

STRUCTURE ELUCIDATION AND STUDIES RELATING TO THE SYNTHESIS
OF PLASMALOPENTAENE-12

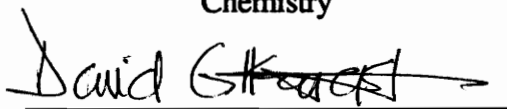
by

Robert F. Keyes

Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

in

Chemistry



D. G. I. Kingston, Chairman



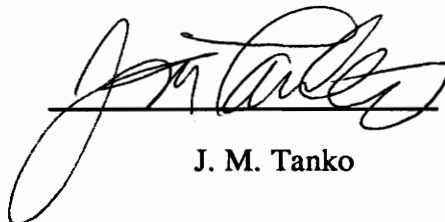
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November 20, 1992

Blacksburg, Virginia

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(ABSTRACT)

The glycerol enol ether, fecapentaene-12, is a direct acting fecal mutagen that is formed in the lower portion of the gastrointestinal tract by anaerobic bacteria. The biological precursor to fecapentaene-12 is a natural product of mammalian origin whose role in the etiology of colon cancer is unknown.

Preliminary evidence indicated that the precursor may be a plasmalogen with an intact pentaenol ether moiety. Further structural studies by means of degradative methods and chromatographic techniques enabled the structure of the precursor to be elucidated. Based on the structure of the precursor, the name plasmalopentaene-12 was coined.

Synthetic methodology was developed for obtaining synthetic plasmalopentaene-12. This was necessary in order to confirm the structure and to determine the precursor's biological role. The synthetic methodology proceeded through a novel "acyl migration" which enables the highly labile pentaenol ether to be generated late in the synthesis. Model studies indicated that this was a feasible pathway. It was also determined that this methodology may be highly adaptable to the synthesis of other plasmalogens and may also provide a new synthetic route to fecapentaene-12.

To My Parents

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to his research director, Dr. David G.I. Kingston for his ideas, support, and patience during the completion of this research project. The suggestions and assistance provided by Dr. Harold Bell, Dr. Harry Dorn, Dr. Joseph Merola, and Dr. James Tanko are also greatly appreciated. Much gratitude is also owed to Dr. Neal Castagnoli for his support. The author also expresses his thanks to the National Cancer Institute for their financial support.

Many thanks are extended to Dr. Leslie Gunatilaka. It was indeed fortunate to have the opportunity to learn about chemistry and life from an outstanding scientist. It made the time spent in Blacksburg much more rewarding.

The members of Dr. Kingston's group are also gratefully acknowledged for their support. The author would especially like to thank Kurt Neidigh and Dr. George Harrigan for continued moral support and for providing an outlet from the stress of graduate school.

Thanks also must be given to some very special friends including Dr. Paul Engen, Dr. Mike Berg, Jim Witsch, Dr. Carl Wild, John Rimoldi, Andrè Melendez and the members of the Blacksburg Rugby Football Club.

It is fitting to acknowledge the support of loved ones when undertaking a project such as this. Therefore, the author wishes to express his sincere gratitude to his parents, Raymond and Louise Keyes and to his sister, Rae Ann Kegley. Without their love, support, patience, and most of all sacrifice, it never would have been possible to accomplish life's goals. This degree is more theirs than the author's.

Finally, the author expresses his most sincere appreciation to Lisa Hamblin. The typing of this manuscript is greatly appreciated, but most of all her love and support made the completion of this project possible. Thank you.

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I. Introduction

1.1 Colon Cancer Incidence.

Cancer is a large group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled or checked, it results in death.

Normally, cells in the body replicate in an orderly fashion, thus bodily repairs can be made and growth will occur. In this respect the body is functioning in a normal way. Occasionally, cells malfunction and begin to grow and spread at an uncontrolled rate giving rise to the masses of abnormal cells called tumors. Initially the tumors are localized, but after time the cancer cells may metastasize, that is, invade other organs or tissues. The spread may occur via transportation of malignant cells through the venous and lymphatic system or the spread may be the result of a direct extension of growth. If metastasis is uncontrolled and becomes advanced then death results.¹ There is a myriad of biological, clinical, and physical causes of cancer. This makes it unsurprising that physicists, microbiologists, toxicologists and chemists all have an active interest in cancer research.

