was warmed to room temperature and stirred in the dark for forty eight hours. The solution was heated to 42 C for three hours. Diethyl ether (500 mL) was added to the mixture and it was washed with 225 mL 0.1N H_2SO_4 four times, followed by washing three times with 250 mL of water, 10% sodium thiosulfate, and water. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The procedure gave 12.8 g of a crude yellow semi-solid. The material was purified in several batches by column chromatography. Approximately 4.0 g of crude product was passed through a silica gel column packed in chloroform (5 x 41 cm). Twenty mL fractions were collected automatically. Fractions 65 through 100 with a single spot on TLC (silica gel in chloroform) R_f=0.55 were combined. The solvent was evaporated under reduced pressure to give a yellow oil as product. NMR in accord with proposed structure. Analysis: $C_{30}H_{57}O_7P$ requires 64.26 % C and 10.25 % H; actual 64.29% C and 10.60 % H. Yield 6.5 g, 0.011m, 78 %.

16. The Synthesis of (S)-(E)-Dipalmiyoylbut-1-enyl-1-phosphonic Acid

To a dry nitrogen flushed system consisting of a 100 mL three necked round bottom flask equipped with addition funnel, condenser and gas inlet tube was added 25 mL of diethyl ether and 5.1 g (0.007 m) of diisopropyl 3,4-dipalmitoylbut-1-enyl-1-phosphonate. A ten-fold excess of trimethylbromosilane (10.7 g, 0.070 m) was added dropwise. The solution was stirred under nitrogen overnight. (Note: solution can be stirred for up to four days with no significant change in yield.) The mixture was washed three times with 35 mL of water (0 C). The ethereal layer was dried over sodium sulfate, filtered and evaporated. The crude solid residue was purified by recrystallization from methanol to give white crystals with melting point 65-67 C. Yield 68.8 %, 3.1 g, 0.0048 m. NMR in accord with proposed structure. Analysis: $C_{36}H_{69}O_7P$ requires 67.05 % C and 10.78 %H, actual results 67.30 % C and 10.89 % H.

17. Synthesis of (S)-(E)-Dicaproylbut-1-enyl-1-phosphonic Acid.

To a dry nitrogen flushed system consisting of a 25 mL round bottom flask, condenser, and gas inlet tube was added 1.76 g (0.0031 m) of the diisopropyl 3,4-dicaproylbut-1-enyl-1-phosphonate. Trimethylbromosilane (0.062 m, 8.95 g) was added dropwise to the yellow oil. The solution darkened in

color when stirred under nitrogen for forty eight hours. Diethyl ether, 50 mL, was added tc the reaction mixture followed by washing with water (35 mL three times). The ethereal layer was dried over sodium sulfate, filtered and evaporated under reduced pressure to give 1.75 g of a yellow semi-solid. The crude product was purified by elution through a column of silica gel (2.5 x 40 cm) with ethyl acetate. This was followed by rechromatographing the material through a second column packed in chloroform and eluted with a chloroform:methanol gradient 100:0 through 0:100. The material containing fractions of a single spot with R_f=0.45 in chloroform were combined and evaporated to give a yellow semisolid. The residue was dissolved in a minimum of chloroform and passeed through a Gelman alpha metricel filter to remove dissolved silica gel from the solvents. This was again evaporated under reduced pressure. NMR in accord with proposed structure. Yield 0.80 g, 0.0017 m, 55 % yield. Analysis $C_{24}H_{45}O_7P$ requires 60.49 % C and 9.52 % H actual results 60.20 % C and 9.43 % H.

18. Synthesis of Diisopropyl 3,4-dipalmitoylbutyl-1-phosphonate.

A 1.00 g (0.0014 m) sample of diisopropyl 3,4-dipalmitoylbut-1-enyl-1-phosphonate was dissolved in 250 mL of absolute ethanol. Five percent paladium/carbon (1.00 g) was added as a catalyst and the system hydrogenated under pressure (56 lbs/in.) for 48 hours until no further uptake of hydrogen could be observed. The solution was filtered through a bed of celite. Solvent was evaporated under reduced pressure to give a white crystalline residue. The product was dissolved in 35 mL of hot methanol and precipitated by the addition of water. The crystals were filtered and washed with methanol-water. Yield 0.570 g, 0.78 mmole, 56 %. Melting point 74-78 C. Spectra in accord with proposed structure. Analysis: $C_{42}H_{83}O_7P$ requires 69.00 % C and 11.44 % H. Experimental results: 68.75 % C and 11.64 % H.

