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Pratt, Clifford, Ph.D.

City University of New York, 1993

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A

ANALOGUES OF PHOSPHATIDYLINOSITOL AND RELATED MATERIALS

by

CLIFFORD PRATT

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

1993

This manuscript has been read and accepted by the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

3/17/93
Date

Chair of Examining Committee

3/23/93
Date

Executive Officer

Supervisory Committee

The City University of New York

ABSTRACT

Analogues of Phosphatidylinositol and Related Materials

by

Clifford Pratt

Advisor: **Professor Robert Engel**

The synthesis of a series of enantiomerically pure, differentially protected, D-*myo*-inositol molecules has been accomplished in which the hydroxyl functionalities on positions #1, #4 and #5 of the *myo*-inositol ring system have been left accessible for future derivatization as phosphonic acid type analogues of phosphatidylinositol and related materials. Also, a convergent synthesis of an isosteric phosphonic acid analogue of phosphatidylinositol has been accomplished in which a non-hydrolyzable P-C-C linkage is present in place of the normal P-O-C esteric linkage joining the phosphate and diacylglycerol portions of the molecule. This analogue also possesses a vinylic linkage present on the lipid backbone which will provide an electron rich site adjacent to phosphorus while maintaining an enzymatically non-labile carbon-phosphorus bond. This species should serve as an excellent analogue for studying the activity of enzymes such as phospholipase C which are known to act on the normal esteric P-O-C linkage of this molecule.

Dedicated to the memory of Richard Henry Hommel and Gordan Mayes.

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I would like to express my sincere appreciation to my mentor Dr. Robert Engel, whose patience, guidance, commitment to excellence and sense of humor helped me overcome the difficult hurdles of the last five years. Dr. Engel helped restore the confidence and faith that I lacked in myself so I could successfully complete this project. I thank you for your time, effort, and especially your friendship, all of which were greatly appreciated and will not be forgotten.

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I wish to express my love, admiration and deepest appreciation to the five most important people in my life; my father Fred, my mother Rose, my brother Doug, my sister-in-law Mary Ann, and my niece Michelle Ann. These people have believed in me from the very beginning, and have always given me their love and support in all of my pursuits through the worst of times, and the best of times. I cannot thank them enough for restoring my confidence and faith in life, God, and myself.

Finally I would like to thank all of my friends who supported me during these endeavors (even though they haven't the faintest idea of what I did!), especially Michael & Christine Schwendemann, Dave Roberto, Joe Reichfeld, Gabrielle & Brian Currie, Anthony Romano, The Nostravos Brothers, The Bridie's Bartenders, Rachel Phillips, Scott Scheidt, and Trish Lucas, all of whom had more faith in me than I had in myself. Your friendships have brightened and enriched my life, I love you all.

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INTRODUCTION

General

In recent years studies on the biochemical importance of phosphorylated inositol species and their role in cellular metabolism have increased dramatically. Phosphorylated inositols and phosphorylated inositol phospholipids have been recognized as indispensable in the mobilization and utilization of intracellular calcium stores, and have been observed in many different types of tissue. This recognition has resulted in an immense amount of work aimed at unraveling the biochemical pathways involving internal calcium release, and the actual involvement of phosphorylated inositols in this mechanism. Many reviews on the fundamental biochemistry have been written.[1-11]

In many types of eucaryotic cells, cells which contain a true nucleus, events that are calcium dependent have been found to involve phosphoinositides present within the plasma membrane. In addition to being structural components of the cell membrane, these phosphoinositides play an important role as second messenger in the mobilization and regulation of calcium from intracellular stores. Second messengers are molecules that convey signals from the receptors present in the plasma membrane to some enzyme or molecular system inside the cell that will carry out the instructions brought by the extracellular agonist. Examples of some of the types of cells utilizing phosphoinositides include: oocytes,[12] blood platelets,[13] pancreatic insulin secreting β-cells,[14] liver,[15] smooth muscle,[16] and brain.[17] The inositides appear to be the first intracellular transmitters for release of calcium due to extracellular stimuli. Extracellular stimuli take many different forms depending on the type of cell being targeted including:

acetylcholine,[18] adrenaline,[19] vassopressin,[20] platelet activating factor,[21] and glucose.[22]

The release of intracellular calcium stores control short term events such as contraction, secretion and metabolism.[23] It may also play a role in long term events such as cell growth and information storage in the brain.[3] An imbalance in the second messenger route may also be one of the reasons normal cells become cancerous.[24]

Basic Structure Of Phosphatidylinositides

Phosphatidylinositides make up less than 10% of the total phospholipids found in animal cells. Of this 10%, phosphatidylinositol makes up approximately 90%, and is found mainly in the endoplasmic reticulum. Only a small amount of phosphatidylinositol is found in the plasma membrane. Phosphatidylinositol has the following structure (Fig. 1).

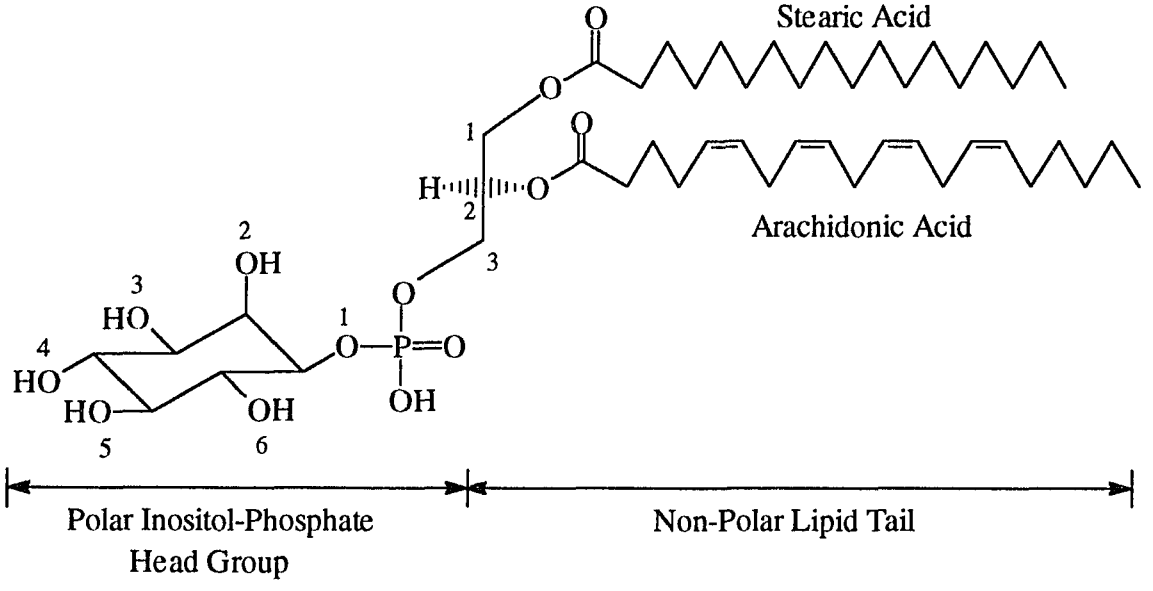


Fig. 1

Phosphatidylinositides contain a polar head group connected to a non-polar lipid tail by way of a phosphate ester linkage. This linkage is attached

to the #1 hydroxyl oxygen atom of the *myo*-inositol, and the #3 hydroxyl oxygen atom of the glycerol backbone. The tail portion consists of (predominantly) stearic acid and arachidonic acid chains which are connected to respectively the #1 and #2 hydroxyl oxygen atoms of the glycerol backbone by way of ester linkages.

As most of the phosphatidylinositol is present in the endoplasmic reticulum, it is not accessible to the proteins and enzymes that participate in extracellular hormonal stimulation. It appears that phosphatidylinositol-4,5-diphosphate (PIP_2) is the first phosphoinositide involved in intracellular calcium release from external stimuli, and it is therefore no surprise that most of the PIP_2 resides in the inner leaflet of the plasma membrane.[11] PIP_2 is structurally similar to phosphatidylinositol, except that the #4 and #5 hydroxyl oxygen atoms on the *myo*-inositol head group are esterified with phosphate groups (Fig. 2).

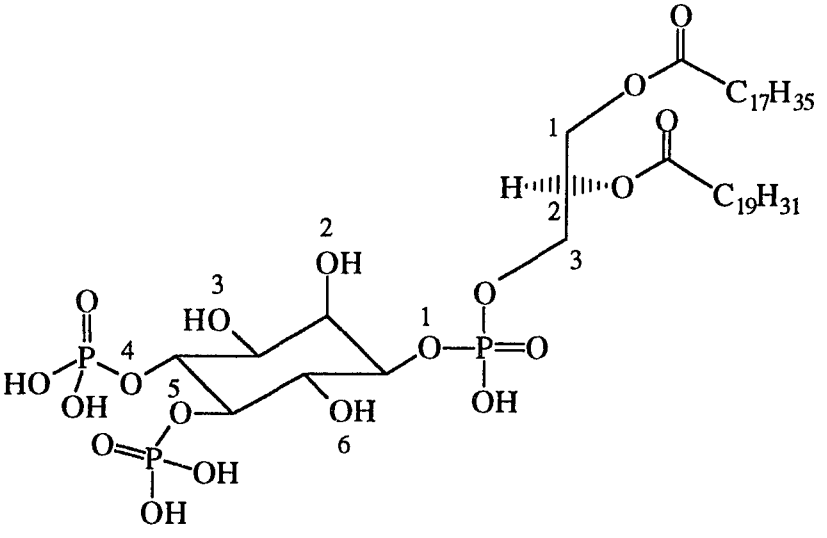


Fig. 2

Mechanism of Calcium Release

Intracellular calcium release begins with the extracellular binding of an agonist, e.g. acetylcholine, to a membrane bound receptor protein (R). Agonists are molecules that fit into and activate specific receptors present on the extracellular surface of the plasma membrane. Conformational changes in the receptor stimulate a GTP binding protein to supply energy to a phosphate ester cleaving enzyme, specifically phospholipase C. Once activated, phospholipase C will bind to PIP_2 and cleave the phosphodiester linkage between the #3 alcohol oxygen atom of the glycerol backbone and the phosphorus atom of the ester. After the bond is broken, a hydroxyl group is added to the phosphorus atom and a proton is added to the oxygen atom. Both the hydroxyl group and proton are supplied by water that is present in the plasma membrane.[25] Complete cleavage leads to inositol-1,4,5-triphosphate (IP_3), and diacylglycerol (DG) (Fig. 3).

The diacylglycerol portion remains in the plasma membrane, while the inositol-1,4,5-triphosphate portion leaves the membrane and enters the protoplasm. The IP_3 travels through the intracellular fluid until it reaches the endoplasmic reticulum. Once there, IP_3 binds to specific receptors that are associated with calcium channels on the surface of the reticulum. This binding causes the calcium channels to open such that calcium is liberated into the cytosol. The released calcium can then be utilized for calcium dependent processes such as cellular contraction and secretion.[26-28]

The release of calcium into the cytosol is a short lived process owing to the rapid degradation of IP_3 into inactive forms. The calcium releasing properties of IP_3 appear to be triggered by the pair of vicinal phosphate groups on the #4 and #5 positions. The phosphate present at the #1 position seems to enhance the affinity of IP_3 for its receptor.[29]

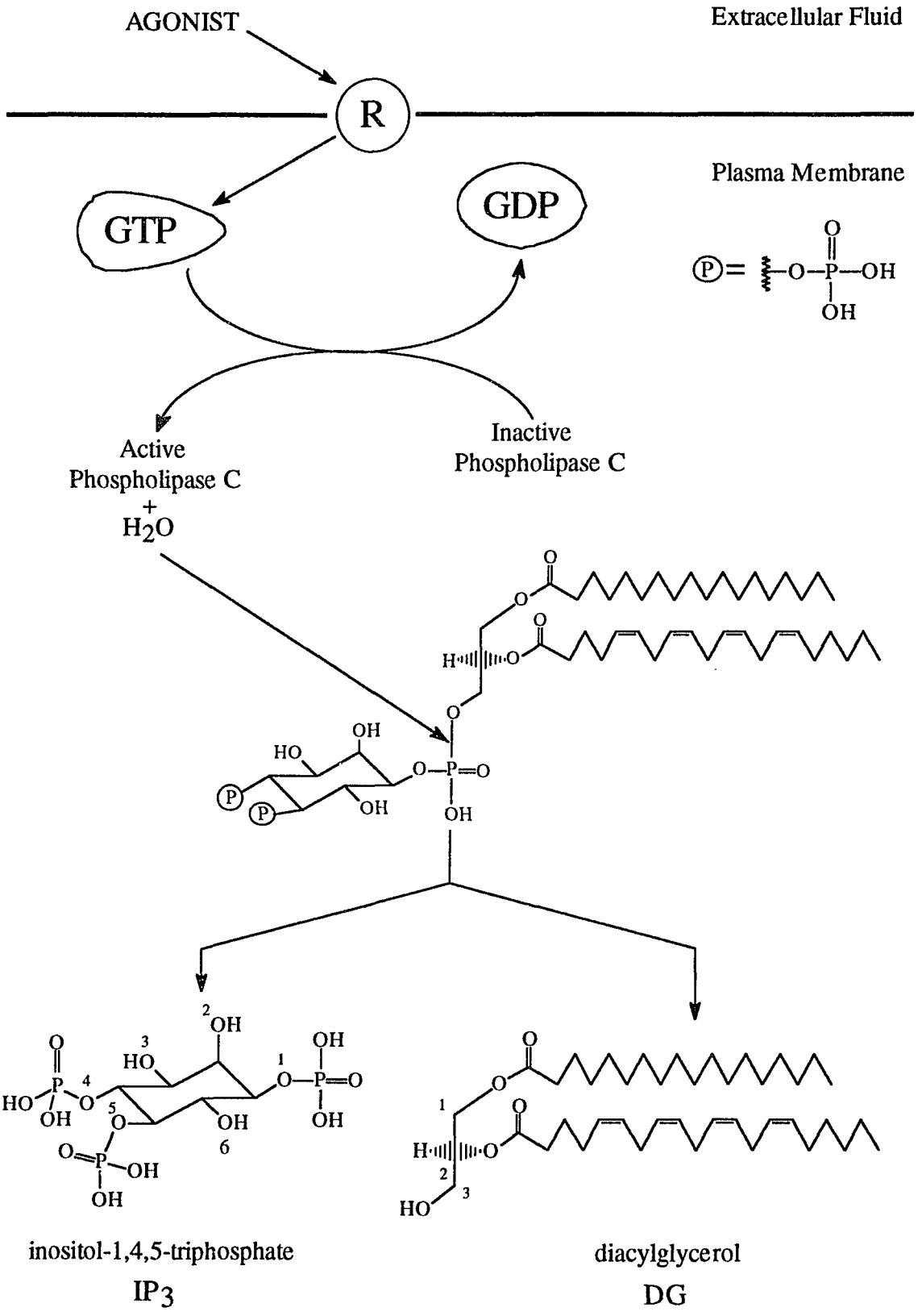

AGONIST
Extracellular Fluid
R
Plasma Membrane
GTP
GDP
Active
Phospholipase C
+
H_2O
Inactive
Phospholipase C
inositol-1,4,5-triphosphate
IP_3
diacylglycerol
DG

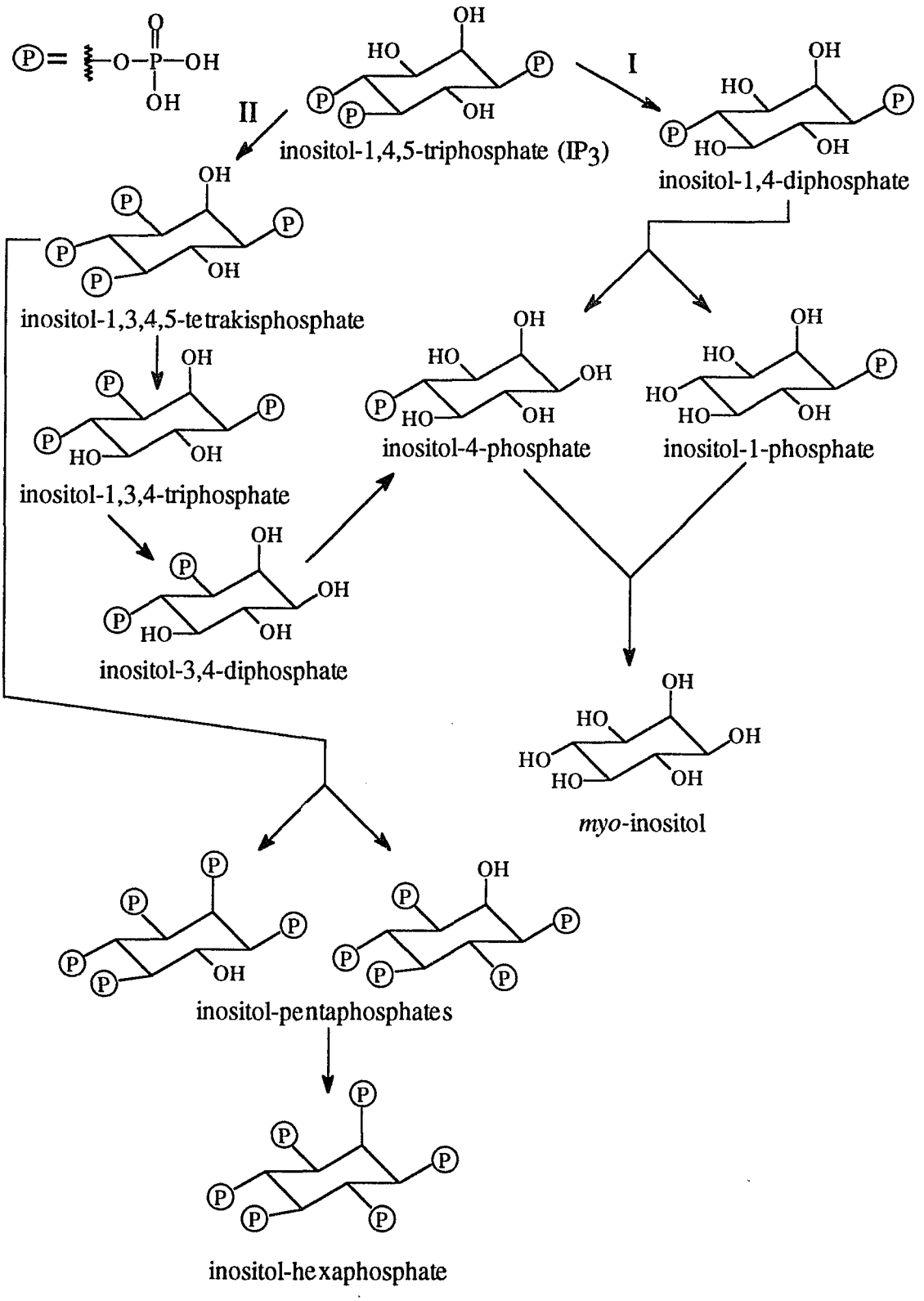
Fig. 3

Metabolic Pathways

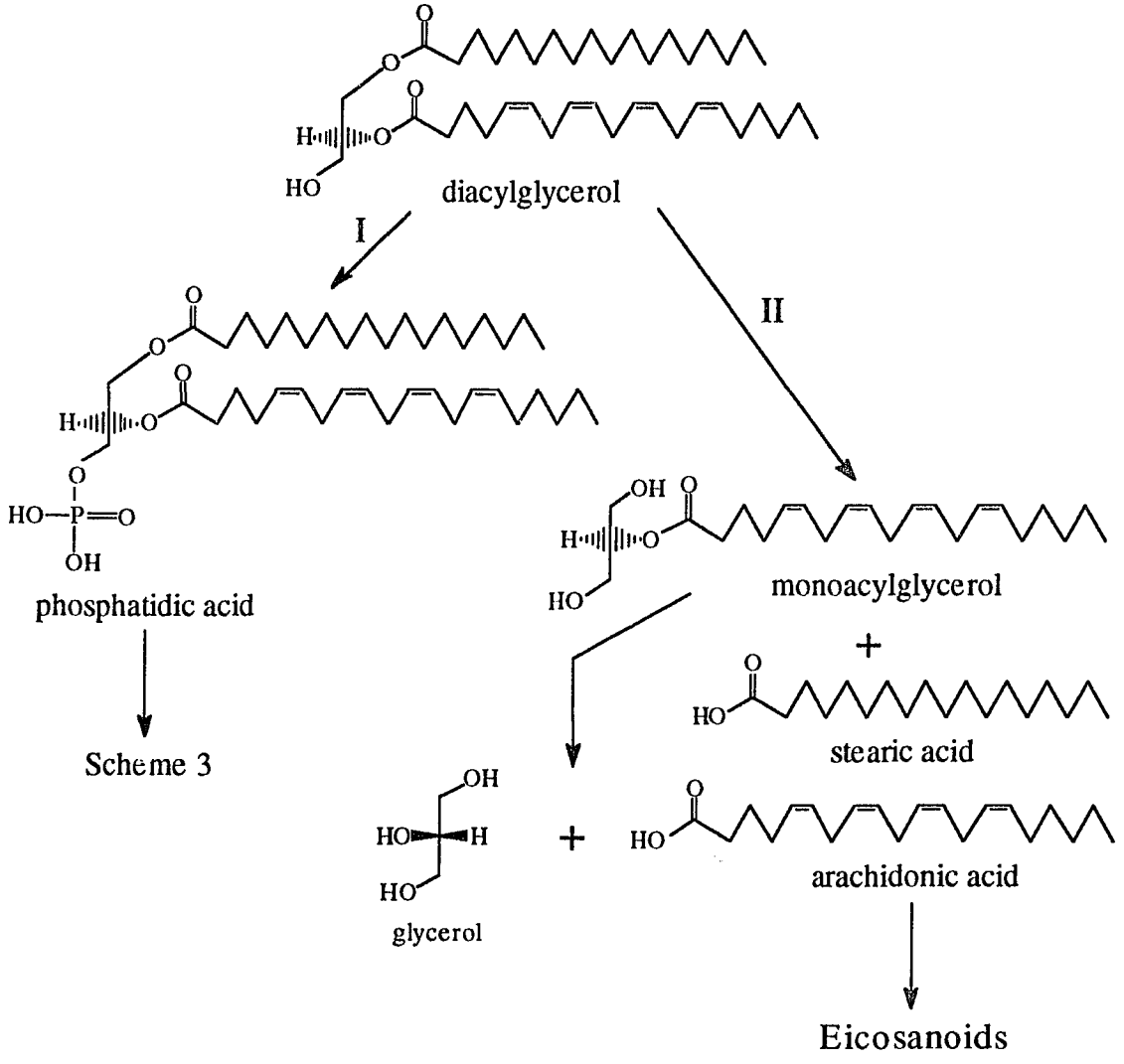
IP_3 is metabolized along either of two existing pathways. One involves hydrolysis of the phosphate ester from the #5 position by the action of inositol-triphosphatase.[30] This attenuates the second messenger activity of IP_3 by removing the phosphate from the #5 position to produce inositol-1,4-diphosphate, which is devoid of further calcium releasing activity. Inositol-1,4-diphosphate is then non-specifically hydrolyzed to inositol-1-phosphate, and inositol-4-phosphate, by an inositol-biphosphatase.[31] The final step involves the hydrolysis of the remaining phosphate ester by inositol-monophosphatases to give *myo*-inositol, which is then combined with phosphatidic acid to form new phosphoinositides.[32]

The second pathway for IP_3 metabolism begins with phosphorylation of IP_3 to inositol-1,3,4,5-tetrakisphosphate by inositol-1,4,5-triphosphate-3-kinase.[33] The inositol-1,3,4,5-tetrakisphosphate can be further phosphorylated to the inositol-pentaphosphates and inositol-hexaphosphate, or degraded via an alternative route to *myo*-inositol.[34,35] Both pathways for IP_3 metabolism are shown in Scheme 1.