19. Synthesis of Diisopropyl 3,4-Dicaproylbutyl-1-phosphonate.

A 1.00 g (0.0018 m) sample of diisopropyl 3,4-dicaprylbut-1-enyl-1-phosphonate was dissolved in 250 mL of absolute ethanol. Five percent palladium/carbon was added as a catalyst and the system hydrogenated at 55 lbs/in for three days until no further uptake of hydrogen was observed. The solution was filtered through a bed of Celite to remove the

catalyst. The filtrate was evaporated under reduced pressure to remove solvent. The residue was dissolved in chloroform and eluted from a silica gel column packed in chloroform (5 x 22 cm). Fractions with a R_f =0.56 were combined. The solvent was evaporated to 3 mL total volume and the solution filtered through a alpha metricel filter system. The remaining solvent was removed. Yield 0.437 g, 0.78 mmole, 44 %. yellow semi-solid. Spectra in accord with proposed structure.

20. Synthesis of 3,4-Dipalmitoylbutyl-1-phosphonic acid.

A 500 mg (0.68 mmole) sample of the diisopropyl 3,4-dipalmitoylbutyl-1-phosphonate was dissolved in 10 mL of diethyl ether in a 25 mL round bottom flask flushed with dry nitrogen. A ten-fold molar excess of trimethylbromosilane (0.0068 m, 1.04 g) was added dropwise and the solution stirred under nitrogen for 48 hours. Ten mL of diethyl ether was added and the solution washed three times with 5 mL of water. The ethereal solution was dried over sodium sulfate, filtered, and evaporated under reduced pressure. The white residue was recrystallized from chloroform:methanol 1:1 followed by recrystallization from methanol. There was thus obtained a white crystalline product of melting point 76-78 C. The product matched data published by Tang, et. al. as

far as chromatography (R_f=0.5 with chloroform:ethanol:formic acid:water 100:10:8:0.5) and spectra. Yield 220 mg, 0.34 mmole, 51 %.

21. Synthesis of 3,4-Dicaprylbutyl-1-phosphonic acid.

In a nitrogen flushed 50 mL round bottom flask equipped with condenser, addition funnel, and gas inlet tube was placed 2.0 g (0.0042 m) diisopropyl 3,4-dicaproylbutyl-1-phosphonate and 15 mL of diethyl ether. A ten-fold excess of trimethylbromosilane (6.4 g, 0.042 m) was added dropwise. The reaction mixture was stirred overnight at room temperature. An additional 15 mL of diethyl ether was added and the solution was three times with 10 mL of water. The organic layer was dried over sodium sulfate, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography using a silica gel column packed in chloroform and eluted with a chloroform-methanol gradient, 100: 0 through 1:10. Fractions with a R_f=0.46-48 were combined to give 1.2 g (0.0025 m, 59.6 %) of a yellow oil. Spectral and chromatographic data matched that of the material synthesized by method 4.

22. Synthesis of Diisopropyl 3,4-dipalmitoyl-1-hydroxybutyl-1-phosphonate.

At 0 C 2.35 g (0.0032 m) of the dipalmitoylvinyl phosphonate diisopropyl ester was dissolved in 20 mL anhydrous ether in a three necked round bottom flask equipped with condenser, gas inlet tube, and addition funnel. The system was flushed with dry nitrogen for thirty minutes. Borane-dimethyl sulfide complex (2 mL, 0.02 m) was added dropwise to the mixture. The solution was stirred at room temperature overnight. Twelve mL of absolute ethanol was added slowly. After significant evolution of hydrogen gas had stopped (1.5 hours) the solution was chilled to 0 C. Five mL of 3M NaOH was added slowly followed by 5 mL of 30 % hydrogen peroxide. The solution was stirred under nitrogen for 48 hours at room temperature. A large amount of white precipitate was evident. Forty mL of ether was added to the flask. The ethereal solution was washed four times with 10 mL portions of water. The precipitate present dissolved in the aqueous layer, and therefore was believed to be inorganic in nature. The aqueous layer was washed three times with small portions of ether. The organics were combined and dried over sodium sulfate. The solution was filtered and evaporated under reduced pressure to give a white solid residue. All of the residue was dissolved in a minimum of chloroform and applied to a silica gel column packed in chloroform (5 x 47 cm) The fractions containing a single spot R_f =0.36 were combined and evaporated. A white crystalline

product of melting point 53-57 C was isolated by recrystallization from methanol. Yield 0.76 g, 0.0010 m, 32 %. Spectra in accord with proposed structure. Analysis: $C_{42}H_{83}O_8P$ requires 67.52 % C and 11.20 % H; actual results 67.38 % C and 10.93 % H.