When phospholipase C cleaves phosphatidylinositol-4,5-diphosphate, the diacylglycerol portion, which remains in the plane of the plasma membrane, will also function as a second messenger by serving to activate an enzyme known as protein kinase C. In addition to diacylglycerol, protein kinase C requires both calcium and phosphatidylserine to become fully activated.[6] When fully active, protein kinase C is responsible for phosphorylation of serine and threonine amino acid residues of specific proteins. These proteins are believed to be involved in the physiological responses caused by calcium release, especially cell secretion and proliferation.[6]


P= O–P–OH
OH
II
I
inositol-1,4,5-triphosphate (IP3)
inositol-1,4-diphosphate
inositol-1,3,4,5-tetrakisphosphate
inositol-4-phosphate
inositol-1-phosphate
inositol-1,3,4-triphosphate
inositol-3,4-diphosphate
myo-inositol
inositol-pentaphosphates
inositol-hexaphosphate

Scheme 1



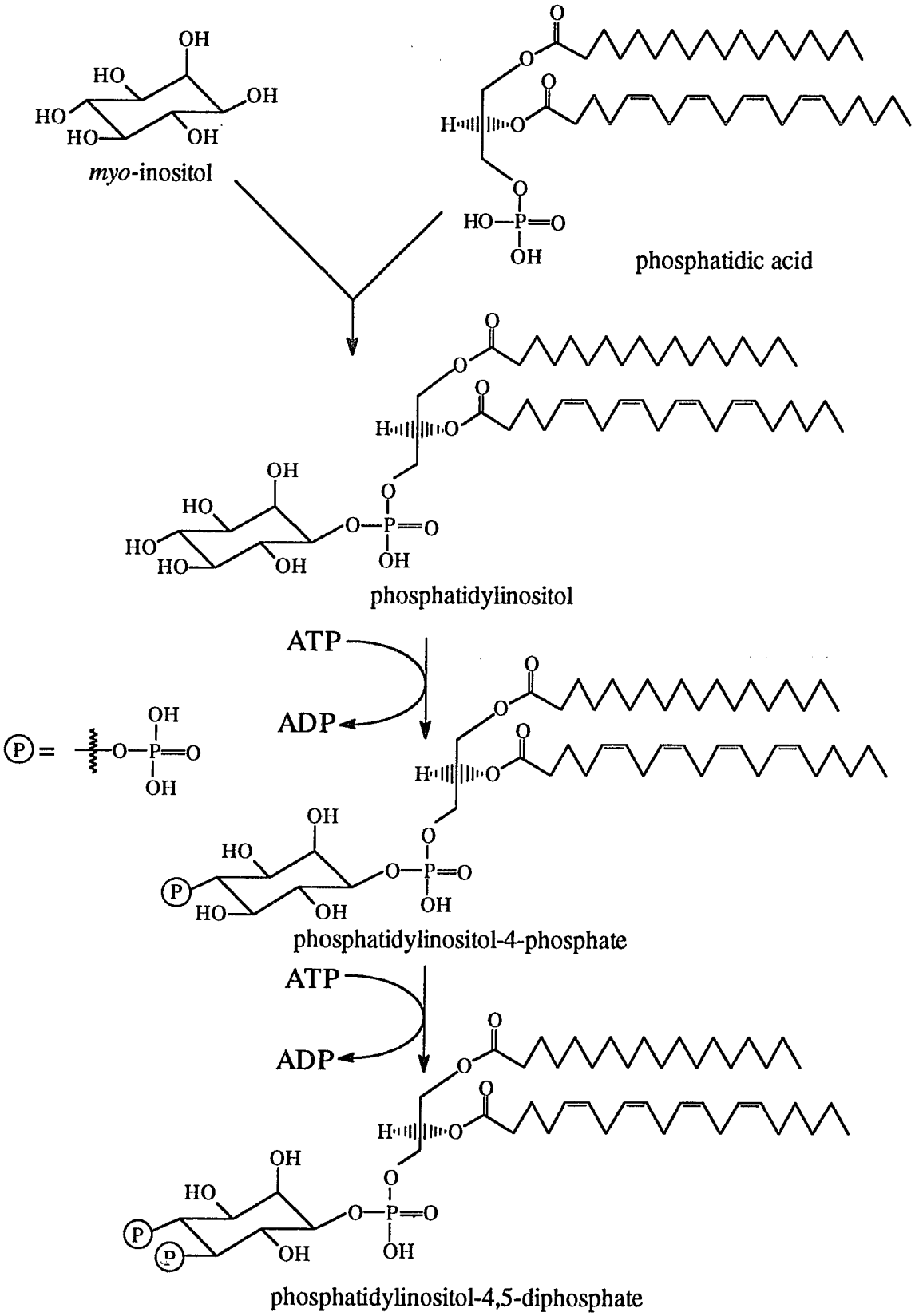
Scheme 2

Metabolism of diacylglycerol can also be accomplished via two pathways. One involves phosphorylation of the diacylglycerol, by way of a diacylglycerol kinase and adenosine triphosphate (ATP), to form phosphatidic acid. Phosphatidic acid is then acted upon by the enzyme CTP-phosphatidate cytidyl transferase which utilizes cytidine triphosphate (CTP) to make diacylglycerol cytidine diphosphate. This compound is then acted upon by the enzyme CDP-diacylglycerol inositol phosphatidate transferase which combines diacylglycerol cytidine diphosphate and *myo*-inositol to give phosphatidylinositol. Phosphatidylinositol is further phosphorylated to,

phosphatidylinositol-4-phosphate by phosphatidylinositol kinase and ATP. Phosphatidylinositol-4-phosphate is then phosphorylated at the #5 inositol position by phosphatidylinositol (4) P kinase and ATP which forms phosphatidylinositol-4,5-diphosphate.[1,2]

The second pathway begins with hydrolysis of the stearic acid group from diacylglycerol by diacylglycerol lipase to form a monoacylglycerol, that is further hydrolyzed to glycerol and arachidonic acid by monoacylglycerol lipase.[6] The stearic acid, arachidonic acid, and glycerol may all be used as building blocks in the reformation of phosphatidic acid. However, the arachidonic acid can be used in the formation of the eicosanoids, which include compounds such as the prostaglandins, thromboxanes, and leukotrienes, all being local hormones that regulate many cellular functions.[3,4] The diacylglycerol metabolic pathways are shown in Schemes 2 and 3.

It is known that the second messenger characteristics of phosphatidylinositol consist of two limbs of a bifurcating signal pathway which control short term cellular responses such as secretion, contraction, and metabolism. Inositol-1,4,5-triphosphate seems to be the central control for internal calcium release, while diacylglycerol seems to function as an internal feedback system, designed to modulate the cellular processes that are activated by this calcium release. The complex feedback interactions between the inositol-1,4,5-triphosphate, and diacyl glycerol pathways lead to an efficient signaling system that will rapidly alter cellular activity in response to appropriate external stimuli.[2]


myo-inositol
phosphatidic acid
phosphatidylinositol
ATP
ADP
phosphatidylinositol-4-phosphate
ATP
ADP
phosphatidylinositol-4,5-diphosphate

Scheme 3

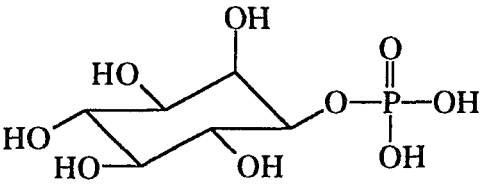
Phosphonates as Antimetabolites

Given the current understanding of the role phosphoinositides and inositol phosphates play in the release and utilization of intracellular calcium stores, the development of structural analogues can serve as probes for the further elucidation of the involved metabolic processes, and as possible regulators for these processes. Preparation of these analogues has been the focus of this research.

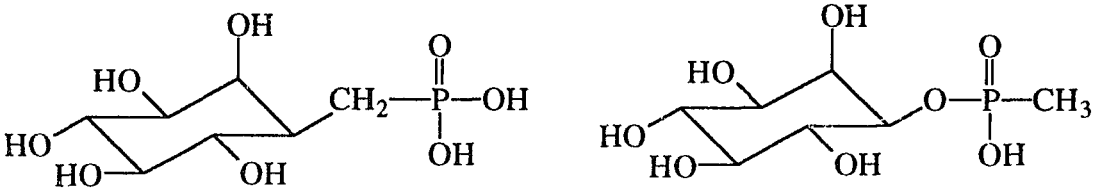
Choosing the correct type of analogue to serve as an effective antimetabolite is a difficult problem. However, a particularly significant group of structural analogues that are well suited for the replacement of phosphoinositides and inositol phosphates are the structurally similar phosphonic acids and their derivatives. The use of isosteric and isopolar phosphonic acid species as analogues for natural phosphates to serve as probes, or antimetabolites, is not new, and has been previously reviewed.[36-38]

The theory behind using this type of analogue focuses on the structure of the phosphate ester portion of the molecule in question. A phosphonic acid analogue is one where a specific oxygen-phosphorus bond is replaced by a carbon-phosphorus bond. The new carbon-phosphorus bond can be in place of either the normal phosphate ester linkage, or a free hydroxyl (Fig. 4).

One presumes that the carbon-phosphorus bond of these analogues will be incapable of being hydrolyzed by the usual enzymes involved in phosphate cleavage, and therefore may be capable of inhibiting one or more enzymatic processes.



typical phosphate ester linkage



phosphonic acid analogues

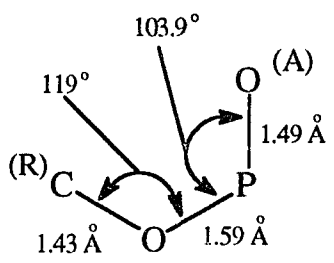
Fig. 4

A major factor that must be considered in the synthesis of phosphonic acid analogues is the physical size and shape of the altered portion of the molecule. If the shape of the altered area is significantly different than that of the natural phosphate ester, one may obtain an analogue that displays unnatural conformations, and thus may result in a large variation of its expected biochemical and physiological activity. For this reason isosteric phosphonic acid analogues are used whenever possible. The term isosteric strictly refers to compounds of identical size and shape. Therefore, the analogues that are synthesized should mimic the natural compound's size, and conformations, as closely as possible.

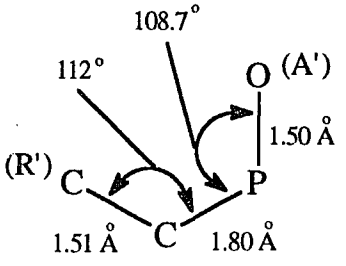
When a methylene group is used in place of a normal esteratic oxygen, one can expect non-trivial differences in both bond length and angles (Fig. 5).

Crystallographic studies [39-43] of this type of analogue reveal that in spite of these length and angle differences, there is only a slight variation in the distance found between the phosphoryl, or phosphonyl oxygen (A & A'),

and the functional groups (R & R'). Therefore this type of analogue will fit reasonably well into the isosteric category.



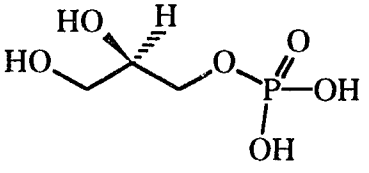
normal phosphate ester



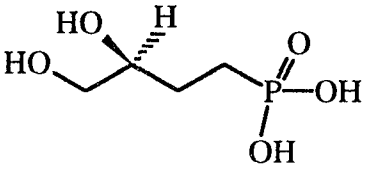
methylene type phosphonic acid

Fig. 5

Methylene phosphonic acids have been widely used as probes and antimetabolites in lipid chemistry. The isosteric chiral phosphonic acid analogue of naturally occurring *sn*-glycerol-3-phosphate was synthesized by Tang, *et al.* [44] starting with optically active malic acid (Fig. 6).



naturally occuring
sn-glycerol-3-phosphate
(*S*)-2,3-dihydroxypropyl-1-phosphate



phosphonic acid analogue
(*S*)-3,4-dihydroxybutyl-1-phosphonic acid

Fig. 6

The rationale behind synthesizing and studying this specific analogue is related to the importance of *sn*-glycerol-3-phosphate as both a branch product of glycolysis and a precursor of lipid biosynthesis. The analogue of glycerol-3-phosphate was tested on bacterial species that showed the capability of transporting it through their plasma membranes, specifically, suitable strains of *Escherichia coli*, and *Bacillus subtilis*. Low external concentrations of this analogue caused significant perturbation of

phosphatidylglycerol synthesis, and bacterial growth inhibition was therefore observed.[45-49]

Growth inhibition is caused by the analogue replacing the naturally occurring phosphate in the reaction catalyzed by CDP-diglyceride:*sn*-glycerol-3-phosphate phosphatidyltransferase, resulting in a polar lipid material to be produced (Fig. 7), which in turn prevents phosphatidylglycerol synthesis, and seriously disrupts the cell's normal lipid composition. [50,51]

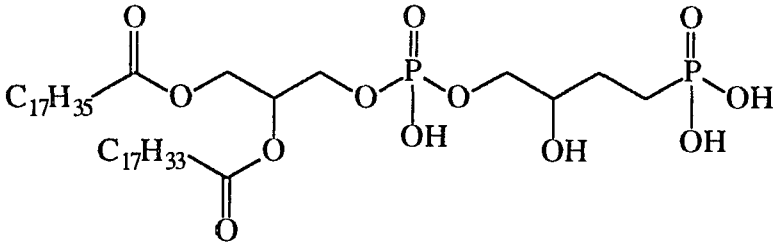
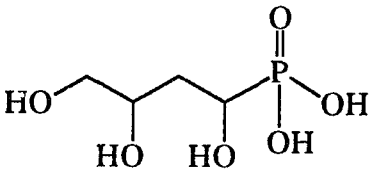


Fig. 7

As well as it may have worked inhibiting the growth of certain bacteria, the glycerol-3-phosphonic acid analogue did not appear to interact with acyl coenzyme A:*sn*-glycerol-3-phosphate acyltransferase, and several other related enzymes, upon in vitro enzymatic studies.[50] This lack of interaction appears to be caused by the loss of enzymatic binding capabilities resulting from the substitution of a methylene group for the esteratic oxygen of the natural substrate.

This type of binding problem prompted the synthesis of another analogue, one which was also isosteric with respect to the natural glycerol, but in addition also contained a hydroxyl group attached to the methylene carbon [44] (Fig. 8).



1,3,4-trihydroxybutyl-1-phosphonic acid

Fig. 8

Enzymatic binding due to the oxygen lone pairs in the natural substrate was hoped to be accomplished by the hydroxyl oxygen of this new analogue. Investigations of 1,3,4-trihydroxybutyl-1-phosphonic acid with acyl coenzyme A:*sn*-glycerol-3-phosphate acyltransferase showed that it did indeed serve as a substrate, and therefore reinforced the theory that prior inert activity was due to loss of proper enzymatic binding.[52]

Lalinde *et al.* [53] have synthesized *sn*-glycerol-3-phosphate analogues involving a vinylic phosphonic acid linkage present on the glycerol backbone (Fig. 9). Enzymatic binding due to electron density adjacent to the phosphorus atom should be maintained with this analogue as it was with the hydroxymethylene analogue shown above. This material has been found to be a weak antibacterial agent.

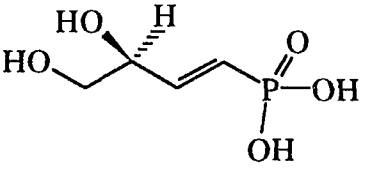


Fig. 9

There has been an exhaustive effort in the preparation of phosphatidic acid analogues containing carbon to phosphorus linkages. Many of these analogues have been prepared by substituting ether and hydrocarbon linkages in place of the usual fatty acid ester linkage.[54] Although these types of linkages exhibit further deviation form normal phospholipids, they

lend an inert character to the molecule without disturbing properties required in normal lipid interactions. Rosenthal *et al.* [55-57] have prepared systems where the carboxylate ester linkage is replaced by an ether linkage and is also isosteric about the phosphorus atom. In another related system all non-hydrocarbon functions of the lipid backbone were eliminated (Fig. 10).

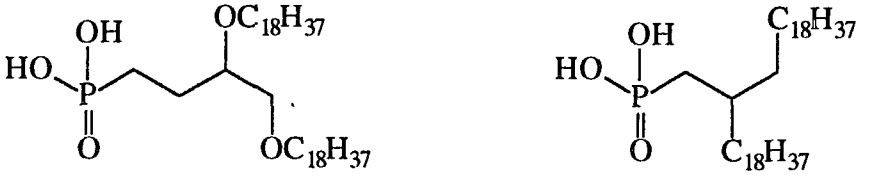
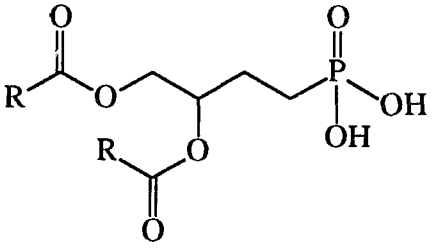


Fig. 10

These analogues are found to be inhibitors of pig kidney phosphatidate phosphohydrolase, and were also reported to cause complete growth inhibition of *Staphylococcus epidermis* and *Streptococcus salivarius*.[58] The degree of inhibition is related to the degree of dispersion, which is greatly diminished when the phosphate esteratic oxygen is removed.

Tang *et al.*[59] has succeeded in preparing phosphatidic acids that incorporated an isosteric backbone, where the length of the fatty acid chains have been both sixteen and eighteen carbons (Fig. 11).



$R = C_{16}H_{33}$ & $C_{18}H_{37}$

Fig. 11

These analogues have been found to exhibit low solubility compared to the natural systems,[59] and no antimetabolic investigations have been performed on them to date.

Rosenthal *et al.*[57,60] have synthesized a diversity of phosphonate species that are related to naturally occurring phosphatidylcholine. One type of analogue contains an isosteric phosphonate linkage, and diether backbone linkages to hydrocarbon chains (Fig. 12). Studies on this analogue proved it to be a significant inhibitor of phospholipase C from the pathogenic bacterium *Clostridium perfringens*.[61,62]

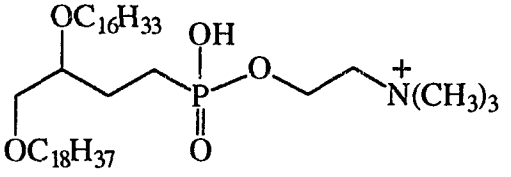


Fig. 12

The synthesis of a totally isosteric phosphonic acid analogue of phosphatidylcholine was accomplished by Braksmayer *et al.*[63] although no biochemical tests have been performed with it (Fig. 13).

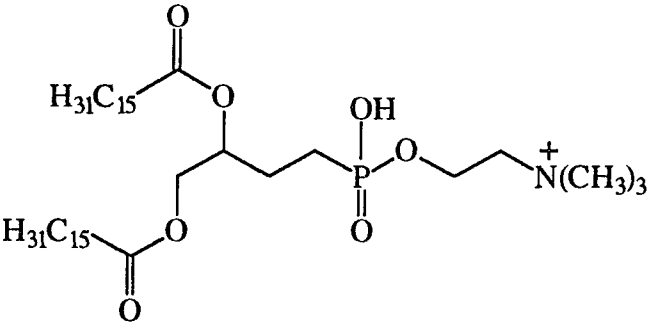


Fig. 13

Rosenthal *et al.*[62] have also succeeded in synthesizing an isosteric phosphonic diether analogue that is completely resistant to hydrolysis catalyzed by any normal phospholipase system (Fig. 14).

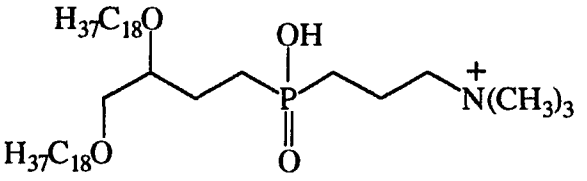


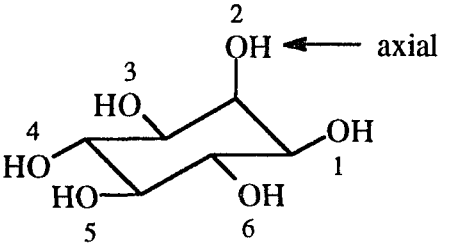
Fig. 14

Studies on this analogue find it to be a competitive inhibitor of phospholipase A from the venom of the cottonmouth pit viper *Agkistrodon piscivorus*,[64] and also an inhibitor of phospholipase C,[62] B and D.[65]

STATEMENT OF PROBLEM

The current research work has been concerned with the synthesis of appropriately protected *myo*-inositol molecules that can be derivatized at specific sites, then subsequently deprotected, to yield suitable phosphonic acid analogues of naturally occurring inositol phosphates, and phosphatidylinositols.

The major problem lies in the *myo*-inositol molecule itself. There are six hydroxyl functional groups contained in the molecule, one on each of the six carbon atoms of the ring. Five of these hydroxyl groups are positioned equatorially, and only one of them, the #2 hydroxyl, bears an axial orientation (Fig. 15).



myo-inositol

Fig. 15

Having the alcohol groups present in this type of arrangement will cause the molecule to have an overall plane of symmetry, and it therefore does not possess any molecular chirality (Fig. 16).

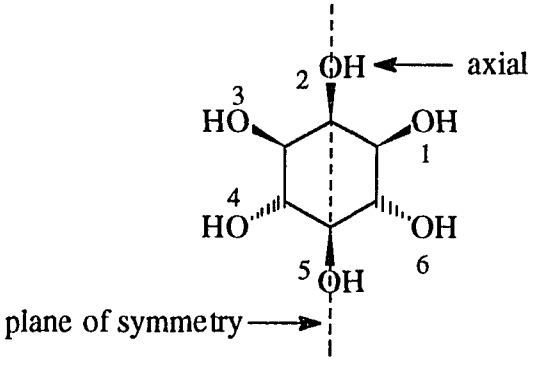


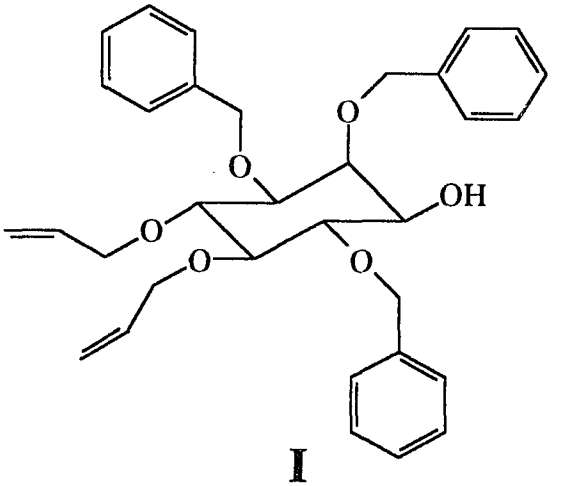
Fig. 16

However, once specific alcohol groups are protected, or derivatized, the plane of symmetry will no longer be present and one will obtain enantiomeric compounds. Because of this, one will need to perform an enantiomeric separation at some point during the synthesis. It is necessary that the enantiomeric molecules be separated so one can isolate the correct enantiomeric form of the *myo*-inositol derivative that is stereochemically equivalent to the one found in biological systems. The naturally occurring form of *myo*-inositol is known as "D-*myo*-inositol".

Once the protecting, derivatizing, and enantiomeric separation steps have been accomplished, the final obstacle will be the deprotection of the blocked hydroxyls without destroying or altering any of the derivatized sites on the analogue.

RESULTS AND DISCUSSION

The original focus of this project was to synthesize an inositol intermediate that would eventually be used in the preparation of several different types of analogues. This "common intermediate" one hoped to create was a selectively pentaprotected *myo*-inositol molecule where only the #1 position contains an open alcohol functionality. The remaining five alcohols would be protected in a selective manner using two different types of protecting groups. Alcohols #4 and #5 were targeted to be protected as allylic ethers, and benzylic ethers were to be made using alcohols #2, #3 and #6. This type of selective protection is desired so the #4 and #5 hydroxyls could be selectively deprotected for future analogue syntheses. Another requirement of this "common intermediate" was that it had to be enantiomerically pure prior to derivatization and deprotection. The structure of this proposed "common intermediate" (−)-2,3,6-tri-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**I**) is shown in Fig. 17.



I

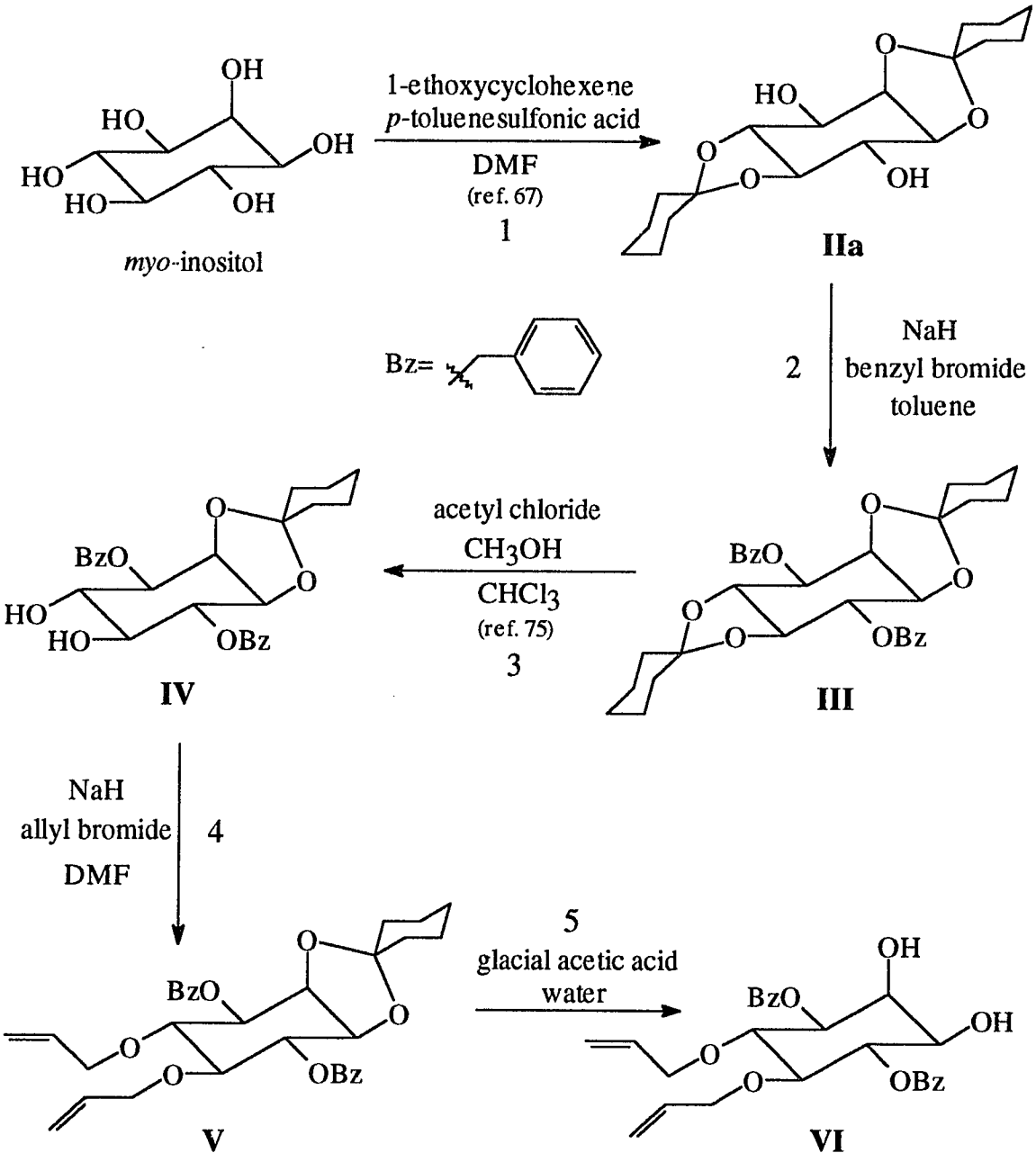
Fig. 17

In order to create the desired inositol intermediate **I**, the effort was begun by following the work reported by Ozaki *et al.*[66] which was

concerned with the total synthesis of optically active *myo*-inositol-1,4,5-triphosphate. By using some of Ozaki's procedures, it was hoped to protect correctly most of the alcohols in this system. Ozaki even reported a supposedly efficient method for the enantiomeric separation of the protected inositols so the isolation of the biologically correct D-*myo*-inositol enantiomer could be accomplished.

Unfortunately Ozaki's procedures were not easily reproduced, and were in fact very inefficient. Modification of these procedures had to be performed in order to obtain acceptable product yields. These experimental modifications enabled one to obtain a racemic differentially protected *myo*-inositol diol **VI** synthesized in five steps with good yield and only minimal experimental difficulty (Scheme 4).

In order to obtain (±)-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**VI**), as shown in Scheme 4, one must play a game of protecting and deprotecting specific alcohols until the desired order is reached. The most difficult and lowest yield reaction occurred in Step #1. This step involves the formation of ketal linkages between *myo*-inositol and cyclohexanone in order to block the reactivity of alcohols #1, #2, #4 and #5. Three structural isomers are formed from this reaction: (±)-1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol (**IIa**), (±)-1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (**IIb**), and (±)-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (**IIc**) (Fig. 18). After the reaction is complete, one is left with a dark brown viscous syrup, and in order to isolate the desired structural isomer **IIa**, a fractional crystallization had to be performed using a mixture of acetone and light petroleum ether. Only the (±)-1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol (**IIa**) crystallizes out, leaving isomers **IIb** and **IIc** in solution.


OH
HO
OH
HO
HO
OH
myo-inositol
1-ethoxycyclohexene
p-toluenesulfonic acid
DMF
(ref. 67)
1
IIa
Bz=
2
NaH
benzyl bromide
toluene
III
acetyl chloride
CH3OH
CHCl3
(ref. 75)
3
IV
NaH
allyl bromide
DMF
4
V
5
glacial acetic acid
water
VI

Scheme 4

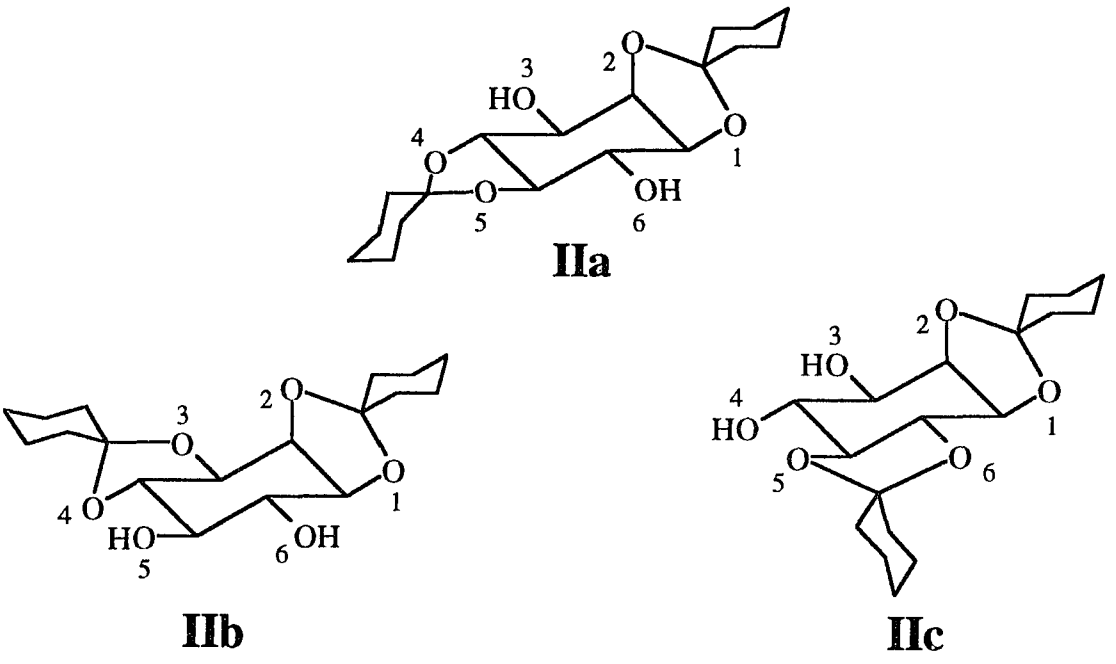
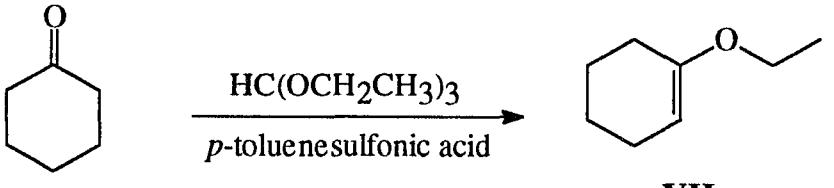


Fig. 18

Another difficulty related to this reaction was the acquisition of the enol ether reagent 1-ethoxycyclohexene (**VII**). This compound is not commercially available, and therefore it had to be synthesized from cyclohexanone and triethyl orthoformate (Scheme 5). Synthetic attempts for the preparation of 1-ethoxycyclohexene were not very efficient at first, but after extensive modifications of published procedures,[68-70] pure 1-ethoxycyclohexene was obtained in very large quantities.

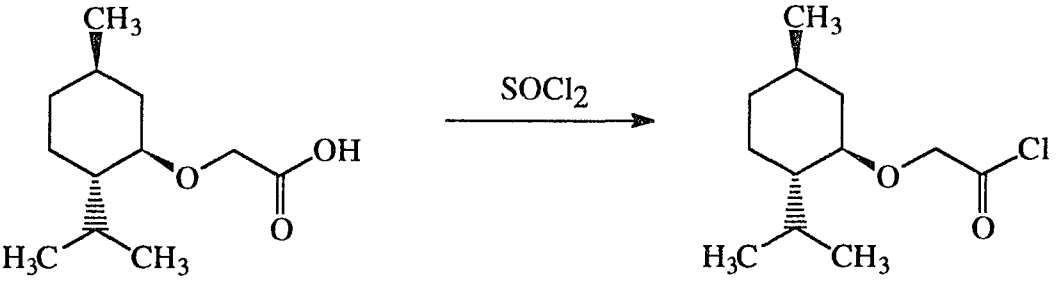


VII

Scheme 5

Since the protected inositol diol **VI** is racemic, it must be separated into its respective enantiomers before it can be used as an analogue. Ozaki's

group [66] reported a supposedly efficient method for enantiomeric separation by first derivatizing the diol with *l*-menthoxyacetyl chloride (**VIII**). By reaction of diol **VI** with this chiral reagent one should form a pair of diastereoisomers that could be easily separated by a combination of fractional crystallization and flash chromatography. The *l*-menthoxyacetyl chloride was not commercially available and was therefore synthesized from *l*-menthoxyacetic acid and thionyl chloride [71,72] (Scheme 6).



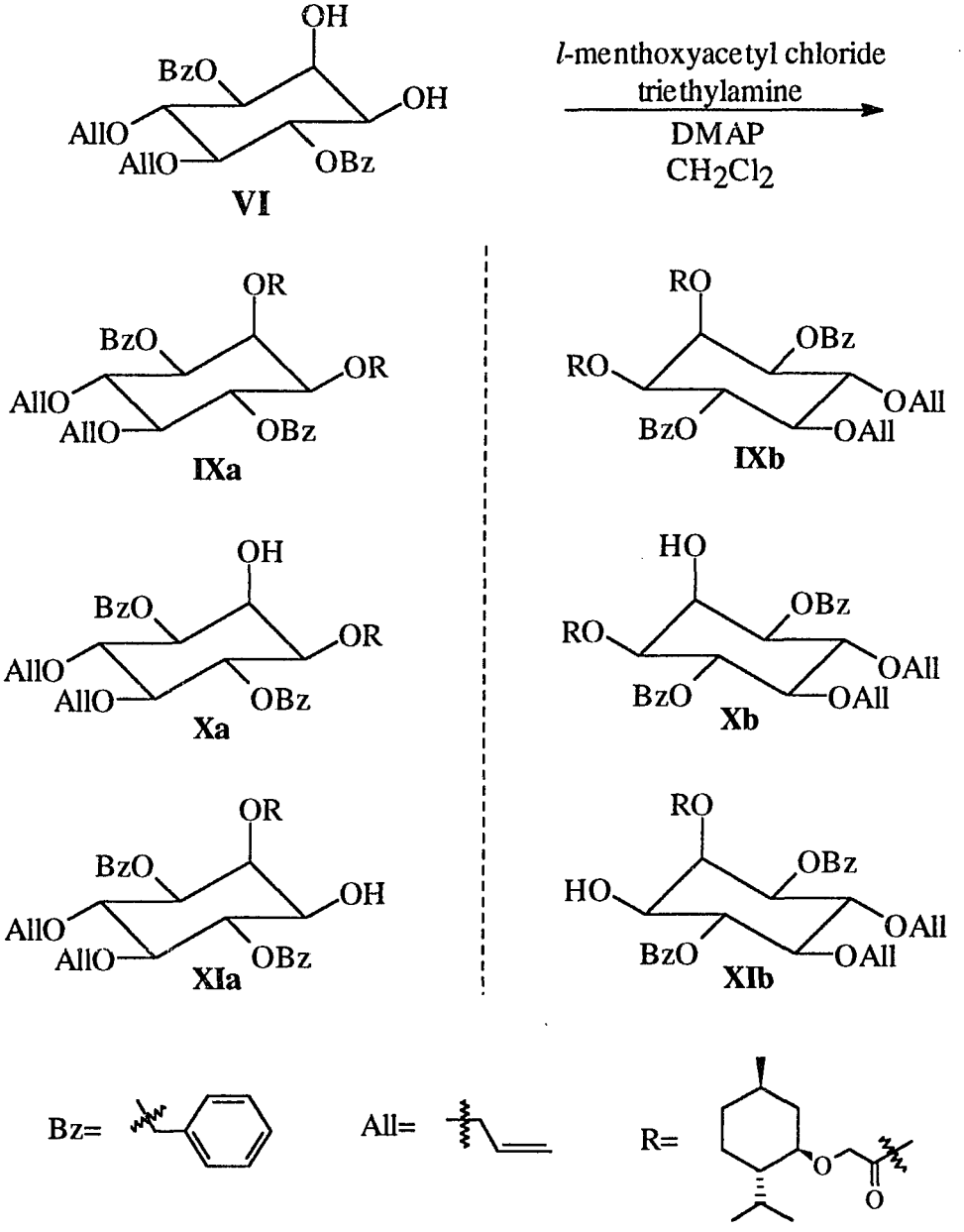
VIII

Scheme 6

Ozaki's group used pyridine as the solvent / base of the reaction, and reported that the *l*-menthoxyacetyl chloride will preferentially add to the #1 alcohol of the inositol diol **VI**. By doing this only a single pair of diastereoisomers, (±)-1-*O*-[*l*-menthoxyacetyl]-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**Xa-b**), will be formed. Unfortunately, under their reported reaction conditions, the reaction did not work very well and only a black tarry substance was obtained that was extremely difficult to purify and analyze.

In order to achieve success in performing this reaction a catalytic amount of 4-(dimethylamino)pyridine (DMAP) had to be added, and the solvent changed to a mixture of methylene chloride and triethylamine.

However, when the reaction was run it was found that the *l*-menthoxyacetyl group attached to both the #1 and #2 alcohols almost equally, and therefore four diastereoisomers were formed instead of two (Scheme 7).

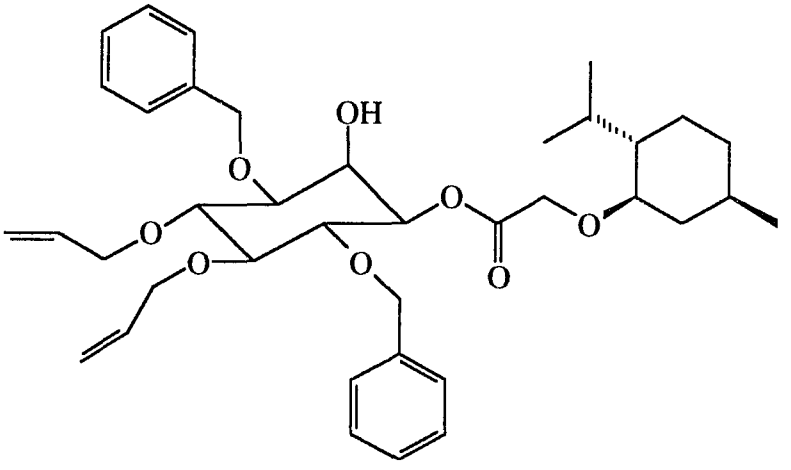


Scheme 7

These diastereoisomers could not be separated by crystallization, and were near impossible to purify by chromatographic means. Some of the 1,2-diadduct (**IXa-b**) was also formed during the reaction, giving an additional

pair of diastereoisomers, but they were easily separated from the mono-derivatized species.

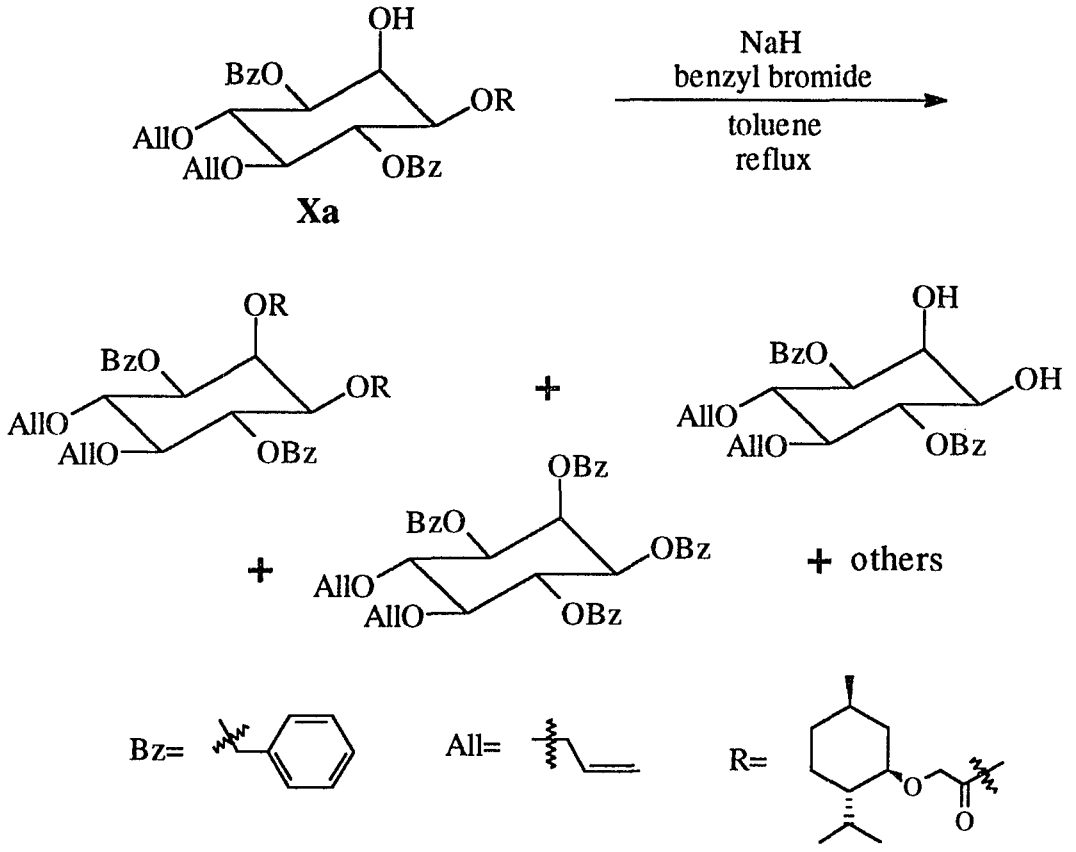
After many attempts at purification it was found that the four monoadduct diastereoisomers (**Xa-b**, **XIa-b**) would all elute partially on top of each other when flash chromatography was used as the separation technique. However success was achieved when a regular phase HPLC prep column (Lichrosorb® Si-60) was used to accomplish the separation. After a long and tedious procedure, 115 mg of (−)-1-*O*-[*l*-menthoxyacetyl]-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**Xa**) was finally obtained (Fig. 19).



Xa

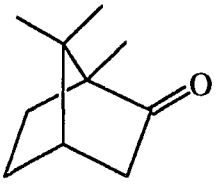
Fig. 19

Once inositol **Xa** was obtained, the next step was to protect the open #2 alcohol with an additional benzyl ether blocking group, followed by saponification of the *l*-menthoxyacetyl ester portion of the molecule to leave the #1 alcohol open for derivatization. This strategy would have been fine had there been no complications in protecting the #2 alcohol. The rather severe reaction conditions used for benzylation damaged the ester linkage of the *l*-menthoxyacetyl group giving a mixture of unwanted products (Scheme 8).



Scheme 8

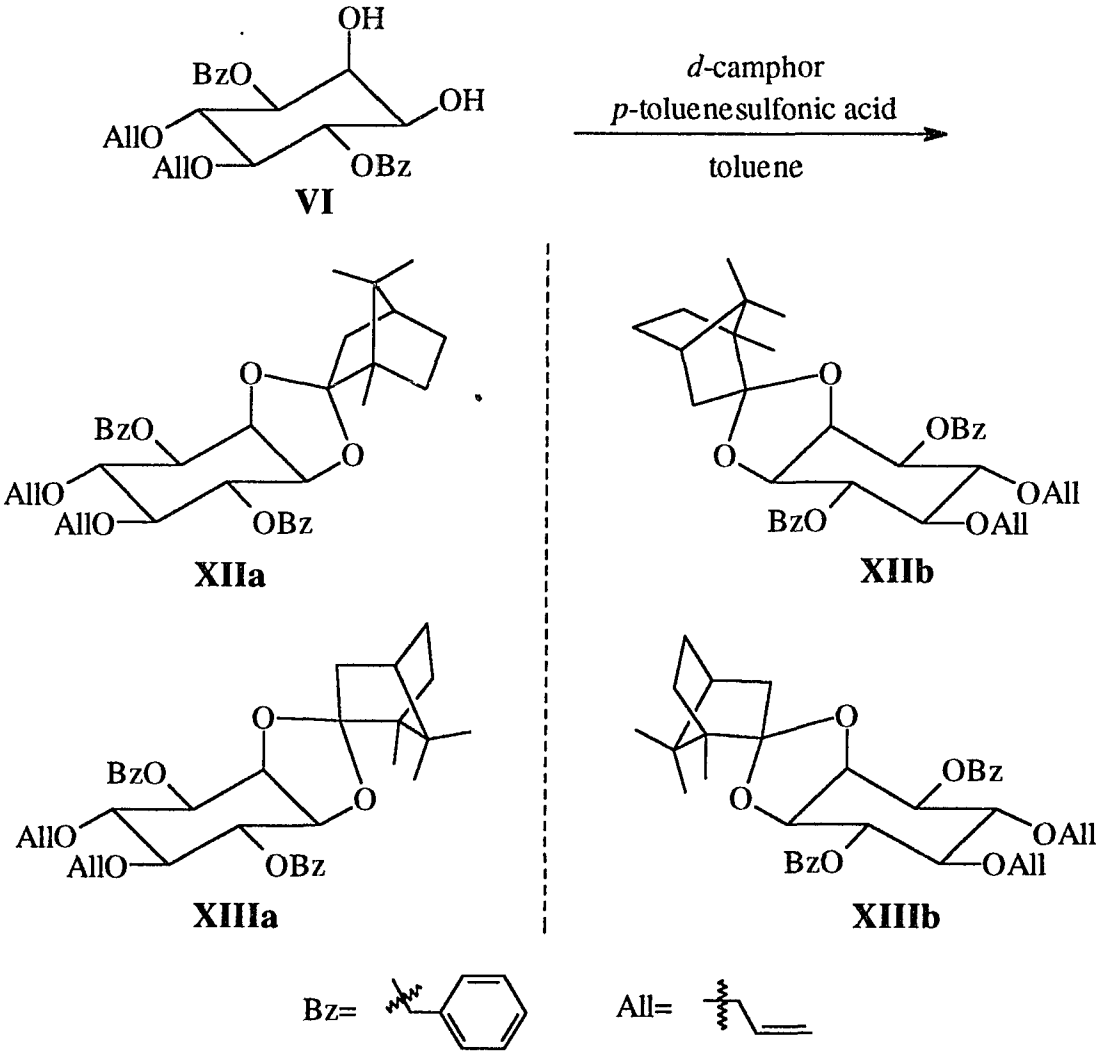
Having failed at successfully protecting the #2 alcohol, and losing all of compound **Xa**, another diastereoisomeric derivatization and separation had to be performed on racemic diol **VI**. Since the separation using the *l*-menthoxyacetyl derivatized inositol was extremely difficult a different approach was attempted. This new approach involved coupling the chiral ketone *d*-camphor (Fig. 20) to the racemic inositol diol **VI** in order to form diastereoisomeric ketals.



d-camphor

Fig. 20

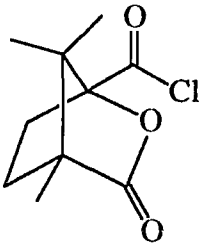
Using *d*-camphor as a chiral derivatizing agent will eliminate the possibility of forming diadducts, and monoadducts of the #2 alcohol, which was a large problem found in the *l*-menthoxyacetyl system. Depending on how the ketal is formed, one will obtain only four diastereoisomers, but if one coupling orientation is favored over another, the amount of each diastereoisomer formed may be skewed, thereby maximizing the formation of only one product. If this occurs, the difficulty associated with diastereoisomeric separation may be greatly decreased (Scheme 9).