23. Synthesis of 3,4-Dipalmitoyl-1-hydroxybutyl-1-phosphonic Acid.

To a 25 mL round bottom flask equipped with addition funnel and gas inlet tube is added 500 mg (0.67 mmole) of 3,4-dipalmitoyl-1-hydroxybutyl-1-phosphonate diisopropyl ester in 10 mL of anhydrous ether. The system is flushed with nitrogen for 30 minutes. Trimethylbromosilane (7.2 mmole, 1.1 g) is added dropwise and the solution allowed to stir for 48 hours at room temperature. An additional 10 mL of ether is added and the solution washed three times with water. The ethereal solution is dried over magnesium sulfate, filtered, and evaporated to give a white solid residue. The residue was recrystallized from chloroform-methanol by addition of water. Yield 365 mg, 0.55 mmole, 82 %. Spectra in accord with proposed structure. Analysis: $C_{36}H_{71}O_8P$ requires 65.23 % C and 10.79 % H; actual results 65.00 % C and 10.86 % H.

24. Synthesis of Di.sopropyl 3,4-Dicapryl-1-hydroxybutyl-1-phosphonate.

In a 50 mL round bottom flask equipped with addition funnel, condenser, and gas inlet tube was placed 3.0 g (0.0053 m) of the diisopropyl 3,4-dicaprylbut-1-enyl-1-phosphonate and 25 mL anhydrous ether. The system was flushed with nitrogen and chilled to 0 C by means of an ice bath. Borane-dimethyl sulfide complex (4.0 mL, 0.04 m) was added to the reaction mixture dropwise. The solution was warmed to room temperature and allowed to stir overnight. Twenty mL of absolute ethanol was added slowly and the mixture allowed to stir for an additional 2 hours. The solution was again chilled to 0 C. Sodium hydroxide, 3M 10 mL, was added dropwise. This was followed by 10 mL of 30 % hydrogen peroxide. The system was allowed to return to room temperature and stirred for an additional 48 hours. Fifty mL of ether was added to the flask. The mixture was extracted four times with 15 mL of water. The organic layer was dried over sodium sulfate, filtered, and evaporated under reduced pressure. A yellow semi-solid was isolated as product. This residue was dissolved in a minimum of chloroform and purified by column chromatography on a 5 x 47 cm silica gel column packed in and eluted with chloroform. Fractions of a single spot with a R_f = 0.32 were combined and evaporated. Yield 1.2

g, 2.1 mmole, 40 %. Spectra in accord with proposed stucture.

25. Synthesis of 3,4-Dicapryl-1-hydroxy-butyl-1-phosphonic Acid.

In a 25 mL round bottom flask equipped with addition funnel and gas inlet tube was placed 500 mg (0.86 mmole) 3,4-dicapryl-1-hydroxybutyl-1-phosphonate diisopropyl ester and 15 mL anhydrous ether. The system was flushed with nitrogen for thirty minutes. A ten-fold excess of trimethylbromosilane (0.0086 m, 1.3 g) was added dropwise. The solution was allowed to stir overnight. Additional ether (10 mL) was added and the solution washed three times with 10 mL water. The ethereal layer was dried over sodium sulfate, filtered, and evaporated under reduced pressure. The yellow oil residue was purified by passage through a silica gel column (2.5 x 27 cm) packed in chloroform and eluted with a chloroform methanol gradient (chloroform:methanol 15:0 through 0:15). Fractions with a R_f=0.44 (chloroform:methanol:water 64:25:8) were combined and volatiles removed. The material was dissolved in a minimum of chloroform and passed through a Gelman alpha metricel filter system twice to remove inorganic particles. The product was a yellow semisolid with spectra in accord with proposed

structure. Yield 190 mg, 0.38 mmole, 44 %. Analysis: $C_{24}H_{47}O_8P$ requires 58.28 % C and 9.58 % H, results 58.19 % C and 9.47 % H.