Scheme 9

Upon completion of this reaction all four the possible *d*-camphor diastereoisomers (**XIIa-b**, **XIIIa-b**) were obtained and they could not be separated by crystallization, flash chromatography or prep HPLC. A new route had to be taken.

Billington *et al.*[73] reported using (1*S*)-(−)-camphanic acid chloride (Fig. 21), to derivatize some of their inositol type molecules into diastereoisomers. They found that in this system the separation of the diastereoisomers could be accomplished with less hassle than using the Ozaki *l*-menthoxyacetyl method. Fortunately this chiral derivatizing agent was able to be purchased, removing the task of having to synthesize it.

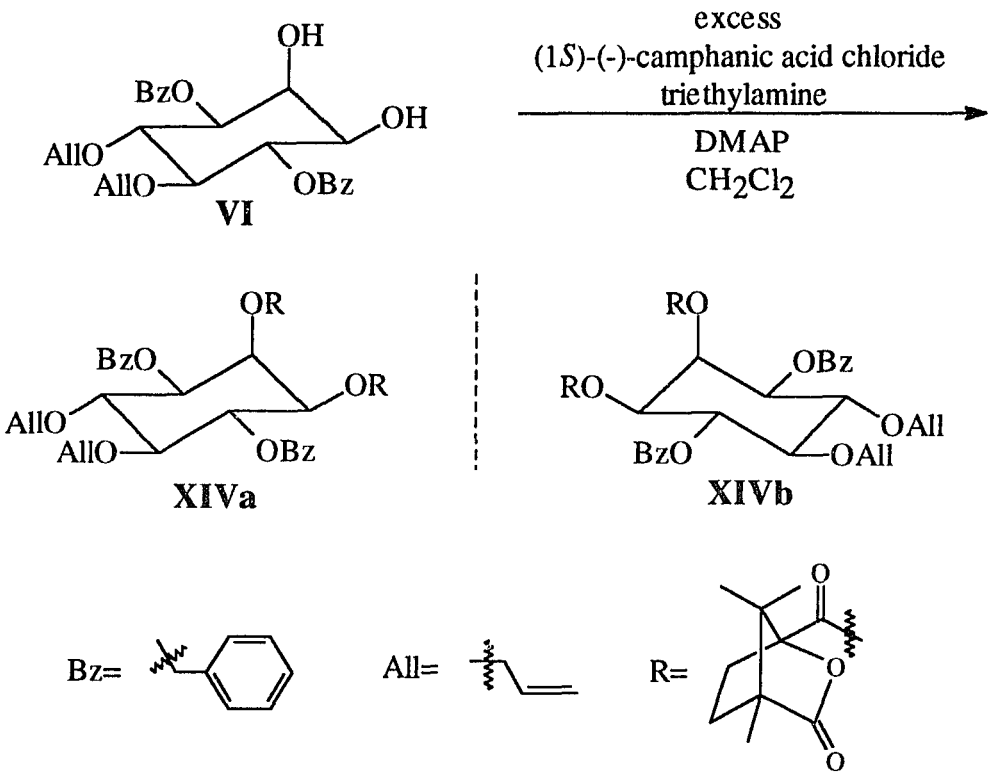


(1*S*)-(-)-camphanic acid chloride

Fig. 21

It was hoped that by deliberately derivatizing racemic inositol diol **VI** in both the #1 and #2 alcohol positions, one would obtain (±)-1,2-di-*O*-[(1*S*)-(−)-camphanoyl]-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XIVa-b**), which would consequently eliminate the possibility of forming any more than two diastereoisomers (Scheme 10).

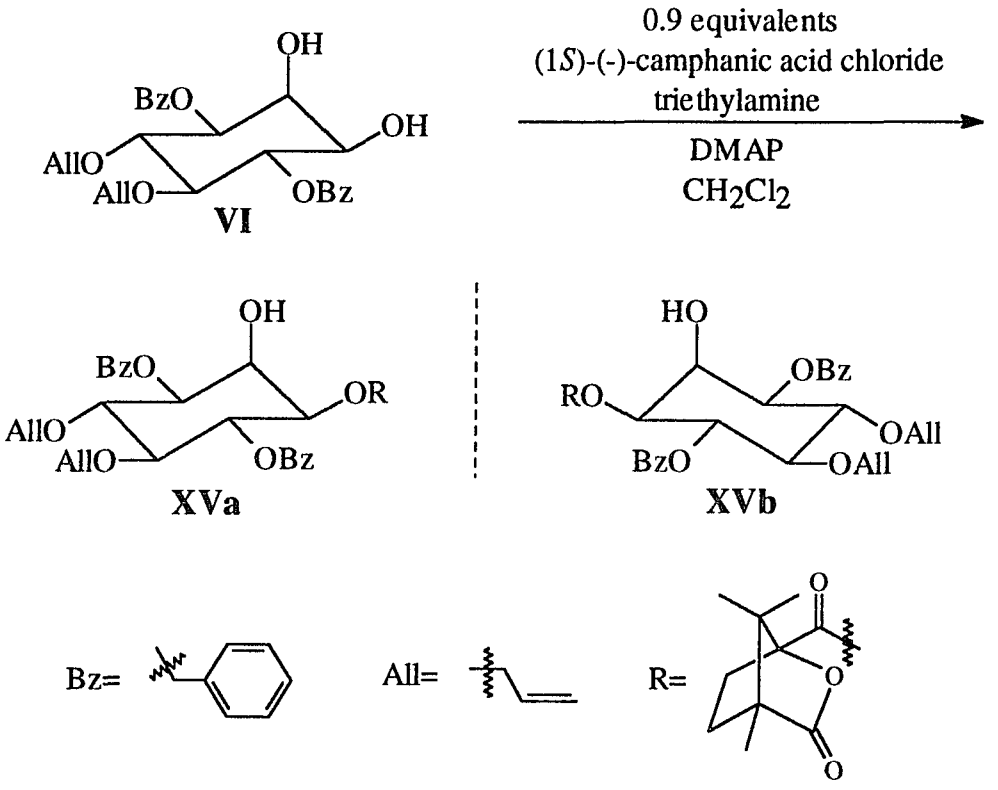
Once the correct diastereoisomer is isolated, saponification of both the (1*S*)-(−)-camphanate esters can be performed and then the problem of selectively blocking the #2 alcohol can be addressed.



Scheme 10

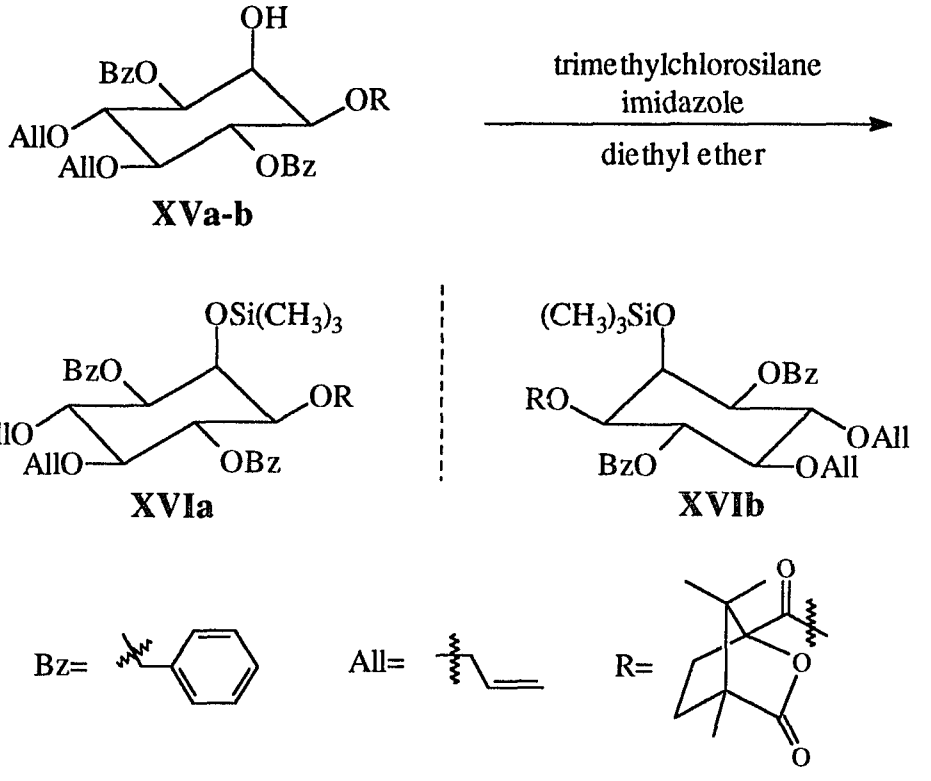
The reaction did indeed yield the predicted diadducts **XIVa** and **XIVb** as the major products, however, the separation of the diastereoisomers was impossible to execute.

The next attempt at diastereoisomeric separation involved monoderivatizing racemic inositol diol **VI** with (1*S*)-(-)-camphanic acid chloride instead of the *l*-menthoxyacetyl chloride which Ozaki's group used. Since the (1*S*)-(-)-camphanic acid molecule is bulkier than the *l*-menthoxyacetyl group, a carefully controlled reaction should succeed in selectively derivatizing the #1 equatorial alcohol over the hindered #2 axial alcohol. The reaction was completed and as predicted only the #1 alcohol was derivatized forming only (±)-1-*O*-[(1*S*)-(-)-camphanoyl]-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XVa-b**), with no diadducts or #2 derivatized species detectable (Scheme 11).



Scheme 11

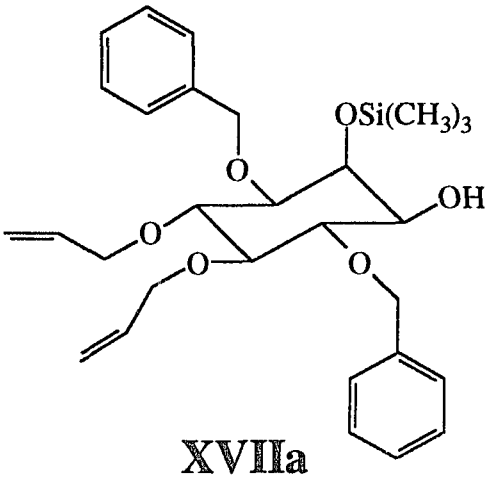
Once again the separation of the diastereoisomers seemed elusive. Failures using flash chromatography and fractional crystallization techniques forced one to proceed by way of the HPLC. Using a regular phase Lichrosorb® Si-60 HPLC prep column all that was obtainable was a double humped peak that corresponded to a large overlap and extremely inefficient separation of the diastereoisomers. Part of the difficulty was due to the free alcohol at the #2 position of the molecule. The polarity of this alcohol was most definitely causing the peak broadening and overlap that was being observed. To compensate for this, the #2 hydroxyl was blocked with a trimethylsilane protecting group in an attempt to decrease the polarity of the molecule and to facilitate separation (Scheme 12).



Scheme 12

The reaction proceeded smoothly yielding the expected diastereoisomeric mixture of (±)-1-*O*-[(1*S*)-(−)-camphanoyl]-2-*O*-trimethylsilyl-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XVIa-b**). The HPLC separation that followed was extremely tedious, but the diastereoisomers were separated efficiently. The next and final step of this synthesis was saponification of the (1*S*)-(−)-camphanate ester to obtain 16.2 mg of (−)-2-*O*-trimethylsilyl-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XVIIa**) (Fig. 22), and 26.1 mg of (+)-2-*O*-trimethylsilyl-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XVIIb**), which corresponds to the biologically incorrect form of the inositol.

The first goal of the project, preparation of a differentially protected *myo*-inositol molecule that can be used in future analogue preparation, was finally achieved.



XVIIa

Fig. 22

Seeing how the above system had plenty of difficulty associated with it, another inositol synthesis was attempted, this time involving different types of protecting groups. The aim of this new synthesis was to create an inositol where the #4 and #5 alcohols would be open for derivatization, and positions #1, #2, #3 and #6 would be selectively protected. Since the #1 alcohol is an important one in inositol metabolism, this position will be protected with a different group than the others, and one that is easily removed. We desire this arrangement so that the #1 alcohol is kept accessible for future derivatization, or coupling type reactions, following the derivatization of the #4 and #5 alcohols. Once again the stereochemistry of the inositol species is of the utmost importance.

This synthesis was begun by referring to the work done by Garegg, *et al.*[74] which conveys procedures for the separation of the two remaining cyclohexylidene positional isomers **IIb** and **IIc** produced from the reaction of *myo*-inositol and 1-ethoxycyclohexene (**VII**). One will recall that three positional isomers are formed from this reaction; (±)-1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol (**IIa**), (±)-1,2:3,4-di-*O*-cyclohexylidene-*myo*-

inositol (**IIb**), and (±)-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (**IIc**). (See Fig. 18).

Originally only one positional isomer **IIa** was isolated, the 1,2:4,5 protected species, from the other two by using fractional crystallization. The remaining two isomers are left behind as a syrupy brown residue after solvent evaporation. These two positional isomers can be numbered two different ways due to the internal plane of symmetry present in the original *myo*-inositol molecule. (See Fig. 16). This new numbering system is shown in Fig. 23.

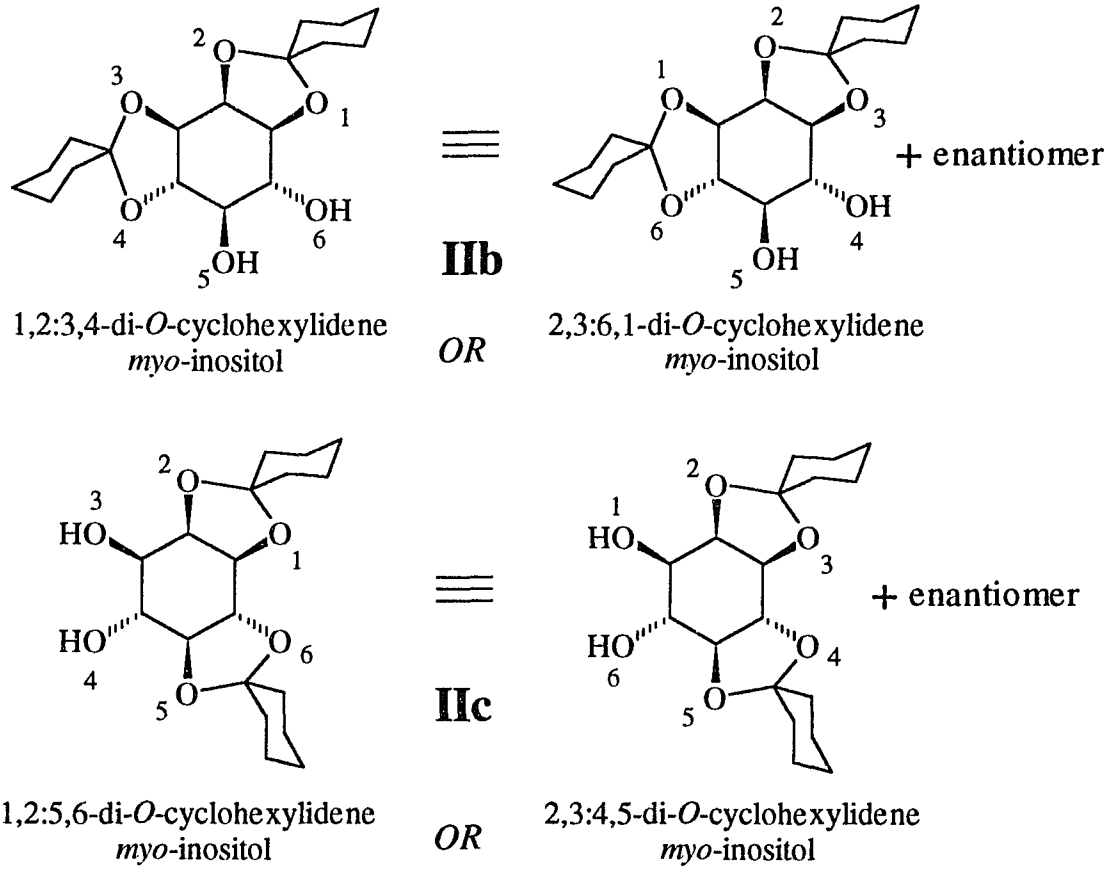


Fig. 23

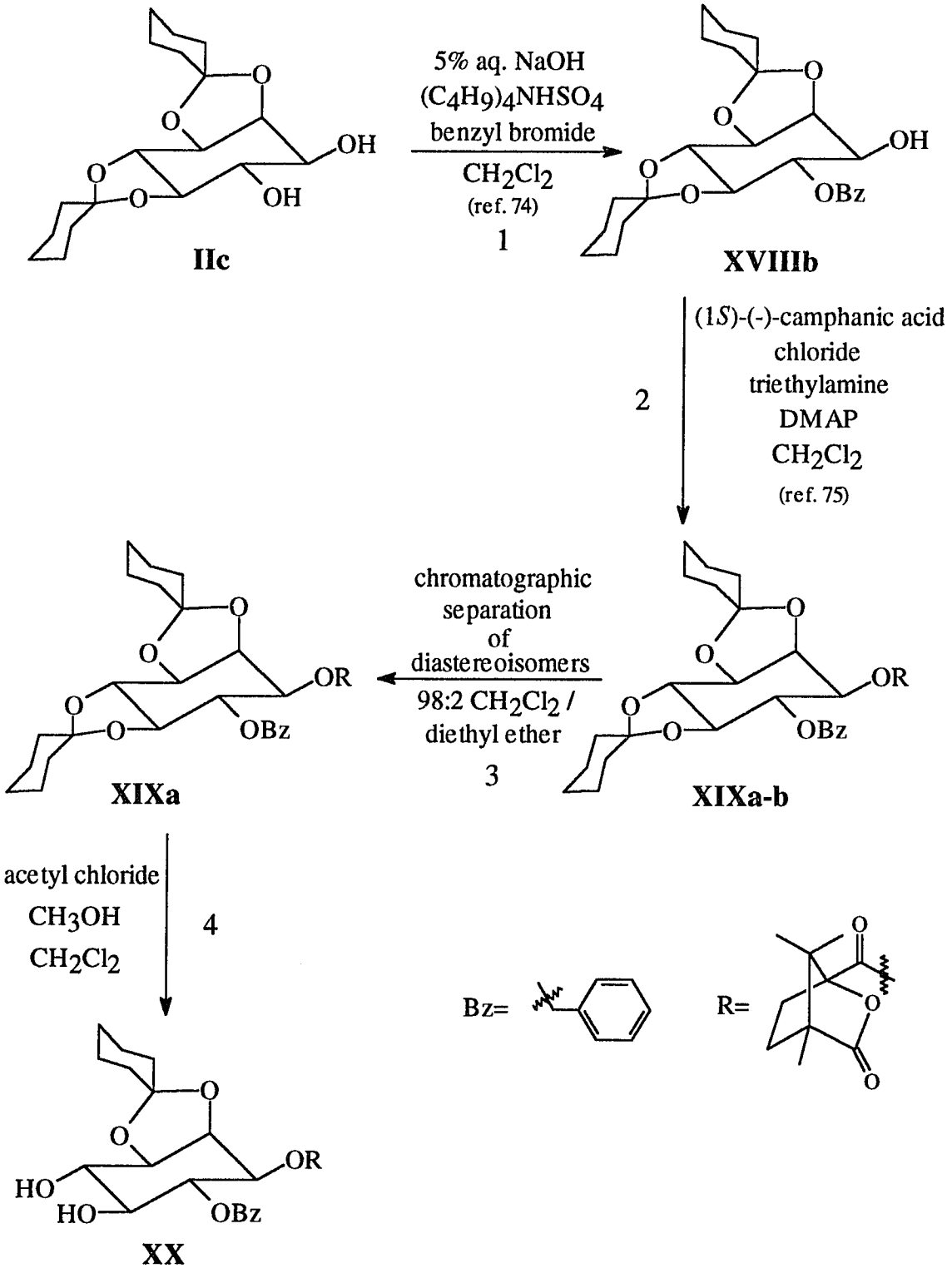
By starting this new synthesis using positional isomer **IIc**, (±)-1,2:5,6(2,3:4,5)-di-*O*-cyclohexylidene-*myo*-inositol, the problem of

protecting the #4 and #5 alcohols with easily removable blocking groups is eliminated, for the compound already has these alcohols blocked with a ketal linkage. After modification of Garegg's procedures isomer **IIc** was isolated very efficiently using by first using a flash chromatography setup with a 2:1 chloroform / acetone mobile phase to remove any residual **IIa**. The material that is isolated is an oil that contains both **IIb** and **IIc**, but **IIc** can be crystallized out by using a 3:1 light petroleum ether / acetone solvent system. Once isomer **IIc** was obtained in decent yields it was used as the starting material for a short synthetic procedure aimed at forming (−)-1-*O*-[(1*S*)-(−)-camphanoyl]-2,3- *O* - cyclohexylidene - 6 - *O* -benzyl-*myo*-inositol (**XX**). Some of the experimental procedures used were modifications of the work reported by Vacca, et al.,[75] and the overall synthetic pathway can be seen in Scheme 13.

The most difficult reaction in this synthetic route is Step 1. This step involves the formation of a benzyl ether at the #6 position of the partially protected inositol **IIc**. However, a total of three products are actually formed from this procedure; (±)-1,6-di-*O*-benzyl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol (**XVIIIa**), (±)-2,3:4,5-di-*O*-cyclohexylidene-6-*O*-benzyl-*myo*-inositol (**XVIIIb**), and (±)-1-*O*-benzyl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol (**XVIIIc**) (Fig. 24).

Fortunately the desired compound **XVIIIb** is formed in the largest yield and was easily separated from the other benzylated species **XVIIIa** and **XVIIIc**, by using flash chromatography with a 50:1 chloroform : acetone mobile phase.

The diastereoisomers formed from reaction Step 2 were so easily and efficiently separated in Step 3 by using flash chromatography conditions, that an HPLC separation was not necessary.


5% aq. NaOH
(C4H9)4NHSO4
benzyl bromide
CH2Cl2
(ref. 74)
1
IIc
XVIIIb
(1S)-(-)-camphanic acid
chloride
triethylamine
DMAP
CH2Cl2
(ref. 75)
2
chromatographic
separation
of
diastereoisomers
98:2 CH2Cl2 /
diethyl ether
3
XIXa
XIXa-b
acetyl chloride
CH3OH
CH2Cl2
4
XX
Bz=
R=

Scheme 13

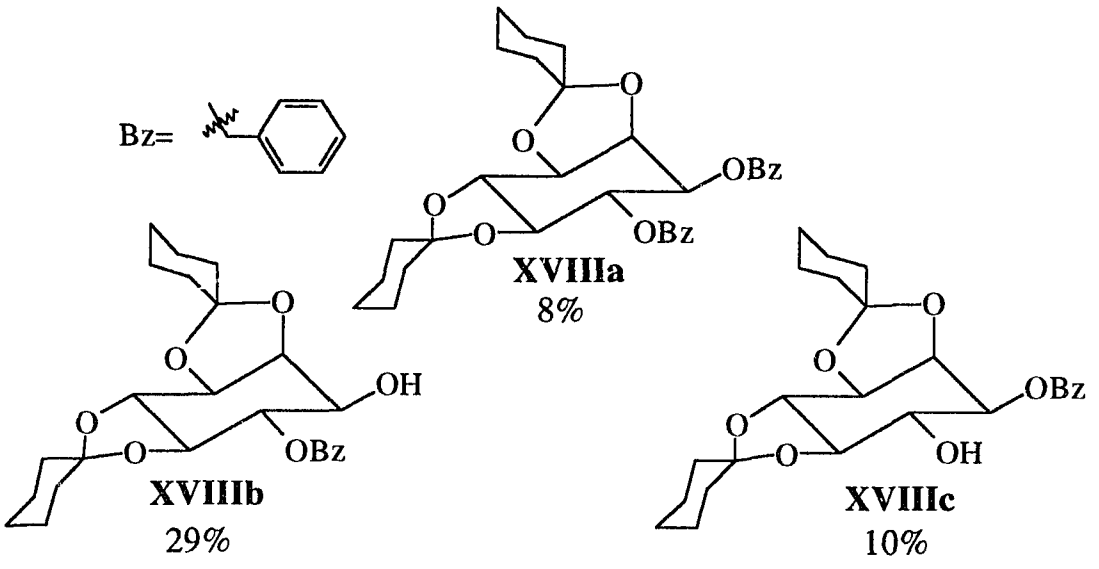
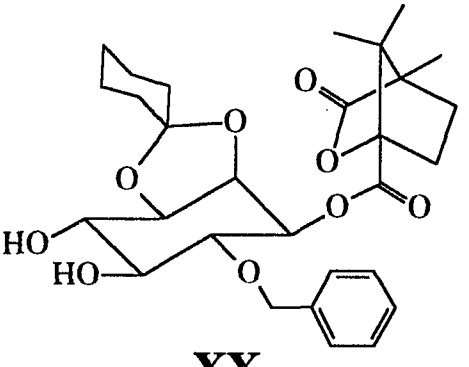


Fig. 24

The second goal of the project was now completed, having synthesized 155 mg of (−)-1-*O*-[(1*S*)-(−)-camphanoyl]-2,3-*O*-cyclohexylidene-6-*O*-benzyl-*myo*-inositol (**XX**), which was ready for further derivatization at the #4 and #5 positions, leaving position #1 blocked, but yet easily accessible for future coupling (Fig. 25).



XX

Fig. 25

The final goal of this project was to synthesize one more protected *myo*-inositol where only the #1 alcohol position is open and all the remaining alcohols are blocked by the same type of protecting groups. Once

this target inositol is obtained it will be coupled to a phosphonolipid that was synthesized by other members of Dr. Engel's research group.

The target *myo*-inositol for this part of the project is one where alcohols #2, #3, #4, #5 and #6 are all protected as benzyl ethers, while alcohol #1 is left open. As always the stereochemistry of the protected *myo*-inositol species must be in the biologically correct "D" form. The overall route for the preparation of this protected *myo*-inositol is one which involves modifications of inositol related, and allyl ether cleavage processes previously reported,[76-78] and is outlined in Scheme 14 and Scheme 15.

The first difficult reaction in this series was Step 4, the preparation of (±)-1-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXIV**). The allylation reaction forms three types of allylic ethers; (±)-1,2-di-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXVIII**), (±)-1-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXIV**), and (±)-2-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXIX**) (Fig. 26).

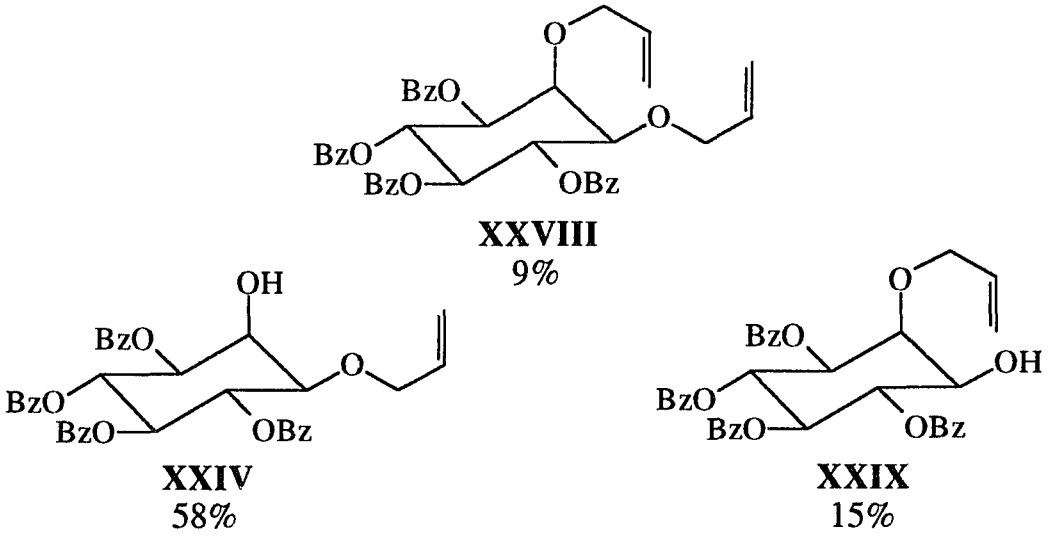
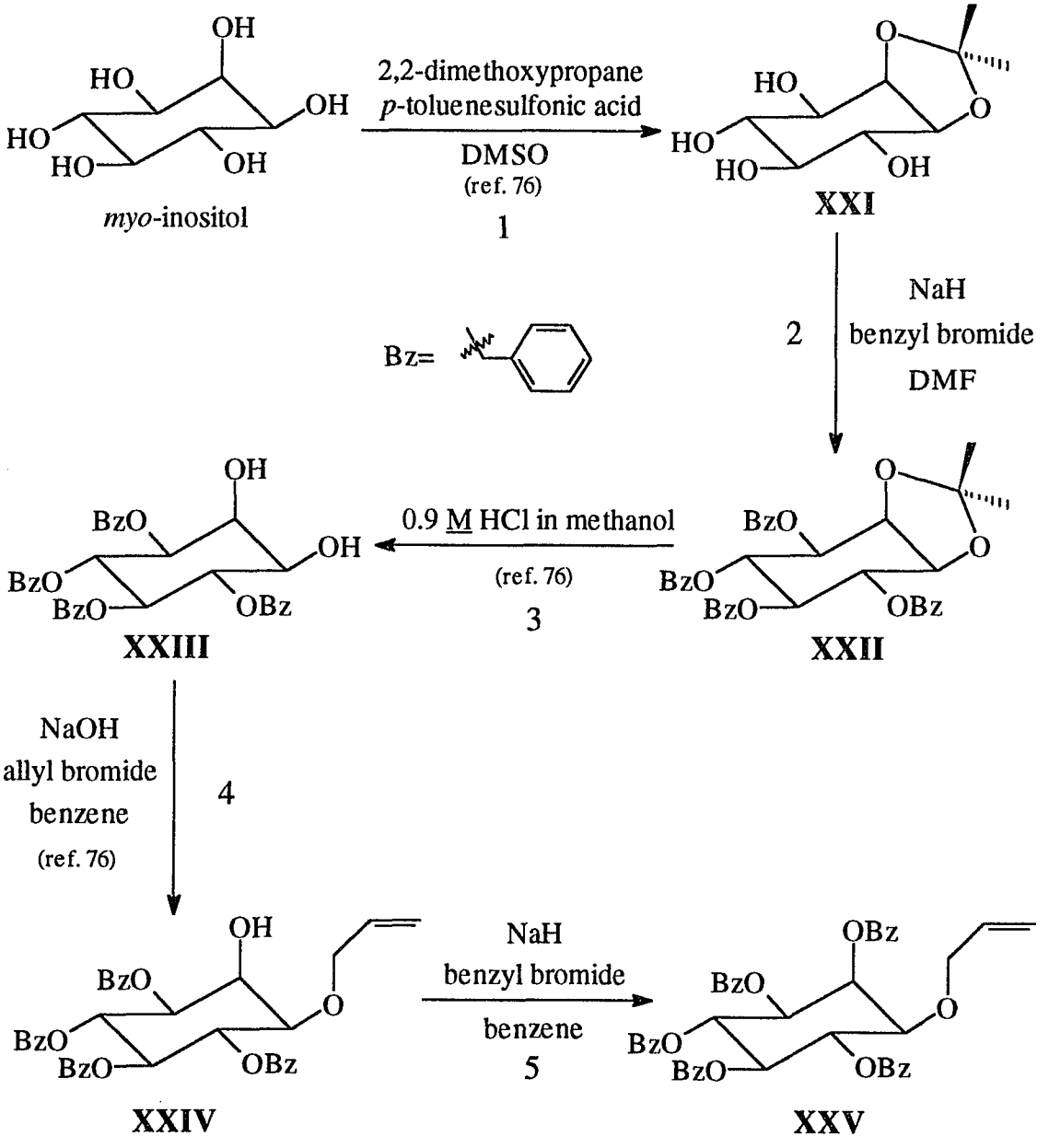


Fig. 26

Purification of the above allyl-inositols by flash chromatography only enables one to separate out the diallyl ether species **XXVIII**, leaving a

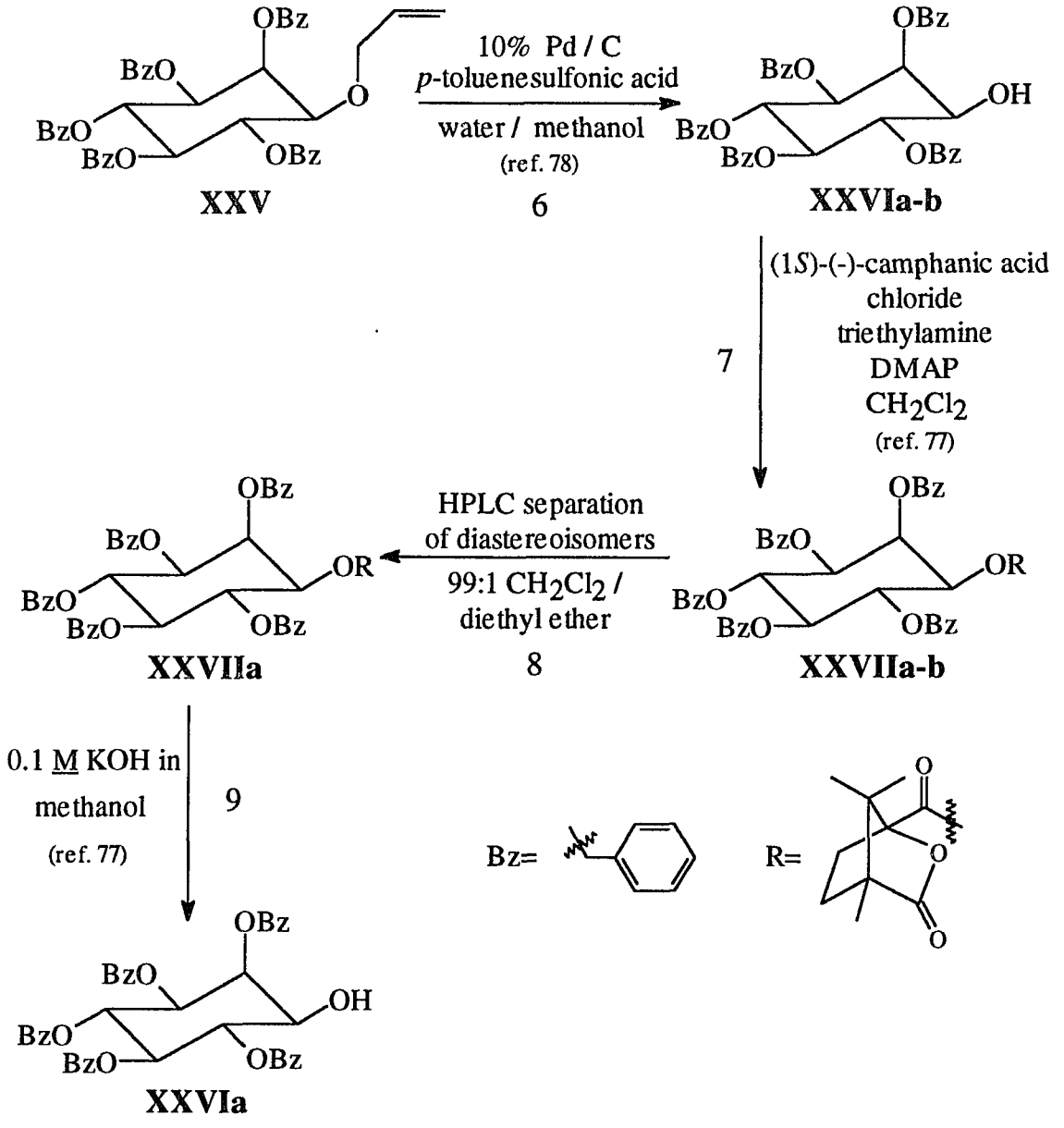
mixture of monoallyl ethers **XXIV** and **XXIX**. The desired compound **XXIV** was finally isolated by using fractional crystallization from a mixture of diethyl ether and light petroleum ether solvents.



Scheme 14

The next troublesome reaction takes place in Step 6, the cleavage of monoallyl ether **XXV** to leave a free alcohol at position #1. In this procedure the allyl ether is cleaved by using palladium on carbon (Pd/C) in

aqueous methanol with a catalytic amount of *p*-toluenesulfonic acid present. The Pd/C works by isomerizing the allyl ether into an enol ether, which is then cleaved by the action of the acid present in the system.



Scheme 15

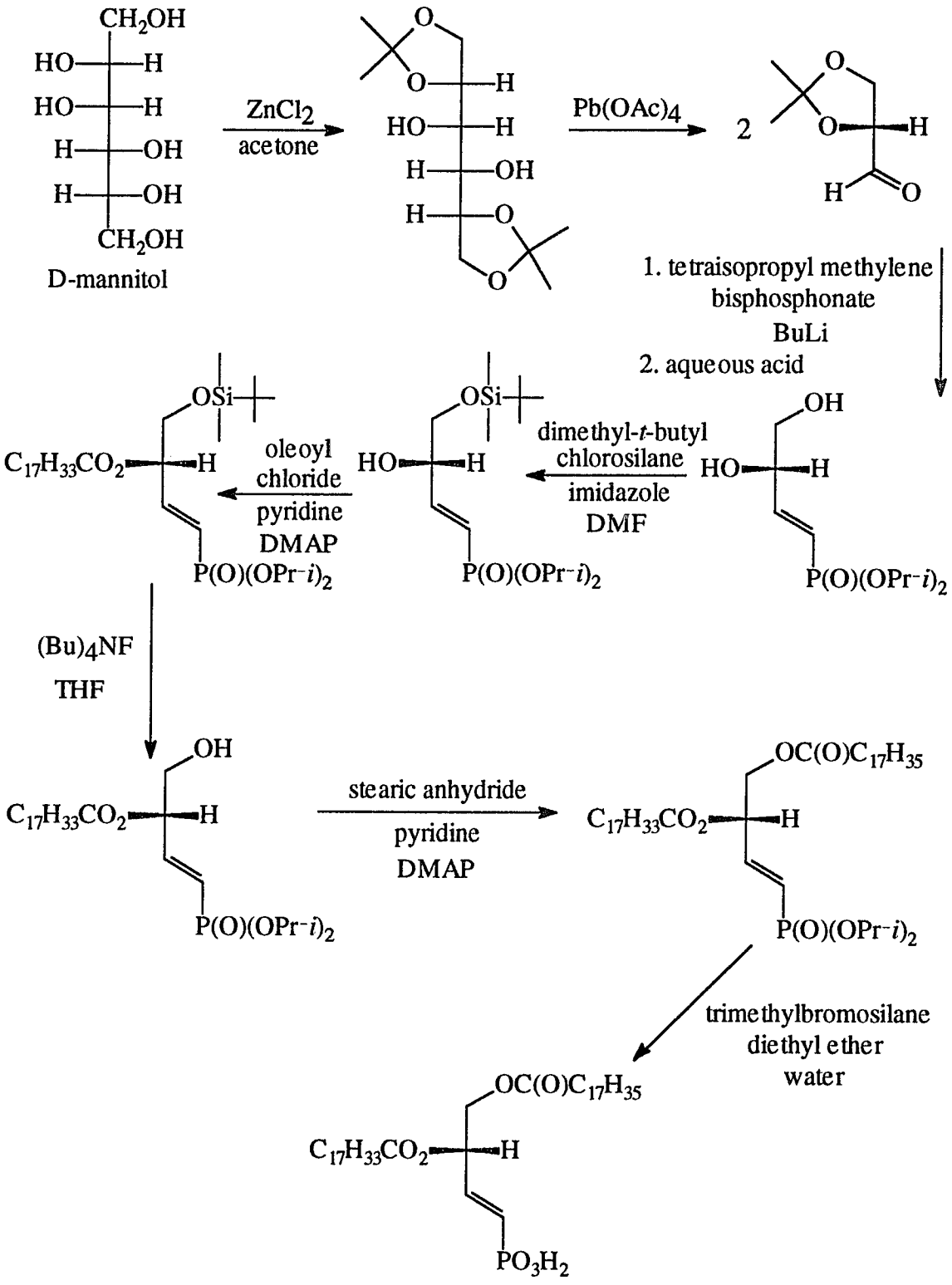
Originally there was concern that the benzyl ethers might somehow be affected by this reaction, but Boss *et al.*[78] reported using these reagents on systems that had both benzyl and allyl ether protecting groups, and claims

the benzyl groups remain untouched. Unfortunately this was not the case for the inositol system. The reaction resulted in cleaving many of the benzyl ethers, as well as the targeted allyl ether, making this step the one with the lowest yield. If this reaction is run again in the future on this or a similar compound it is suggested that one try using Wilkinson's catalyst (tris[triphenylphosphine] rhodium chloride) as an alternate to Pd/C, for it may reduce the benzyl ether cleavage problem.[83]

The remaining steps are rather straightforward. However, it should be noted that the HPLC separation of the diastereoisomers, Step 8, was an extremely tedious one. Only about 20 mg of the diastereoisomeric mixture could be injected into the prep column per run, resulting in excellent baseline separation. Unfortunately each run takes about twenty minutes, and injecting any more than 20 mg per run causes overlap to occur.

During the preparation of the (-)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIa**), another member of Dr. Engel's research group, Ting-yi Chu, was completing the synthesis of a vinylic phosphonolipid (**XXX**). The exact procedure used to synthesize this phosphonolipid can be found in Pratt *et al.*[79] and is shown in Scheme 16.

Once the syntheses of both the vinylic phosphonate lipid (**XXX**) and pentaprotected inositol (**XXVIa**) were completed, the next step was to link the oxygen atom of the #1 alcohol of the inositol to the phosphorus atom of the lipid. Lipid **XXX** was supplied in very crude form and had to be purified by flash chromatography with ethyl acetate as the mobile phase before coupling could be attempted. After isolating the pure lipid it was then linked to the inositol using trichloroacetonitrile in pyridine as the coupling reagents [80] (Scheme 17).



XXX

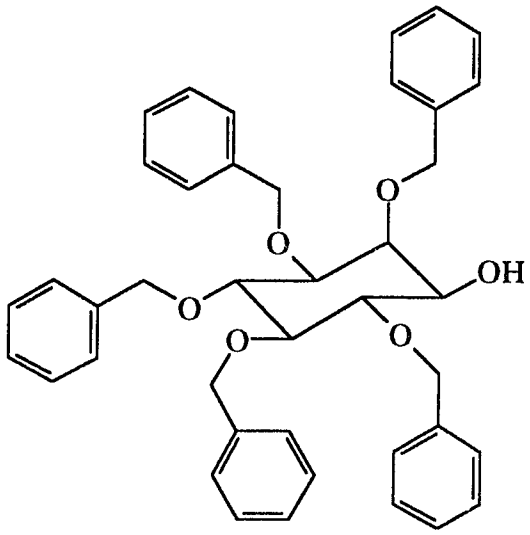
Scheme 16

It is extremely important that both the trichloroacetonitrile and the pyridine be freshly distilled and dried immediately prior to the reaction. Both the lipid and the inositol must be thoroughly dried as well, and the reaction mixture kept under a nitrogen atmosphere.

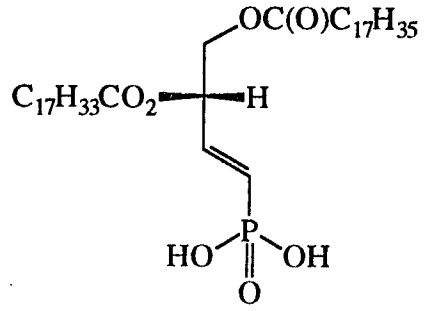
Due to the bulkiness of each of the reactants, one must consider that there are massive steric factors inhibiting the success of this reaction. These steric factors are most likely the reason for the low (20%) yield of this coupling. Purification of the coupled material **XXXI** proceeded smoothly using flash chromatography with a mobile phase consisting of 50:1 methylene chloride / diethyl ether.

The final step in this synthetic pathway is the cleavage of the benzyl ether protecting groups. To accomplish this, trimethyliodosilane was used as the cleaving agent.[81] The deprotection using trimethyliodosilane rapidly cleaves the benzyl ethers selectively in only twenty minutes, compared to other ether or ester linkages, giving one the target molecule **XXXII** without reduction of the olefinic linkages present on the lipid backbone and oleoyl portions of the compound (Scheme 18).

By means of a convergent synthesis one has obtained an isosteric phosphonic acid analogue of phosphatidylinositol where a non-hydrolyzable P-C-C linkage is present in place of the normal P-O-C esteric linkage joining the phosphate and diacylglycerol portions of the molecule. The vinylic linkage present on the backbone will provide an electron rich site adjacent to phosphorus while maintaining an enzymatically non-labile carbon-phosphorus bond, which should be an excellent analogue for studying the activity of enzymes such as phospholipase C which are known to act on the normal esteric P-O-C linkage of this molecule.