26. Attempted Synthesis of Diisopropyl 3,4-Dipalmitoyl-1,2-epoxybutyl-1-phosphonate by m-Chloroperbenzoic Acid.

In a 50 mL round bottom flask was mixed 2.6 g (0.0036 m) diisopropyl 3,4-dipalmitoylbut-1-enylphosphonate and 15 mL methylene chloride. m-Chloroperbenzoic acid (0.75 g, 0.0043 m) in 15 mL methylene chloride was added dropwise over 20 minutes while the temperature of the system was maintained at 23 C by the addition of an external water bath. The solution was stirred overnight at room temperature. Excess peracid was destroyed by the addition of solid sodium sulfite. The solution was filtered and the precipitate washed with methylene chloride to give a total volume of 50 mL. The filtrate was washed three times with 50 mL of 5 % sodium bicarbonate, water, saturated sodium chloride and water. The organic layer was dried over potassium carbonate and filtered. Volatiles were evaporated under reduced pressure to give 1.53 g residue. Spectral studies clearly showed the presence of vinylic peaks in the NMR which indicated epoxidation had not taken place.

The above procedure was repeated with an increase in reaction temperature to 50 C and stirring for 24 hours with the same negative results.

27. Attempted Synthesis of Diisopropyl 3,4-Dipalmitoyl-1,2-epoxybutyl-1-phosphonate by the method of Marmor.

To a 0 C solution of 3.6 g (0.0048 m) 3,4-dipalmitoylbut-1-enyl-1-phosphonate diisopropyl ester in 10 mL pyridine was added 1.8 mL 4-6 % NaOCl (Chlorox). The solution was allowed to warm to room temperature and stirred for one hour. Twentyfive mL of water was added to the solution followed by 25 mL of chloroform. The system was stirred for 10 minutes. An additional 25 mL of chloroform was used to transfer the solution to a separatory funnel. The phases were separated and the aqueous layer washed with 25 mL of chloroform. The organic phases were combined and dried over potassium carbonate and filtered. The volatiles were evaporated under reduced pressure. NMR of the residue indicated the presence only of starting material with no significant reaction.

28. Synthesis of (S)-4-Hydroxy-1,2-Dipalmitoyloxybutane

To 2.25 g (0.0033 m) of (S)-4-benzyloxy-1,2-dipalmitoyloxybutane in 200 mL ethyl acetate was added 500 mg

10 % Pd on carbon. The solution was hydrogenated in a Parr apparatus at 56 psig overnight until no more hydrogen was taken up. The solution was filtered through a bed of celite to remove the catalyst. The solvent was evaporated to give a white solid residue. The product was recrystallized from hot methanol. Yield 93 %, 1.83 g of white crystals mp 57-60 C. Spectral data in accord with proposed structure.

29. Synthesis of (S)-3,4-dipalmitoyloxy-1-butanal.

To a solution of 1.10 g (0.0051 m) CrO_3-C_5H_5NHCl in 50 mL dichloromethane was added 0.082 g (0.00102 m) sodium acetate and 1.0 g of celite. The solution was stirred quickly while a solution of 2.0 g (0.0034 m) of the 1,2-dipalmitoyloxy-4-hydroxybutane in 15 mL dichloromethane was added dropwise. The reaction mixture was stirred overnight. Fifty mL of diethyl ether was added and the solution filtered through a bed of Florisil. The precipitate was washed four times with 50 mL of ether. The filtrate was evaporated to give a white solid material. Recrystallization from methanol yielded 1.0 g (0.0017 m), 33% white crystals. Spectra in accord with proposed structure.

30. Attempted Synthesis of (S)-3,4-Dipalmitoyloxy-2-chloro-butanal.

A 50 mL round bottom flask equipped with magnetic stirrer and addition funnel is chilled to -78 C with a dry ice-isopropanol bath. Twentyfive mL of dry dichloromethane was added followed by 500 mg (0.00086 m) 3,4-dipalmitoyloxybutanal. Eightythree uL of sulfuryl chloride (0.0010 m) in 15 mL methylene chloride added dropwise. The solution was gradually warmed to room temperature and allowed to stir for one hour. Solvent was then evaporated under reduced pressure. The 0.6 g of crude residue rapidly decomposed to a black gum.

The synthesis was repeated with several changes: use of an inert nitrogen atmosphere and stirring at either -25 C and 0 C. In all attempts, the synthesis failed. No starting material could be recovered.