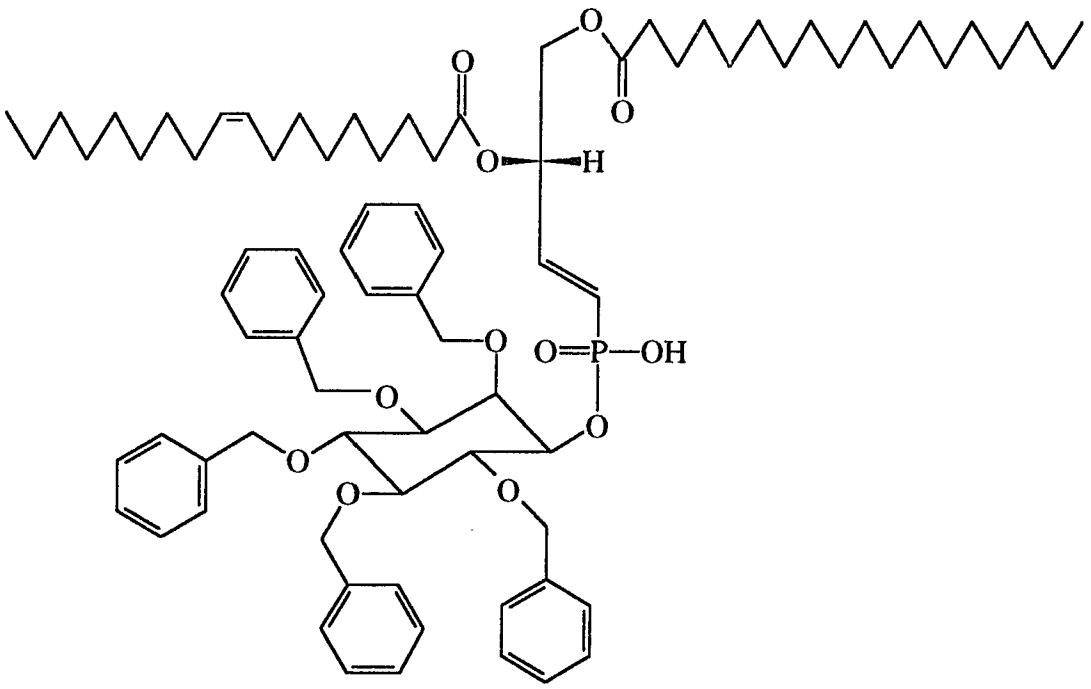


XXVIa



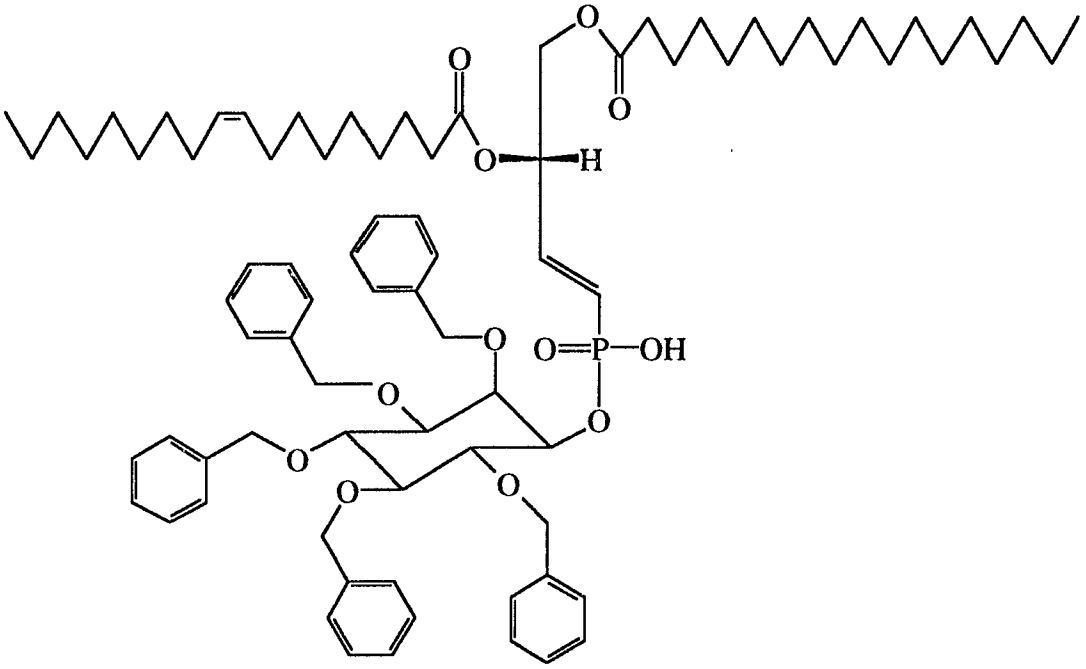
XXX

trichloroacetonitrile
pyridine
50° C
(ref. 80)



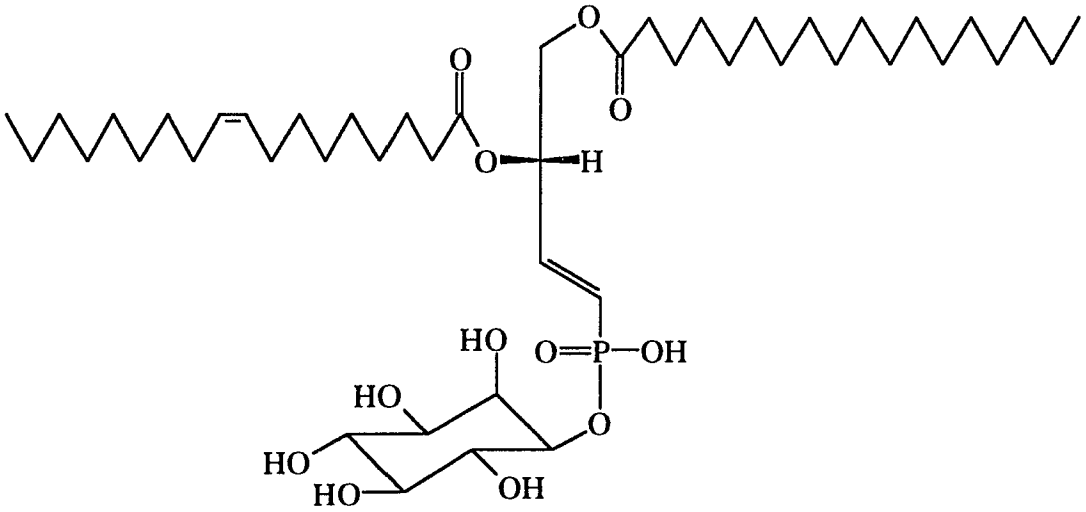
XXXI

Scheme 17



XXXI

trimethyliodosilane
chloroform
(ref. 81)



XXXII

Scheme 18

FUTURE RESEARCH

The first priorities for future research should be biochemical studies of the action of analogue **XXXII**, (*S*)-(*E*)-3-oleoyloxy-4-stearoyloxybut-1-enylphosphonic acid *myo*-inosit-1-yl ester on phospholipase C, and other related type enzymes. It would be instructive to observe the perturbation of the whole phosphatidylinositol metabolic pathway when this analogue replaces the natural lipid.

As far as future analogue preparation is concerned, the focus should be on the synthesis of methylphosphonic acid and hydroxymethylphosphonic acid analogues of inositol mono-, di-, and triphosphates (Fig. 27). Preparation of these analogues can be accomplished by starting with differentially protected inositols **XVIIa**, **XX** and **XXVIa**, all of which were prepared during the course of my research.

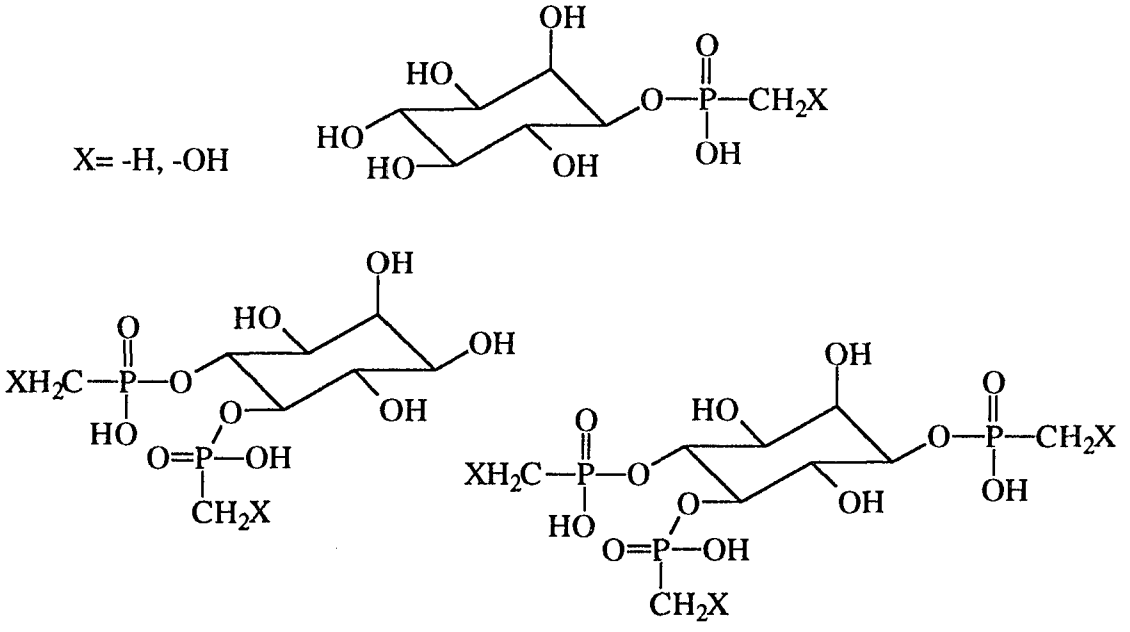


Fig. 27

These analogues can be used to study the receptors which control the calcium channels on the endoplasmic reticulum, and as probes to investigate

the hydrolysis process responsible for terminating the calcium releasing activity of inositol-1,4,5-triphosphate.

Another interesting class of analogues that warrants synthesis and testing would be a series of deoxyinositol phosphates, phosphatidyldeoxyinositols, and phosphatidyldeoxyinositol phosphates (Fig. 28).

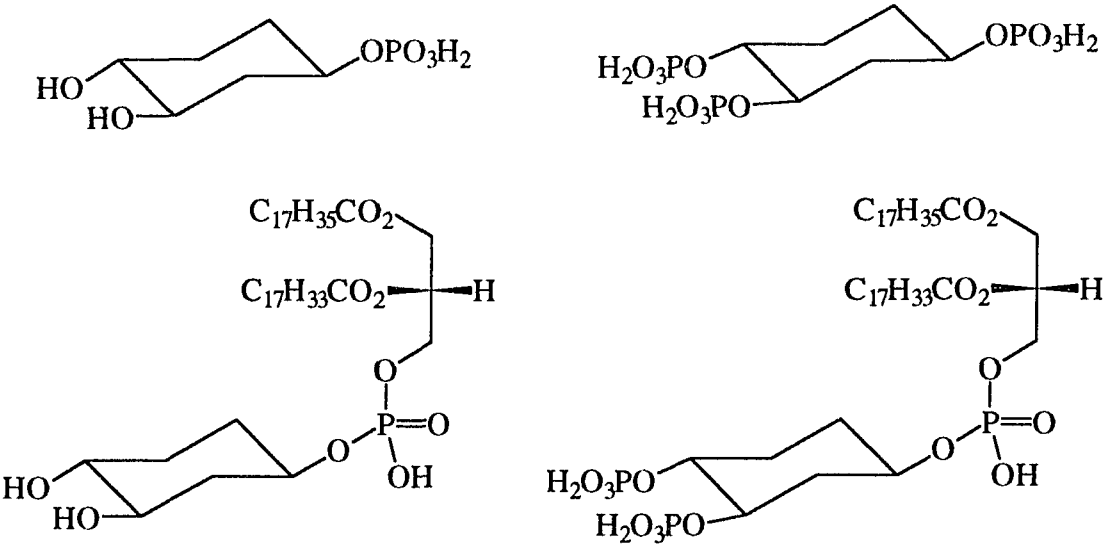


Fig. 28

These materials differ from the natural species by the lack of oxygen attached to the inositol ring except for those oxygens involved in esteric linkages to phosphorus. These analogues would be synthesized for the purpose of exploring their potential for perturbation of the normal phosphorylation / dephosphorylation processes, and for elucidation of competing metabolic pathways.

EXPERIMENTAL SECTION

General

All chemicals were of commercial reagent quality and were used without further purification with the following exceptions: chloroform was distilled over phosphorus pentoxide; pyridine was distilled over potassium hydroxide immediately prior to use; dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were distilled over calcium hydride and stored over molecular sieves 4A prior to use; benzyl bromide and allyl bromide were distilled prior to use.

Thin Layer Chromatography (TLC) was performed using Kodak Chromagram sheets and visualized by either iodine or UV methods. Phosphorus containing compounds were visualized by spraying the TLC plate with "phospho-spray", which is a mixture of molybdenum trioxide, sulfuric acid and water. Visualization is accomplished by the appearance of a blue color caused by a complex formed between phosphate, or phosphonic, acids and molybdenum.[82] Silica gel for flash chromatography was from EM Science (230-400 mesh). HPLC was performed using a Waters 6000A instrument with a Lichrosorb® Si-60 (10μm) preparative column and refractive index detector. All solvents used for HPLC mobile phases were of HPLC grade quality and degassed prior to use. Infrared spectra were measured using a Perkin-Elmer 1600 FTIR instrument. NMR spectra were measured using either a Varian EM360 (60 MHz) or IBM-Bruker WP200SY (200 MHz) instrument. Optical rotations were measured using a JASCO DIP-140 digital polarimeter. Elemental analyses were performed by Desert Analytics of Tucson, Arizona, and by Schwarzkopf Microanalytical Laboratories of Woodside, New York.

*Preparation of 1-ethoxycyclohexene (**VII**)*

To cyclohexanone (150.0 g, 1.53 mol) was added triethylorthoformate (237.0 g, 1.60 mol), and *p*-toluenesulfonic acid monohydrate (1.5 g, 7.9 mmol). The reaction vessel was continuously flushed with a nitrogen atmosphere and stirred for 24 hours at room temperature. The resulting solution was heated over an oil bath (180° C) in order to distill off any ethylformate and ethanol formed during the reaction. The residue was then refluxed for 45 minutes over an oil bath (200° C). The resulting solution was distilled and the fraction boiling from 155-160° C was collected as pure 1-ethoxycyclohexene (**VII**) (109.6 g, 57%). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.05-1.37, triplet, 3H; δ 1.37-2.65, multiplet, 8H; δ 3.30-3.80, quartet, 2H; δ 4.32-4.69, broad triplet, 1H.

*Preparation of (±)-1,2:4,5(2,3:5,6)-di-*O*-cyclohexylidene-*myo*-inositol (**IIa**), (±)-1,2:3,4(2,3:6,1)-di-*O*-cyclohexylidene-*myo*-inositol (**IIb**), (±)-1,2:5,6(2,3:4,5)-di-*O*-cyclohexylidene-*myo*-inositol (**IIc**)*

To dimethylformamide (DMF) (500 mL) was added *myo*-inositol (33.0 g, 183.2 mmol), 1-ethoxycyclohexene (**VII**) (60.0 g, 475.4 mmol), and *p*-toluenesulfonic acid monohydrate (1.0 g, 5.3 mmol). The resulting solution was heated at 95° C for 2.25 hours under a nitrogen atmosphere. The mixture was cooled to room temperature and methylene chloride (750 mL) was added. This mixture was washed with 5% aqueous sodium bicarbonate solution (500 mL), distilled water (500 mL), and saturated aqueous sodium chloride solution (500 mL), dried ($MgSO_4$), filtered , and evaporated under reduced pressure to leave a viscous dark brown residue. The residue was dissolved in a minimum amount of acetone, and light petroleum ether (30 - 60° C) was slowly added until an opaque solution was

obtained. The solution was placed in an acetone / dry ice bath with stirring for 2 hours, and then placed in the freezer for 3 days. The crystals that were formed were collected and washed with cold acetone / pet. ether (1:2) and air dried to give pure (±)-1,2:4,5(2,3:5,6)-di-*O*-cyclohexylidene-*myo*-inositol (**IIa**) (14.2 g 23%) as a white crystal with a melting point of 173-174° C. The filtrate was evaporated under reduced pressure and the viscous brown residue was subjected to flash chromatography on silica gel (140 g) in 1.35 g portions using a 2:1 chloroform / acetone mixture as the eluent, which gave a mixture of **IIb** and **IIc** free of any residual **IIa**. The columns were run until 8.03 g of the **IIb** - **IIc** mixture was collected as a viscous oil. The oil was dissolved in acetone (3 mL), then light petroleum ether (30 - 60° C) (9 mL) was added to induce crystallization. The mixture was placed in the freezer for 4 days, then filtered to give (±)-1,2:5,6(2,3:4,5)-di-*O*-cyclohexylidene-*myo*-inositol (**IIc**) (3.6 g) as a white solid with a melting point of 158 - 159° C. The filtrate was evaporated under reduced pressure to an oil and crystallized on standing to (±)-1,2:3,4(2,3:6,1)-di-*O*-cyclohexylidene-*myo*-inositol (**IIb**) (4.3 g) as a white solid with a melting point of 133 - 134° C. ^{1}H NMR (**IIa**) (200 MHz, $CDCl_3$): δ 1.22-1.85, multiplet, 20H; δ 2.75-2.90, broad doublet, 1H; δ 3.22-3.53, multiplet, 2H; δ 3.71-3.96, multiplet, 2H; δ 3.96-4.13, multiplet, 2H; δ 4.39-4.54, triplet, 1H. ^{1}H NMR (**IIb**) (200 MHz, $CDCl_3$): δ 1.22 - 2.16, multiplet, 20H; δ 3.25 - 3.63, broad singlet, 2H; δ 3.63 - 3.88, multiplet, 3H; δ 3.89 - 4.11, multiplet, 1H; δ 4.15 - 4.32, multiplet, 1H; δ 4.57 - 4.78, multiplet, 1H. ^{1}H NMR (**IIc**) (200 MHz, $CDCl_3$): δ 1.22 - 2.15, multiplet, 20H; δ 3.06 - 3.31, broad singlet, 1H; δ 3.32 - 3.50, multiplet, 1H; δ 3.77 - 4.18, multiplet, 4H; δ 4.25 - 4.43, multiplet, 1H; δ 4.43 - 4.58, multiplet, 1H.

*Preparation of (±)-1,2:4,5-di-*O*-cyclohexylidene-3,6-di-*O*-benzyl-*myo*-inositol (III)*

To toluene (170 mL) was added (±)-1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol (**IIa**) (5.0 g, 14.7 mmol). The resulting solution was stirred for 15 minutes at room temperature giving an opaque solution. Sodium hydride (97%) (0.9 g, 37.5 mmol) was slowly added to this solution and the mixture was stirred for 15 minutes at room temperature under a helium atmosphere. Benzyl bromide (6.3 g, 36.8 mmol) was then added dropwise and the solution was refluxed under a helium atmosphere for 6 hours, and then was left stirring overnight at room temperature. The reaction mixture was washed with distilled water (2 x 130 mL), and saturated aqueous sodium chloride solution (130 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give 8.7 grams of a viscous yellow oil. The oil was purified by flash chromatography on silica gel (215 g) using a 1:3 diethyl ether / light petroleum ether (30-60^{o} C) mixture as the eluent, which gave pure (±)-1,2:4,5-di-*O*-cyclohexylidene-3,6-di-*O*-benzyl-*myo*-inositol (**III**) (6.4 g, 84%) as a white crystal with a melting point of 112-113^{o} C. TLC in diethyl ether / light petroleum ether (1:3) gave R_f = 0.84. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95-2.05, multiplet, 20H; δ 3.18-3.44, multiplet, 1H; δ 3.52-3.85, multiplet, 2H; δ 3.85-4.19, multiplet, 2H; δ 4.19-4.45, multiplet, 1H; δ 4.60-5.05, multiplet, 4H; δ 7.03-7.64, multiplet, 10H.

*Preparation of (±)-1,2-*O*-cyclohexylidene-3,6-di-*O*-benzyl-*myo*-inositol (IV)*

To chloroform (100 mL) was added dry methanol (100 mL), and (±)-1,2-*O*-cyclohexylidene-3,6-di-*O*-benzyl-*myo*-inositol (**III**) (6.3 g, 12.1 mmol). The resulting mixture was stirred under a helium atmosphere for 5

minutes at room temperature. Seven drops (Pasteur pipet) of acetyl chloride was then added and the mixture was stirred for 1 hour at room temperature under a helium atmosphere. The reaction mixture was neutralized by the dropwise addition of saturated aqueous sodium bicarbonate. The solvents were evaporated under reduced pressure leaving behind a white solid residue. This residue was partitioned between methylene chloride (100 mL), and distilled water (100 mL). The methylene chloride layer was collected and washed with saturated aqueous sodium chloride solution (100 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure to leave crude (±)-1,2-*O*-cyclohexylidene-3,6-di-*O*-benzyl-*myo*-inositol (**IV**) (5.2 g, 98%) as a white crystal with a melting point of 147.5-148^0 C. No purification was necessary and the synthesis was continued using the crude crystal. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.18-1.86, multiplet, 10H; δ 2.83-3.21, broad doublet, 2H; δ 3.23-3.42, triplet, 1H; δ 3.42-3.60, multiplet, 2H; δ 3.78-4.13, multiplet, 2H; δ 4.18-4.34, triplet, 1H; δ 4.53-4.84, multiplet, 3H; δ 4.85-5.03, doublet, 1H; δ 7.15-7.50, multiplet, 10H.

*Preparation of (±)-1,2-*O*-cyclohexylidene-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (V)*

To dimethylformamide (DMF) (160 mL) was added (±)-1,2-*O*-cyclohexylidene-3,6-di-*O*-benzyl-*myo*-inositol (**IV**) (5.2 g, 11.8 mmol). The mixture was stirred at room temperature under a helium atmosphere until all the solid was dissolved. Sodium hydride (97%) (1.8 g, 75.0 mmol) was slowly added and the resulting mixture was stirred for 1.5 hours at room temperature under a helium atmosphere. The mixture will turn a dull green color. Allyl bromide (8.6 g, 71.1 mmol) was then added and the solution was heated between 50-60^0 C for 6 hours. The heat was then removed and

the mixture was left to stir for 16 hours at room temperature under a helium atmosphere, leaving a cloudy dark brown solution. A distilled water / ice mixture (175 mL) was added to the reaction solution, which was then extracted with methylene chloride (175 mL). The methylene chloride layer was washed with distilled water (175 mL), aqueous saturated sodium chloride solution (175 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure leaving 6.0 grams of a dark brown oil. The oil was purified by flash chromatography on silica gel (200 g) using a 1:10 ethyl acetate / n-hexane mixture as the eluent, which gave pure (±)-1,2-*O*-cyclohexylidene-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**V**) (5.1 g, 83%) as a light yellow oil. TLC in ethyl acetate / n-hexane (1:3) gave $R_f = 0.86$. ^1H NMR (200 MHz, $CDCl_3$): δ 1.17-1.90, multiplet, 10H; δ 3.13-3.85, multiplet, 5H; δ 3.90-4.45, multiplet, 5H; δ 4.53-5.00, multiplet, 4H; δ 5.04-5.40, multiplet, 4H; δ 5.77-6.14, multiplet, 2H; δ 7.05-7.65, multiplet, 10H.

*Preparation of (±)-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (VI)*

To (±)-1,2-*O*-cyclohexylidene-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inoitol (**V**) (5.0 g, 9.6 mmol) was added a distilled water (45 mL) / glacial acetic acid (45 mL) mixture. The resulting mixture was refluxed for 1 hour, cooled down to room temperature and ice cold distilled water (50 mL) was added to it. The reaction vessel was placed in an ice bath for 15 minutes and the resulting crystals were isolated by filtration and washed with small portions of ice cold distilled water. The crude crystals were recrystallized form hot n-hexane (150 mL), which gave pure (±)-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**VI**) (4.0 g, 95%) as a white crystal with a melting point of 105° C. ^1H NMR (200 MHz, $CDCl_3$): δ 2.55-3.06, broad singlet, 2H; δ 3.16-3.58, multiplet, 3H; δ 3.60-3.97, multiplet, 2H; δ 4.01-4.55, multiplet,

5H; δ 4.55-5.07, multiplet, 4H; δ 5.07-5.52, multiplet, 4H; δ 5.82-6.23, multiplet, 2H; δ 7.17-7.68, multiplet, 10H.

Preparation of l-*menthoxyacetyl chloride (**VIII**)*

To *l*-menthoxyacetic acid (3.0 g, 14.0 mmol) was added thionyl chloride (9.1 g, 76.5 mmol). The resulting mixture was placed under a nitrogen atmosphere, stirred and heated to 50° C for 3 hours. The excess thionyl chloride was removed under reduced pressure to give a yellow oil residue. The residue was vacuum distilled at 9 mm Hg using an oil bath at 170° C as the heat source. The fraction distilling off at 135° C as a yellow oil was collected as pure *l*-menthoxyacetyl chloride (**VIII**) (2.5 g, 77%). The ^{1}H NMR (60 MHz) spectra for **VIII** was identical to the one for *l*-menthoxyacetic acid with the exception of the upfield acid -OH peak, which, as expected, was no longer present.

*Preparation of (-)-1-*O*-[*l*-menthoxyacetyl]-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**Xa**)*

To methylene chloride (20 mL) was added (±)-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**VI**) (0.6 g, 1.4 mmol), 4-(dimethylamino)pyridine (DMAP) (8.0 mg, 6.5 x 10^{-5} mol), triethylamine (0.7 g, 6.9 mmol), and *l*-menthoxyacetyl chloride (**VIII**) (0.3 g, 1.3 mmol). The resulting mixture was stirred for 24 hours at room temperature under a nitrogen atmosphere. The reaction mixture was then washed with distilled water (2 x 20 mL), saturated aqueous sodium chloride solution (20 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure leaving 1.3 grams of an oily residue. The oil was purified by flash chromatography on silica gel (40 g) using a 1:1 n-hexane / diethyl ether mixture as the eluent, which gave pure

(±)-1-*O*-[*l*-menthoxyacetyl]-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**Xa-b**) (0.4 g, 45%) as an oil. TLC of **Xa-b** in n-hexane / diethyl ether (1:1) gave R_f = 0.66. The diastereoisomeric mixture of **Xa-b** was separated by using HPLC with a Lichrosorb® prep column and a 2:1 n-hexane / diethyl ether mobile phase at a flow rate of 8.5 mL / min. Approximately 5 mg of the mixture was injected into the column per run, adding up to about 70 injections total. Diastereoisomer **Xb** eluted from the column 6 - 8 minutes after injection, and diastereoisomer **Xa** eluted from the column 8.2 - 12 minutes after injection. The HPLC separation yielded (-)-1-*O*-[*l*-menthoxyacetyl]-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**Xa**) (115 mg) as an oil. Specific rotation $[\alpha]_D$ = -47.1 (0.036 M, $CHCl_3$). ^1H NMR (60 MHz, $CDCl_3$): δ 0.70 - 2.52, multiplet, 16H; δ 2.55 - 2.73, broad singlet, 3H; δ 3.04 - 3.92, multiplet, 5H; δ 3.95 - 4.51, multiplet, 8H; δ 4.60 - 4.94, multiplet, 4H; δ 5.05 - 5.63, multiplet, 4H; δ 5.65 - 6.44, multiplet, 2H; δ 7.13 - 7.68, multiplet, 10H.

*Preparation of (±)-1,2-*O*-[d-camphoryl]-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XIIa-b**) (**XIIIa-b**)*

To toluene (35 mL) was added (±)-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**VI**) (1.2 g, 2.7 mmol), *d*-camphor (0.4 g, 2.6 mmol), and *p*-toluenesulfonic acid monohydrate (50.0 mg, 2.6 x 10^{-4} mol). The resulting mixture was refluxed for 2 hours using a Dean-Stark condenser apparatus. The reaction mixture was cooled to room temperature, neutralized by adding solid sodium bicarbonate (20 mg), dried ($MgSO_4$), filtered, and evaporated under reduced pressure leaving 1.3 grams of a light brown crystal. The crystal was purified by flash chromatography on silica gel (35 g) using diethyl ether as the eluent, which gave (±)-1,2-*O*-[*d*-camphoryl]-3,6-di-*O*-

benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XIIa-b**) (**XIIIa-b**) (0.6 g, 43%). TLC in diethyl ether gave R_f = 0.73. The four diastereoisomers synthesized by this reaction could not be separated. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.82 - 0.92, singlet, 3H; δ 0.95 - 1.00, singlet, 3H; δ 1.02 - 1.08, singlet, 3H; δ 1.17 - 2.90, multiplet, 7H; δ 3.13 - 4.12, multiplet, 4H; δ 4.21 - 4.75, multiplet, 6H; δ 4.75 - 5.20, multiplet, 4H; δ 5.23 - 5.85, multiplet, 4H; δ 5.85 - 6.70, multiplet, 2H; δ 7.40 - 7.93, multiplet, 10H.

*Preparation of (±)-1,2-di-*O*-[(1S)-(-)-camphanoyl]-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XIVa-b**)*

To methylene chloride (25 mL) was added (±)-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**VI**) (0.6 g, 1.4 mmol), 4-(dimethylamino)pyridine (DMAP) (20.0 mg, 1.6 x 10^{-4} mol), triethylamine (0.4 g, 4.0 mmol), and (1*S*)-(-)-camphanic acid chloride (0.7 g, 3.2 mmol). The resulting mixture was stirred for 24 hours at room temperature under a nitrogen atmosphere. The reaction mixture was then washed with distilled water (2 x 30 mL), saturated aqueous sodium chloride solution (20 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure leaving 1.3 grams of a light brown oil. The oil was purified by flash chromatography on silica gel (33 g) using a 30:1 methylene chloride / ethyl acetate mixture as the eluent, which gave pure (±)-1,2-di-*O*-[(1*S*)-(-)-camphanoyl]-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XIVa-b**) (0.9 g, 80%) as a light yellow oil. TLC in methylene chloride / ethyl acetate (30:1) gave R_f = 0.87. The two diastereoisomers synthesized by this reaction could not be separated. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.66 - 1.20, multiplet, 18H; δ 1.37 - 2.61, multiplet, 10H; δ 3.27 - 3.98, multiplet, 3H; δ 4.02 - 4.33, multiplet, 3H; δ

4.36 - 4.55, singlet, 2H; δ 4.55 - 4.77, doublet, 2H; δ 4.78 - 5.35, multiplet, 5H; δ 5.44 - 6.12, multiplet, 3H; δ 6.87 - 7.30, multiplet, 10H.

*Preparation of (±)-1-*O*-[(1S)-(-)-camphanoyl]-3,6-di-*O*-benzyl-4,5-di-*O*-allyl*-myo*-inositol **(XVa-b)***

To methylene chloride (27 mL) was added (±)-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**VI**) (1.0 g, 2.3 mmol), 4-(dimethylamino)pyridine (DMAP) (30.0 mg, 2.5 x 10^{-4} mol), triethylamine (0.7 g, 6.9 mmol), and (1*S*)-(-)-camphanic acid chloride (0.5 g, 2.3 mmol). The resulting mixture was stirred for 48 hours at room temperature under a nitrogen atmosphere. The reaction mixture was then washed with distilled water (2 x 20 mL), saturated aqueous sodium chloride solution (20 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure leaving 1.8 g of a viscous light brown residue. The residue was purified by flash chromatography on silica gel (50 g) using a 3:1 chloroform / ethyl acetate mixture as the eluent, which gave pure (±)-1-*O*-[(1*S*)-(-)-camphanoyl]-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XVa-b**) (0.8 g, 56%) as a light yellow oil. TLC in chloroform / ethyl acetate (3:1) gave R_f = 0.73. ^1H NMR (60 MHz, $CDCl_3$): δ 0.73 - 1.34, multiplet, 9H; δ 1.50 - 2.53, multiplet, 4H; δ 2.54 - 2.92, broad singlet, 1H; δ 3.19 - 4.02, multiplet, 6H; δ 4.23 - 4.65, multiplet, 4H; δ 4.65 - 4.91, multiplet, 2H; δ 4.91 - 5.17, multiplet, 2H; δ 5.18 - 5.79, multiplet, 4H; δ 5.83 - 6.61, multiplet, 2H; δ 7.36 - 7.81, multiplet, 10H.

*Preparation of (-)-1-*O*-[(1S)-(-)-camphanoyl]-2-*O*-trimethylsilyl-3,6-di-*O*-benzyl-4,5-di-*O*-allyl*-myo*-inositol **(XVIa)**, and (+)-1-*O*-[(1S)-(-)-camphanoyl]-2-*O*-trimethylsilyl-3,6-di-*O*-benzyl-4,5-di-*O*-allyl*-myo*-inositol **(XVIb)***

To diethyl ether (15 mL) was added (±)-1-*O*-[(1*S*)-(−)-camphanoyl]-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XVa-b**) (0.7 g, 1.1 mmol), imidazole (75.0 mg, 1.1 mmol), and trimethylchlorosilane (0.5 g, 4.6 mmol). The resulting mixture was stirred for 19 hours at room temperature under a nitrogen atmosphere. The reaction mixture was filtered to remove the solid imidazole chloride, and was then evaporated under reduced pressure to yield 0.7 grams of a light brown oil. The oil was purified by flash chromatography on silica gel (20 g) using a 99:1 methylene chloride / diethyl ether mixture as the eluent, which gave (±)-1-*O*-[(1*S*)-(−)-camphanoyl]-2-*O*-trimethylsilyl-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XVIa-b**) (256.0 mg, 34%) as a light yellow oil. TLC using methylene chloride / diethyl ether (99:1) gave $R_f = 0.85$. The diastereoisomers **XVIa-b** were separated using HPLC with a Lichrosorb® prep column and a 99:1 methylene chloride / diethyl ether mobile phase at a flow rate of 1.9 mL / min. Approximately 3 mg of compound was injected per run, adding up to about 92 injections total. Compound **XVIa** eluted from the column 16 - 18 minutes after injection, and compound **XVIb** eluted from the column 18.4 - 20 minutes after injection. The HPLC separation yielded (−)-1-*O*-[(1*S*)-(−)-camphanoyl]-2-*O*-trimethylsilyl-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XVIa**) (101.3 mg), and (+)-1-*O*-[(1*S*)-(−)-camphanoyl]-2-*O*-trimethylsilyl-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XVIb**) (99.4 mg) as oils.

*Preparation of (−)-2-*O*-trimethylsilyl-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XVIIa**)*

To absolute ethanol (14 mL) was added potassium hydroxide (80.0 mg, 1.4 mmol), and (−)-1-*O*-[(1*S*)-(−)-camphanoyl]-2-*O*-trimethylsilyl-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XVIa**) (101.3 mg, 1.5×10^{-4} mol).

The resulting mixture was stirred for 24 hours at room temperature. The reaction mixture was evaporated under reduced pressure, and the residue was partitioned between diethyl ether (20 mL) and distilled water (14 mL). The water layer was collected and extracted with an additional portion of diethyl ether (7 mL). The diethyl ether layers were combined, dried ($MgSO_4$), filtered, and evaporated under reduced pressure leaving 60.0 mg of a brown solid. The solid was purified by flash chromatography on silica gel (2.5 g) using a 1:3 n-hexane / diethyl ether mixture as the eluent, which gave (−)-2-*O*-trimethylsilyl-3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol (**XVIIa**) (16.2 mg, 21%) as a light brown solid. TLC in n-hexane / diethyl ether (1:3) gave R_f = 0.57. Specific rotation $[\alpha]_D$ = -10.3 (0.0158 M, $CHCl_3$). ^1H NMR (60 MHz, $CDCl_3$): δ -0.05 - 0.15, singlet, 9H; δ 2.50 - 2.80, broad singlet, 1H; δ 3.29 - 4.39, multiplet, 5H; δ 4.40 - 4.70, multiplet, 5H; δ 4.75 - 5.19, multiplet, 4H; δ 5.20 - 5.80, multiplet, 4H; δ 5.82 - 6.77, multiplet, 2H; δ 7.51 - 7.85, multiplet, 10H.

*Preparation of (±)-2,3:4,5-di-*O*-cyclohexylidene-6-*O*-benzyl-*myo*-inositol (**XVIIIb**)*

To methylene chloride (180 mL) was added (±)-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol (**IIc**) (3.6 g, 10.6 mmol), benzyl bromide (2.7 g, 15.8 mmol), 5% aqueous sodium hydroxide solution (180 mL), and tetrabutylammonium hydrogen sulfate (3.6 g, 10.6 mmol). The resulting mixture was refluxed for 6 hours, then cooled to room temperature. The methylene chloride layer was collected and washed with distilled water (150 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure leaving 6.2 grams of a light brown oil. The oil was purified by flash chromatography on silica gel (200 g) using a 50:1 chloroform / acetone

mixture as the eluent, which gave pure (±)-2,3:4,5-di-*O*-cyclohexylidene-6-*O*-benzyl-*myo*-inositol (**XVIIIb**) (1.4 g, 31%) as a light yellow oil. TLC in chloroform / acetone (50:1) gave $R_f = 0.79$. ^1H NMR (200 MHz, $CDCl_3$): δ 1.23 - 1.96, multiplet, 20H; δ 2.65 - 2.85, singlet, 1H; δ 3.44 - 3.68, triplet, 1H; δ 3.85 - 3.97, multiplet, 1H; δ 3.97 - 4.10, singlet, 1H; δ 4.10 - 4.52 multiplet, 3H; δ 4.60 - 4.91, quartet, 2H; δ 7.19 - 7.53, multiplet, 5H.

*Preparation of (-)-1-*O*-[(1S)-(-)-camphanoyl]-2,3:4,5-di-*O*-cyclohexylidene-6-*O*-benzyl-*myo*-inositol (**XIXa**), (+)-1-*O*-[(1S)-(-)-camphanoyl]-2,3:4,5-di-*O*-cyclohexylidene-6-*O*-benzyl-*myo*-inositol (**XIXb**)*

To methylene chloride (50 mL) was added (±)-2,3:4,5-di-*O*-cyclohexylidene-6-*O*-benzyl-*myo*-inositol (**XVIIIb**) (1.3 g, 3.0 mmol), 4-(dimethylamino)pyridine (DMAP) (40.0 mg, 3.3×10^{-4} mol), triethylamine (0.9 g, 8.9 mmol), and (1*S*)-(-)-camphanic acid chloride (0.8 g, 3.7 mmol). The resulting mixture was stirred for 24 hours at room temperature under a nitrogen atmosphere. The reaction mixture was washed with distilled water (2 x 40 mL), saturated aqueous sodium chloride solution (40 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure leaving 1.7 grams of a light yellow glass. The glass was purified and the diastereoisomers separated in one step by using flash chromatography on silica gel (200 g) with a 98:2 methylene chloride / diethyl ether mixture as the eluent, which gave pure (+)-1-*O*-[(1*S*)-(-)-camphanoyl]-2,3:4,5-di-*O*-cyclohexylidene-6-*O*-benzyl-*myo*-inositol (**XIXb**) (686 mg, 37%) as a yellow solid with a melting point of 122 - 123° C, and (-)-1-*O*-[(1*S*)-(-)-camphanoyl]-2,3:4,5-di-*O*-cyclohexylidene-6-*O*-benzyl-myo-inositol (**XIXa**) (798 mg, 44%) as a yellow solid with a melting point of 150 - 151° C. TLC in methylene chloride / diethyl ether (98:2) gave R_f(**XIXb**) = 0.35, and R_f(**XIXa**) = 0.22.

Specific rotation (**XIXa**) $[\alpha]_D$ = -32.9 (0.0418 **M**, $CHCl_3$), (**XIXb**) $[\alpha]_D$ = +17.1 (0.0379 **M**, $CHCl_3$). ^1H NMR (**XIXa**) (200 MHz, $CDCl_3$): δ 0.83 - 0.96, singlet, 3H; δ 0.97 - 1.06, singlet, 3H; δ 1.06 - 1.17, singlet, 3H; δ 1.18 - 2.13, multiplet, 23H; δ 2.25 - 2.51, multiplet, 1H; δ 3.43 - 3.60, triplet, 1H; δ 3.74 - 3.95, multiplet, 2H; δ 4.23 - 4.40, triplet, 1H; δ 4.55 - 4.65, triplet, 1H; δ 4.67 - 4.92, quartet, 2H; δ 5.16 - 5.31, triplet, 1H; δ 7.20 - 7.45, multiplet, 5H. ^1H NMR (**XIXb**) (200 MHz, $CDCl_3$): δ 0.85 - 0.95, singlet, 3H; δ 0.96 - 1.05, singlet, 3H; δ 1.06 - 1.17, singlet, 3H; δ 1.25 - 2.22, multiplet, 23H; δ 2.35 - 2.56, multiplet, 1H; δ 3.46 - 3.62, triplet, 1H; δ 3.76 - 3.94, multiplet, 2H; δ 4.27 - 4.40, triplet, 1H; δ 4.55 - 4.67, triplet, 1H; δ 4.72 - 4.94, quartet, 2H; δ 5.22 - 5.35, triplet, 1H; δ 7.18 - 7.45, multiplet, 5H.

*Preparation of (-)-1-*O*-[(1S)-(-)-camphanoyl]-2,3-*O*-cyclohexylidene-6-*O*-benzyl-*myo*-inositol (**XX**)*

To methylene chloride (28 mL) was added (-)-1-*O*-[(1*S*)-(-)-camphanoyl]-2,3:4,5-di-*O*-cyclohexylidene-6-*O*-benzyl-*myo*-inositol (**XIXa**) (335.0 mg, 5.5 x 10^{-4} mol), dry methanol (6 mL), and acetyl chloride (57 μL). The resulting mixture was stirred for 3 hours at room temperature under a nitrogen atmosphere. The reaction mixture was evaporated under reduced pressure leaving 240 mg of a white solid. The solid was purified by flash chromatography on silica gel (8 g) using a 7:3 ethyl acetate / n-hexane mixture as the eluent, which gave pure (-)-1-*O*-[(1*S*)-(-)-camphanoyl]-2,3-*O*-cyclohexylidene-6-*O*-benzyl-*myo*-inositol (**XX**) (155.3 mg, 53%) as a white solid with a melting point of 176 - 178° C. TLC in ethyl acetate / n-hexane (7:3) gave R_f = 0.52. Specific rotation $[\alpha]_D$ = -33.7 (0.0585 **M**, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ 0.91 -1.04, singlet, 3H; δ 1.06 -

1.23, singlet, 6H; δ 1.25 - 1.82, multiplet, 11H; δ 1.82 - 2.12, multiplet, 2H; δ 2.21 - 2.47, multiplet, 1H; δ 3.35 - 3.57, multiplet, 2H; δ 3.57 - 3.94, multiplet, 3H; δ 3.98 - 4.16, multiplet, 1H; δ 4.47 - 4.61, multiplet, 1H; δ 4.69 - 4.90, multiplet, 2H; δ 5.15 - 5.30, multiplet, 1H; δ 7.22 - 7.45, multiplet, 5H.

*Preparation of (±)-1,2-*O*-isopropylidene-*myo*-inositol (XXI)*

To dimethyl sulfoxide (DMSO) (75 mL) was added *myo*-inositol (20.0 g, 111.0 mmol), *p*-toluenesulfonic acid monohydrate (154.0 mg, 8.1 x 10^{-4} mol), and 2,2-dimethoxypropane (63.5 g, 609.7 mmol). The resulting mixture was refluxed for 2 hours at 110^{0} C under a nitrogen atmosphere, allowing 40 mL of distillate (methanol, 2,2-dimethoxypropane) to be collected. The reaction mixture was then placed under reduced pressure to remove any additional methanol or unreacted 2,2-dimethoxypropane. Additional *myo*-inositol (20.0 g, 111.0 mmol) was added to the mixture, and was then heated for 1.5 hours at 120^{0} C under a nitrogen atmosphere. The reaction mixture was cooled down to room temperature and solid anhydrous potassium carbonate (154.0 mg, 1.1 mmol) was added and the solution was stirred vigorously for 5 minutes. The DMSO was removed under reduced pressure leaving a light brown syrupy residue. The residue was dissolved in absolute ethanol (1.5 L) and refluxed for 30 minutes. The mixture was then cooled to room temperature, filtered, and evaporated under reduced pressure to a volume of approximately 300 mL. Crystallization occurred at room temperature and the vessel was placed in the freezer for 2 days. The crystal was collected by vacuum filtration and washed with cold absolute ethanol (50 mL). The white solid was purified by recrystallization from 1-propanol, which gave pure (±)-1,2-*O*-isopropylidene-*myo*-inositol (**XXI**) (18.4 g, 38%)

as a white solid with a melting point of 184 - 186° C. ^{1}H NMR (200 MHz, D_2O): δ 1.38 - 1.50, singlet, 3H; δ 1.55 - 1.69, singlet, 3H; δ 3.22 - 3.40, triplet, 1H; δ 3.52 - 3.76, multiplet, 2H; δ 3.83 - 3.97, multiplet, 1H; δ 4.05 - 4.20, multiplet, 1H; δ 4.46 - 4.59, triplet, 1H.

*Preparation of (±)-1,2-*O*-isopropylidene-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (XXII)*

To dimethylformamide (DMF) (135 mL) was added (±)-1,2-*O*-isopropylidene-*myo*-inositol (**XXI**) (5.0 g, 22.7 mmol). The resulting mixture was stirred for 30 minutes at room temperature under a helium atmosphere. Sodium hydride (97%) (3.3 g, 137.5 mmol) was slowly added, and the mixture stirred for 1 hour at room temperature under a helium atmosphere. Benzyl bromide (25.0 g, 146.2 mmol) was added and the resulting mixture was refluxed for 6 hours, cooled to room temperature and stirred overnight. An ice / distilled water mixture (200 mL) was slowly added to the reaction vessel, followed by methylene chloride (200 mL). The aqueous layer was collected and extracted with additional methylene chloride (2 x 20 mL). The methylene chloride layers were combined and washed with distilled water (200 mL), saturated aqueous sodium chloride solution (200 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure, leaving 16.4 grams of a dark brown non-viscous residue. The residue was purified by flash chromatography on silica gel (400 g) using a 15:1 toluene / diethyl ether mixture as the eluent, which gave (±)-1,2-*O*-isopropylidene-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXII**) (9.1 g, 69%) as a light yellow non-viscous oil. TLC in toluene / diethyl ether (15:1) gave R_f = 0.49. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30 - 1.45, singlet, 3H; δ 1.46 - 1.58, singlet, 3H; δ 3.33 - 3.50, triplet, 1H; δ 3.64 - 3.87, multiplet, 2H; δ 3.90 -

4.03, triplet, 1H; δ 4.05 - 4.18, triplet, 1H; δ 4.20 - 4.34, multiplet, 1H; δ 4.65 - 5.00, multiplet, 8H; δ 7.13 - 7.48, multiplet, 20H.

*Preparation of (±)-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXIII**)*

To methanol (86 mL) was added concentrated hydrochloric acid (7 mL), and (±)-1,2-*O*-isopropylidene-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXII**) (11.9 g, 20.5 mmol). The resulting mixture was refluxed for 30 minutes and cooled down to room temperature. Anhydrous sodium bicarbonate was slowly added until the evolution of carbon dioxide was no longer observed, and the mixture was evaporated under reduced pressure to leave a white residue. The residue was extracted with chloroform (150 mL), dried (K_2CO_3), filtered, and evaporated under reduced pressure leaving 13.0 grams of a light brown solid. The solid was purified by recrystallization form methanol (40 mL), which gave pure (±)-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXIII**) (8.3 g, 75%) as a white solid with a melting point of 109 - 110° C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.35 - 2.58, broad singlet, 2H; δ 3.35 - 3.56, multiplet, 3H; δ 3.77 - 3.91, multiplet, 1H; δ 3.91 - 4.06, multiplet, 1H; δ 4.13 - 4.24, multiplet, 1H; δ 4.63 - 5.04, multiplet, 8H; δ 7.15 - 7.50, multiplet, 20H.