31. Synthesis of 2,3-dipalmitoyloxy-1-propanal.

To a solution of dipalmitin (0.430 g, 0.000756 m) in 10 mL of dry methylene chloride was added 0.19 g (0.00227 m) anhydrous sodium acetate and 0.244 g (001134 m) pyridinium chlorochromate. The dark orange solution was allowed to stir at room temperature for two hours. Twentyfive mL anhydrous ether was added and the mixture stirred an additional 5-10 minutes. The solution was filtered through a bed of Florisil. The precipitated material was washed free of

product by three 10 mL portions of ether. The filtrate was evaporated under reduced pressure. The residue was bathed in dry nitrogen and frozen overnight. The material was dissolved in 25 mL ether and washed three times with 10 mL 5 % sodium bicarbonate and water. The ethereal solution was evaporated and the residue washed three times with 10 mL isopropanol. The white solid was recrystallized from cold isopropanol. Yield 0.300 g, 0.000529 m, 70 %. Spectral data in accord with proposed structure. The material was stored frozen in an inert atmosphere.

32. Synthesis of Diethyl 3,4-Dipalmitoyloxy-1,2-epoxybutyl-1-phosphonate.

A 25 mL round bottom flask equipped with septum, addition funnel, gas inlet tube, and stirrer was flushed with dry nitrogen for one hour. n-Butyl lithium in hexanes (0.51 mL, 0.00794 m) was added and the system chilled to -20 C with a dry ice-isopropanol bath. Five mL of dry THF was added and the system further chilled to -70 C. After stirring for 15 minutes, 0.15 g (0.000756 m) diethyl chloromethylphosphonate was added. An addition 5 mL of THF was added through the funnel as a rinse. The solution was stirred for one hour. The 3,4-dipalmitoyloxypropanal (0.43 g, 0.000758 m) was dissolved in 10 mL THF and added to the

system dropwise. The reaction mixture was stirred overnight. Five mL of water was added to quench and anion left. An additional 10 mL of THF was added and the solution washed four times with ether:methylene chloride 1:1. The organic layer was dried over magnesium sulfate, filtered, and evaporated under reduced pressure. Yield 0.538 g crude residue. The material was recrystallized from chloroform:methanol 1:1. Yield 0.401 g, 0.56 mmole, 71 %. Melting point 27-30 C. Spectra in accord with proposed structure. Analysis $C_{40}H_{77}O_8P$ requires 67.01 % C and 10.82 % H, actual 67.34 % C and 11.04 % H.

33. Attempted synthesis of 3,4-dipalmitoyloxy-1,2-epoxybutyl-1-phosphonate.

In a 25 ml round bottom was added 0.250 g (0.35 mmole) diethyl 3,4-dipalmitoyloxy-1,2-epoxybutyl-1-phosphonate and 10 mL anhydrous diethyl ether. Trimethylbromosilane (0.70 mmole, 0.11g) in 5 mL ether was added dropwise. The solution was stirred at room temperature for one hour. The solvent was removed under reduced pressure. NMR of the residue indicated that the proton signals of the oxirane system had shifted. It was concluded the epoxide has undergone nucleophilic attack by the bromide.

34. Attempted Synthesis of 3,4-Dipalmitoyloxy-1,2-epoxy-butyl-1-phosphonate.

To a 0.250 g (0.35 mmole) diethyl 3,4-dipalmitoyloxy-1,2-epoxy-butylphosphonate under nitrogen was added 0.16 g (1.05 mmole) trimethylbromosilane. This was stirred for one hour at room temperature. This was evaporated under high vacuum. To the residue was added sodium methoxide (1.05 mmole, 0.57 g) in 5 mL methanol. This was stirred under nitrogen overnight at room temperature. Evaporation of the material under reduced pressure followed by the addition of 0.12 g (1.05 mmole) chlorotrimethylsilane resulted in the decomposition of the product.

35. Synthesis of 3,4-dipalmitoyloxy-but-1-enylphosphonyl choline.

To 1.3 g (0.0020 m) 3,4-dipalmitoyloxy-but-1-enyl-1-phosphonate was added 37 mL dry pyridine and 3.3 g (0.012 m) choline tosylate. The temperature of the system was warmed to 50 C and 7.1 mL (10.2 g, 0.071 m) trichloroacetonitrile was added. The solution was stirred for 48 hours. Acetonitrile was added dropwise to precipitate a tan solid. This material was recrystalized from acetone to yield 1.2 g, 0.0016 m, 80 %. NMR in accord with proposed structure. Analysis $C_{41}H_{81}O_7NP$ requires 67.36 % C and 11.16 % H, actual 67.18 % C and 11.05 %H.

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