*Preparation of (±)-1-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXIV**)*

To (±)-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXIII**) (8.0 g, 14.8 mmol) was added dry benzene (80 mL), powdered sodium hydroxide (9.0 g, 225.0 mmol), and allyl bromide (2.7 g, 22.3 mmol). The resulting mixture was refluxed for 2 hours under a nitrogen atmosphere, then cooled down to room temperature. The reaction mixture was washed with distilled water (80 mL),

dried (K_2CO_3), filtered, and evaporated under reduced pressure leaving 9.0 grams of a light brown oily residue. The residue was purified by flash chromatography on silica gel (200 g) using a 2:1 diethyl ether / n-hexane mixture as the eluent, which gave a 6.3 gram mixture of (±)-1-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXIV**), and (±)-2-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXIX**) as a light yellow oil. TLC in diethyl ether / n-hexane (2:1) gave $R_f = 0.57$. The desired compound **XXIV** was isolated by fractional crystallization by first dissolving the mixture in a minimum amount of diethyl ether, followed by the slow addition of light petroleum ether (30 - 60° C) until a precipitate formed. The crystallization mixture was placed in the freezer for 2 days, and then vacuum filtered to give (±)-1-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXIV**) (5.0 g, 58%) as a white solid with a melting point of 64 - 64° C. ^1H NMR (200 MHz, $CDCl_3$): δ 2.40 - 2.63, broad singlet, 1H; δ 3.0 - 3.55, multiplet, 3H; δ 3.88 - 4.10, multiplet, 2H; δ 4.10 - 4.30, multiplet, 3H; δ 4.64 - 5.00, multiplet, 8H; δ 5.13 - 5.38, multiplet, 2H; δ 5.82 - 6.07, multiplet, 1H; δ 7.10 - 7.48, multiplet, 20H.

*Preparation of (±)-1-*O*-allyl-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (XXV)*

To (±)-1-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**XXIV**) (4.3 g, 7.4 mmol) was added dry benzene (55 mL) and stirred until all the solid was dissolved. Sodium hydride (97%) (0.7 g, 29.2 mmol) was slowly added and stirred for 20 minutes under a helium atmosphere. Benzyl bromide (4.5 g, 26.3 mmol) was added and the resulting mixture was refluxed for 3 hours under a helium atmosphere. The reaction mixture was cooled to room temperature and washed with distilled water (65 mL). The water layer was

collected and extracted with additional benzene (15 mL). The benzene layers were combined, dried (K_2CO_3), filtered, and evaporated under reduced pressure, leaving 7.7 grams of a light yellow oily residue. The residue was purified by flash chromatography on silica gel (190 g) using a 3:1 n-hexane / diethyl ether mixture as the eluent, which gave (±)-1-*O*-allyl-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXV**) (4.4 g, 89%) as a colorless oil. TLC in n-hexane / diethyl ether (3:1) gave $R_f = 0.71$. ^1H NMR (200 MHz, $CDCl_3$): δ 3.15 - 3.56, multiplet, 3H; δ 3.87 - 4.20, multiplet, 5H; δ 4.44 - 5.00, multiplet, 10H; δ 5.00 - 5.40, multiplet, 2H; δ 5.75 - 6.05, multiplet, 1H; δ 7.05 - 7.53, multiplet, 25H.

*Preparation of (±)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIa-b**)*

To methanol (50 mL) was added (±)-1-*O*-allyl-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXV**) (4.4 g, 6.6 mmol), distilled water (10 mL), 10% palladium on activated carbon (Pd/C) (440.0 mg), and *p*-toluenesulfonic acid monohydrate (440.0 mg, 2.3 mmol). The resulting mixture was refluxed for 24 hours, then cooled down to room temperature. The Pd/C was filtered off and the solvents evaporated under reduced pressure, leaving a white oily residue. The residue was partitioned between distilled water (80 mL) and diethyl ether (80 mL). The water layer was collected and extracted with additional diethyl ether (15 mL). The diethyl ether layers were combined and evaporated under reduced pressure, leaving 3.6 grams of a waxy white solid. The solid was purified by flash chromatography on silica gel (90 g) using a 50:1 chloroform / diethyl ether mixture as the eluent, which gave pure (±)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIa-b**) (1.6 g, 38%) as a white solid with a melting point of 92 - 93° C. TLC in chloroform / diethyl ether (50:1) gave $R_f = 0.62$. ^1H NMR (200 MHz, $CDCl_3$): δ 2.05 - 2.46,

broad singlet, 1H; δ 3.33 - 3.62, multiplet, 3H; δ 3.74 - 3.94, triplet, 1H; δ 3.96 - 4.20, multiplet, 2H; δ 4.56 - 5.13, multiplet, 10H; δ 7.10 - 7.62, multiplet, 25H.

*Preparation of (-)-1-*O*-[(1S)-(-)-camphanoyl]-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIIa**), (+)-1-*O*-[(1S)-(-)-camphanoyl]-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIIb**)*

To methylene chloride (37 mL) was added (±)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIa-b**)(1.6g, 2.5 mmol),4-(dimethylamino)pyridine (DMAP) (33.0 mg, 2.7 x 10^{-4} mol), triethylamine (0.8 g, 7.9 mmol), and (1*S*)-(-)-camphanic acid chloride (0.7 g, 3.2 mmol). The resulting mixture was stirred for 24 hours at room temperature under a nitrogen atmosphere. The reaction mixture was washed with distilled water (2 x 30 mL), saturated aqueous sodium chloride solution (20 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure, leaving 2.1 grams of a light brown solid. The solid was purified by flash chromatography on silica gel (50 g) using a 99:1 methylene chloride / diethyl ether mixture as the eluent, which gave a mixture of diastereoisomeric esters **XXVIIa-b** (2.0 g, 99%). TLC in methylene chloride / diethyl ether (99:1) gave R_f(**XXVIIa**) = 0.60, and R_f(**XXVIIb**) = 0.73. The diastereoisomeric mixture (**XXVIIa-b**) was separated by using HPLC with a Lichrosorb® prep column and a 99:1 methylene chloride / diethyl ether mobile phase at a flow rate of 2.5 mL / min. Approximately 20 mg of the mixture was injected into the column per run, adding up to 95 injections total. Diastereoisomer **XXVIIb** eluted from the column at 8.5 - 12.5 minutes after injection, and diastereoisomer **XXVIIa** eluted from the column at 14 - 17 minutes after injection. After the HPLC separation one obtained (-)-1-*O*-[(1*S*)-(-)-camphanoyl]-2,3,4,5,6-

penta-*O*-benzyl-*myo*-inositol (**XXVIIa**) (990.0 mg) as a white solid with a melting point of 158 - 160° C, and (+)-1-*O*-[(1*S*)-(−)-camphanoyl]-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIIb**) (1.0 g) as a white solid with a melting point of 144 - 146° C. ^{1}H NMR (**XXVIIa**) (200 MHz, $CDCl_3$): δ 0.74 - 0.90, singlet, 3H; δ 0.90 - 1.03, singlet, 3H; δ 1.03 - 1.22, singlet, 3H; δ 1.43 - 2.10, multiplet, 3H; δ 2.15 - 2.46, multiplet, 1H; δ 3.43 - 3.80, multiplet, 2H; δ 3.90 - 4.40, multiplet, 3H; δ 4.44 - 5.70, multiplet, 11H; δ 6.93 - 7.68, multiplet, 25H. ^{1}H NMR (**XXVIIb**) (200 MHz, $CDCl_3$): δ 0.83 - 0.96, singlet, 3H; δ 0.96 - 1.05, singlet, 3H; δ 1.05 - 1.19, singlet, 3H; δ 1.48 - 2.02, multiplet, 3H; δ 2.15 - 2.43, multiplet, 1H; δ 3.50 - 3.73, multiplet, 2H; δ 4.01 - 4.32, multiplet, 3H; δ 4.50 - 5.17, multiplet, 11H; δ 6.90 - 7.54, multiplet, 25H.

*Preparation of (−)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIa**), (+)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIb**)*

To absolute ethanol (100 mL) was added potassium hydroxide (0.7g, 12.5 mmol), and (−)-1-*O*-[(1*S*)-(−)-camphanoyl]-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIIa**) (990.0 mg, 1.2 mmol). The resulting mixture was stirred for 24 hours at room temperature. The solvents were removed under reduced pressure, leaving a solid yellow-white residue. The residue was partitioned between diethyl ether (100 mL) and distilled water (70 mL). The water layer was collected and extracted with additional diethyl ether (35 mL). The diethyl ether layers were combined, dried ($MgSO_4$), filtered, and evaporated under reduced pressure, leaving 0.9 grams of an oily residue. The residue was purified by flash chromatography on silica gel (25 g) using a 1:3 n-hexane / diethyl ether mixture as the eluent, which gave (−)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIa**) (724.6 mg, 96%). TLC in

n-hexane / diethyl ether (1:3) gave R_f = 0.81. The exact same procedure was performed on (+)-1-*O*-[(1*S*)-(−)-camphanoyl]-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIIb**), which gave (+)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIb**) (725.6 mg, 91%). Specific rotation (**XXVIa**) $[\alpha]_D$ = -9.0 (0.1078 **M**, $CHCl_3$), (**XXVIb**) $[\alpha]_D$ = +8.8 (0.1268 **M**, $CHCl_3$). ^1H NMR (**XXVIa**) (200 MHz, $CDCl_3$): δ 2.02 - 2.26, broad doublet, 1H; δ 3.25 - 3.60, triplet, 3H; δ 3.60 - 3.87, triplet, 1H; δ 3.87 - 4.14, multiplet, 2H; δ 4.46 - 5.08, multiplet, 10H; δ 6.94 - 7.55, multiplet, 25H. ^1H NMR (**XXVIb**) (200 MHz, $CDCl_3$): δ 2.18 - 2.35, broad doublet, 1H; δ 3.32 - 3.63, triplet, 3H; δ 3.70 - 3.94, triplet, 1H; δ 3.96 - 4.18, multiplet, 2H; δ 4.50 - 5.14, multiplet, 10H; δ 6.98 - 7.57, multiplet, 25H.

*Preparation of (S)-(E)-3-oleoyloxy-4-stearoyloxybut-1-enylphosphonic acid 2,3,4,5,6-penta-*O*-benzyl-*myo*-inosit-1-yl ester (**XXXI**)*

To freshly distilled pyridine (0.5 mL) was added (*S*)-(*E*)-3-oleoyloxy-4-stearoyloxybut-1-enylphosphonic acid (**XXX**) (70.0 mg, 0.1 mmol), (−)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**XXVIa**) (50.0 mg, 8.0 x 10^{-5} mol), and trichloroacetonitrile (450.0 mg, 3.1 mmol). The resulting mixture was stirred for 3 days under a nitrogen atmosphere using a temperature controlled water bath at 51° C. The reaction mixture was evaporated under reduced pressure, leaving a reddish-brown residue. The residue was purified by flash chromatography on silica gel (10 g) using a 50:1 methylene chloride / diethyl ether mixture as the eluent, which gave pure (*S*)-(*E*)-3-oleoyloxy-4-stearoyloxybut-1-enylphosphonic acid 2,3,4,5,6-penta-*O*-benzyl-*myo*-inosit-1-yl ester (**XXXI**) (21.0 mg, 20%). TLC in methylene chloride / diethyl ether (50:1) with phosphomolybdate spray visualization gave R_f = 0.85. Specific rotation $[\alpha]_D$ = -2.47 (0.0014 **M**, CCl_4). ^1H NMR (200 MHz,

$CDCl_3$): δ 0.9, broad multiplet, 6H; δ 1.1 - 1.4, broad multiplet, 52H; δ 1.9 - 2.5, multiplet, 8H; δ 3.3 - 3.6, multiplet, 3H; δ 3.8 - 4.0, multiplet 2H; δ 4.0 - 5.0, multiplet, 13H; δ 5.2, multiplet, 1H; δ 5.4, triplet, 2H; δ 5.6 - 5.8, multiplet, 2H; δ 7.0 - 7.4, multiplet, 25H; δ 12.1, singlet, 1H. Anal. calcd. for $C_{81}H_{115}O_{12}P$: C 74.17, H 8.84%; found C 73.91, H 9.14%.

Preparation of (S)-(E)-3-oleoyloxy-4-stearoyloxybut-1-enylphosphonic acid myo-*inosit-1-yl ester (XXXII)*

To (*S*)-(*E*)-3-oleoyloxy-4-stearoyloxybut-1-enylphosphonic acid 2,3,4,5,6-penta-*O*-benzyl-*myo*-inosit-1-yl ester (**XXXI**) (7.3 mg, 5.6 x 10^{-6} mol), was added chloroform (0.5 mL), and iodotrimethylsilane (0.25 mL). The resulting mixture was stirred for 15 minutes at room temperature, then quenched by the addition of methanol (0.75 mL). The volatile materials were removed under reduced pressure and diethyl ether (1 mL) was added to the residue. Saturated aqueous sodium bisulfite solution (2 drops, Pasteur pipet) was added and the material became clear. Volatile materials were again removed under reduced pressure, leaving a white residue. The residue was purified by flash chromatography on silica gel (20 g) using chloroform as the eluent, which gave pure (*S*)-(*E*)-3-oleoyloxy-4-stearoyloxybut-1-enylphosphonic acid *myo*-inosit-1-yl ester (**XXXII**) (3.7 mg, 77%). TLC in chloroform with phosphomolybdate spray visualization gave R_f = 0.31. Specific rotation $[\alpha]_D$ = -6.38 (0.0021 **M**, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ 0.9, broad multiplet, 6H; δ 1.0 - 1.6, broad multiplet, 52H; δ 1.6 - 2.5, multiplet, 8H; δ 3.6, singlet, 6H; δ 3.7 - 4.5, broad multiplet, 9H; δ 5.4, triplet, 2H; δ 5.7 - 5.8, multiplet, 2H. Anal. calcd. for $C_{46}H_{85}O_{12}P$: C 64.16, H 9.95%; found: C 63.90, H 10.10%.

REFERENCES

1. M. J. Berridge and R. F. Irvine, *Nature*, **312**, 315 (1984).

2. M. J. Berridge, *Biochem. J.*, **220**, 345 (1984).

3. M. J. Berridge, *Ann. Rev. Biochem.*, **56**, 159 (1987).

4. L. E. Hokin, *Ann. Rev. Biochem.*, **54**, 205 (1985).

5. Y. Nishizuka, *Science*, **233**, 305 (1986).

6. Y. Nishizuka, *Nature*, **308**, 693 (1984).

7. J. R. Williamson, R. H. Cooper, S. K. Joseph, and A. P. Thomas, *Am. J. Physiol.*, **248**, C203 (1985).

8. S. R. Nahorski, D. A. Kendall, and I. R. Batty, *Biochem. Pharmacol.*, **35**, 2447 (1986).

9. P. W. Majerus, E. J. Neufeld, and D. B. Wilson, *Cell*, **37**, 701 (1984).

10. A. R. Hughes, D. A. Horstman, H. Takemura, and J. W. Putney, *Am. Rev. Respir. Dis.*, **141**, 5115 (1990).

11. C. P. Downes and R. H. Michell In *Molecular Mechanisms of Transmembrane Signaling*, P. Cohen and M. Houslay, Eds., Elsevier: New York, 1985; pp 3-56.

12. L. F. Jaffe, *Devl. Biol.*, **99**, 265 (1983).

13. T. J. Hallam, A. Sanchez, and T. J. Rink, *Biochem. J.*, **218**, 819 (1984).

14. C. B. Wollheim and G. W. G. Sharp, *Physiol. Rev.*, **61**, 914 (1981).

15. P. F. Blackmore, B. P. Hughes, E. A. Shuman, and J. H. Exton, *J. Biol. Chem.*, **257**, 190 (1984).

16. R. D. Brown, K. D. Berger, and P. Taylor, *J. Biol. Chem.*, **259**, 7554 (1984).

17. M. J. Berridge, R. M. C. Dawson, C. P. Downes, J. P. Heslop, and R. F. Irvine, *Biochem. J.*, **212**, 473 (1983).

18. A. A. Abdel-Latif, R. A. Akhtar, and J. N. Hawthorne, *Biochem. J.*, **162**, 61 (1977).

19. J. A. Creba, C. P. Downes, P. T. Hawkins, G. Brewster, R. H. Michell, and C. J. Kirk, *Biochem. J.*, **212**, 733 (1983).

20. A. P. Thomas, J. Alexander, and J. R. Williamson, *J. Biol. Chem.*, **259**, 5574 (1984).

21. S. D. Shukla, D. B. Buxton, M. S. Olson, and D. J. Hanahan, *J. Biol. Chem.*, **258,** 10212 (1983).

22. S. G. Laychock, *Biochem. J.*, **216**, 101 (1983).

23. M. R. Hokin and L. E. Hokin, *J. Biol. Chem.*, **203**, 967 (1953).

24. J. Downward, Y. Yarden, E. Mayes, G. Scrace, N. Totty, P. Stockwell, A. Ullrich, J. Schlessinger, and M. D. Waterfield, *Nature*, **307**, 521 (1984).

25. D. B. Wilson, T. M. Connolly, T. E. Bross, P. W. Majerus, W. R. Sherman, A. N. Tyler, L. J. Rubin, and J. E. Brown, *J. Biol. Chem.*, **260**, 13496 (1985).

26. L. F. Bass, and S. K. Joseph, *J. Biol. Chem.*, **260**, 15172 (1985).

27. J. B. Smith, L. Smith, and B. L. Higgins, *J. Biol. Chem.*, **260**, 14413 (1985).

28. S. K. Joseph and J. R. Williamson, *J. Biol. Chem.*, **261**, 14658 (1986).

29. R. F. Irvine, K. D. Brown, and M. J. Berridge, *J. Biol. Chem.*, **222**, 269 (1984).

30. C. P. Downes, M. C. Mussat, and R. H. Michell, *Biochem. J.*, **203**, 169 (1982).

31. D. J. Storey, S. B. Shears, C. J. Kirk, and R. H. Michell, *Nature*, **312**, 374 (1984).

32. L. M. Hallcher and W. R. Sherman, *J. Biol. Chem.*, **255**, 1089 (1980).

33. R. F. Irvine, A. J. Letcher, J. P. Heslop, and M. J. Berridge, *Nature*, **320**, 631 (1986).

34. P. T. Hawkins, L. Stephens, and C. P. Downes, *Biochem. J.*, **238**, 507 (1986).

35. I. R. Batty, S. R. Nahorski, and F. R. Irvine, *Biochem. J.*, **232**, 211 (1985).

36. R. Engel, *Chem. Rev.*, **77**, 349 (1977).

37. R. Engel In *The Role of Phosphonates in Living Systems*, R. L. Hilderbrand, Ed., CRC Press, Inc.; Boca Raton, Fl., 1983; pp 97-138.

38. A. Holy, *Nucleosid. Nucleotid.*, **6**, 147 (1987).

39. J. Kraut, *Acta Crystallogr.*, **14**, 1146 (1961).

40. W. G. Ferrier, A. R. Lindsay, and D. W. Young, *Acta Crystallogr.*, **15**, 616 (1962).

41. Y. Okaya, *Acta Crystallogr.*, **20**, 712 (1966).

42. S. Abrahamsson and T. Pascher, *Acta Crystallogr.*, **21**, 79 (1966).

43. M. A. Viswamitra, B. S. Reddy, G. H. -Y. Lin, and M. Sundaralingam, *J. Am. Chem. Soc.*, **93**, 4565 (1971).

44. K. C. Tang, B. E. Tropp, and R. Engel, *Tetrahedron*, **34**, 2873 (1978).

45. J. Kabak, L. DeFilippe, R. Engel, and B. Tropp, *J. Med. Chem.*, **15**, 1074 (1972).

46. C. S. Shopis, R. Engel, and B. E. Tropp, *J. Bacteriol.*, **112**, 408 (1972).

47. C. S. Shopis, W. D. Nunn, R. Engel, and B. E. Tropp, *Antimicrob. Agents Chemother.*, **4**, 467 (1973).

48. C. S. Shopis, R. Engel, and B. E. Tropp, *J. Biol. Chem.*, **249**, 2473 (1974).

49. D. A. Klein, R. Engel, and B. E. Tropp, *J. Bacteriol.*, **129**, 550 (1977).

50. P. -J. Cheng, W. D. Nunn, R. J. Tyhach, S. L. Goldstein, R. Engel, and B. E. Tropp, *J. Biol. Chem.*, **250**, 1633 (1975).

51. R. J. Tyhach, R. Engel, and B. E. Tropp, *J. Biol. Chem.*, **251**, 6716 (1976).

52. K. C. Tang, B. Mildnener, L. Eisikowitz, B. E. Tropp, and R. Engel, Paper #54, Division of Biological Chemistry, American Chemical Society/Chemical Society of Japan Joint Meeting, April 1979, Honolulu.

53. N. Lalinde, B. E. Tropp, and R. Engel, *Tetrahedron*, **39**, 2369 (1983).

54. F. Paltauf, *Chem. Phys. Lipids*, **11**, 270 (1973).

55. A. F. Rosenthal, G. M. Kosolapoff, and R. P. Geyer, *Recl. Trav. Chim. Pays-Bas*, **83**, 1273 (1964).

56. A. F. Rosenthal, *J. Chem. Soc.*, 7345 (1965).

57. A. F. Rosenthal, *J. Lipid Res.*, **7**, 779 (1966).

58. A. F. Rosenthal and M. Prousada, *Biochim. Biophys. Acta*, **125**, 265 (1966).

59. J. C. Tang, B. E. Tropp, and R. Engel, *Chem. Phys. Lipids*, **17**, 169 (1976).

60. A. F. Rosenthal and M. Pousada, *Biochim. Biophys. Acta*, **164**, 226 (1968).

61. A. F. Rosenthal and M. Pousada, *Lipids*, **4**, 37 (1969).

62. A. F. Rosenthal and S. V. Chodsky, *Chem. Commun.*, 1504 (1968).

63. D. Braksmayer, B. E. Tropp, and R. Engel, *Chem. Phys. Lipids*, **19**, 93 (1977).

64. A. F. Rosenthal and A. C. -H. Han, *Biochim. Biophys. Acta*, **218**, 213 (1970).

65. H. S. Hendrickson, D. G. Rustard, E. M. Scattergood, and D. E. Engle, *Chem. Phys. Lipids*, **13**, 63 (1974).

66. S. Ozaki, Y. Watanabe, T. Ogasawara, Y. Kondo, N. Shiotani, H. Nishii, and T. Matsuki, *Tetrahedron Lett.*, **27**, 3157 (1986).

67. S. J. Angyal, M. E. Tate, and S. D. Gero, *J. Chem. Soc.*, 4116 (1961).

68. S. A. Patwadhan and S. Dev, *Synth. Commun.*, 348 (1973).

69. U. Schmidt and P. Grafen, *Liebigs Ann. Chem.*, **656**, 97, (1962).

70. R. A. Wohl, *Synthesis*, 38 (1974).

71. D. F. Holmes and R. Adams, *J. Am. Chem. Soc.*, **56**, 2093 (1934).

72. R. Adams, Ed., *Organic Reactions*, Vol. 2, John Wiley and Sons: New York, 1944; pp 137-139.

73. D. C. Billington, R. Baker, J. J. Kulagowski, and I. M. Mawer, *J. Chem. Soc. Chem. Commun.*, 314 (1987).

74. P. J. Garegg, T. Iverson, R. Johansson, and B. Lindberg, *Carbohydr. Res.*, **130**, 322 (1984).

75. J. P. Vacca, S. J. DeSolms, J. R. Huff, D. C. Billington, R. Baker, J. J. Kulagowski, and I. M. Mawer, *Tetrahedron*, **45**, 5679 (1989).

76. R. Gigg and C. D. Warren, *J. Chem. Soc.*, 2367 (1969).

77. D. C. Billington, R. Baker, J. J. Kulagowski, and I. M. Mawer, *J. Chem. Soc. Chem. Commun.*, 314 (1987).

78. R. Boss and R. Scheffold, *Angew. Chem., Int. Ed. Engl.*, **15**, 558 (1976).

79. C. Pratt, Y.-j. Liu, T.-y. Chu, K. Melkonian, B. E. Tropp, and R. Engel, *Can. J. Chem.*, **8**, 2135 (1992).

80. P. W. Schwartz, B. E. Tropp, and R. Engel, *Chem. Phys. Lipids*, **48**, 1, (1988).

81. M. E. Jung and M. A. Lyster, *J. Org. Chem.*, **42**, 3761 (1977).

82. J. C. Dittmer and R. L. Lester, *J. Lipid Res.*, **5**, 126 (1964).

83. E. J. Corey and J. W. Suggs, *J. Org. Chem.*, **38**, 3224 (1973).