The mortality rate of cancer has increased from 3.3% in 1900 to 20% in 1988.² Since the quiescent period for cancer is greater than 50 years, it has been postulated that the increased mortality rate observed is due to the increasing age of the population.³ Between 1950 and 1977 there were more deaths from neoplasia than can be explained by the increasing age of the population (Table 1-1). Thirty year trends in age-adjusted cancer death rates per 100,000 population (1956-58 to 1986-88) also show that there has been an increase in cancer death rates (Table 1-2).⁴

Table 1-1. Distribution of the Most Common Causes of Death in the United States from 1950 to 1977.

CAUSE	DEATH RATE ^a			
	1950	1960	1970	1977
All disease	841.5	760.9	714.3	612.3
Heart disease	307.6	286.2	253.6	210.4
Neoplasms	125.4	125.8	129.9	133.0
Cerebrovascular disease	88.8	79.7	66.3	48.2
Accidents	57.5	49.9	53.7	43.8
Tuberculosis	21.7	5.4	2.2	1.0

^aDeath rates (deaths/100,000 population) are adjusted to the age structure of the population.

**Table 1-2. 30-Year Trends in Cancer Death Rates
By Site and Sex, 1956-58 to 1986-88.**

SITES	SEX	1956-58	1986-88	PERCENT CHANGE
ALL SITES	Male	180.1	216.4	+ 20
	Female	139.9	139.4	*
ESOPHAGUS	Male	4.8	5.7	+ 20
	Female	1.2	1.5	+ 25
STOMACH	Male	18.7	7.0	- 62
	Female	9.7	3.2	- 67
COLON & RECTUM	Male	25.3	24.0	- 5
	Female	23.0	16.8	- 27
RECTUM	Male	8.4	3.6	- 57
	Female	5.7	2.1	- 36
LIVER†	Male	6.0	5.0	- 16
	Female	6.3	3.2	- 49
PANCREAS	Male	9.9	9.9	*
	Female	6.2	7.1	+ 15
LUNG	Male	33.4	73.7	+ 121
	Female	5.4	28.2	+ 425
MELANOMA OF SKIN	Male	1.3	2.9	+ 126
	Female	1.0	1.5	+ 55
BREAST	Male	0.3	0.2	- 22
	Female	26.0	27.2	*
OTHER UTERUS	Female	7.4	3.6	- 52
OVARY	Female	8.8	7.9	- 10
PROSTATE	Male	21.4	24.0	+ 12
BLADDER	Male	7.2	5.6	- 22
	Female	2.9	1.7	- 42
KIDNEY	Male	3.7	5.0	+ 35
	Female	1.9	2.4	+ 22
BRAIN	Male	4.0	5.0	+ 24
	Female	2.6	3.3	+ 25
LEUKEMIA	Male	8.7	8.1	*
	Female	5.8	4.8	- 16

*Percent changes not listed because they are not meaningful.

†Primary and non-specified.

Excluding nonmelanoma skin cancer and carcinoma, in site colon and rectum cancer has the third highest incidence and death rate of all cancers by 1992 estimates.⁵ The incidence of colorectal cancer tends to increase with age. More than 94% of all cases occur after the age of 50.⁶ It has been proposed that the high mortality rate of colorectal cancer can be explained by the low nerve cell density at the epithelial cells of the intestine. The disease, in most cases, is at an advanced stage when symptoms appear. Early detection of small cancers and polyps is of paramount importance in reducing the likelihood of major surgery and in helping to eliminate the disease's progression.

Cancer epidemiology may give insights into mechanisms of carcinogenesis, but it cannot determine low levels of risk.⁷ Epidemiological studies involving colon cancer suggest that individuals with diets high in fat and low in fiber have a higher incidence of colon cancer.^{8,9} Since cancer is a chronic disease with an undetectable starting point and a long latent period, individuals may be exposed to many other hazards before the cancer becomes manifest. Exposure to only one hazard is unlikely therefore distinguishing between the different exposures or hazards would be impractical.

It is now possible to screen for carcinogenic activity by making use of a host of available tests that have recently been developed. Of these tests the Ames test is probably the most popular.¹⁰ The Ames test measures back-mutation to histidine independence of histidine-requiring mutants of *Salmonella typhimurium*.¹¹ This allows mutagenicity to be quantitatively determined by counting the number of relevant colonies observed after incubation with the suspected mutagen. Since many carcinogens are also mutagenic, a correlation can be developed thus providing a method to determine the possible carcinogenicity of a particular substance.

It is a relatively simple task to utilize the Ames test on colonic tissue since the tissue is easily biopsied. This makes it possible to indirectly study the developing disease

microscopically. Many times the progression of colon cancer can be observed directly. This is made possible by several techniques. The first is a method called proctosigmoidoscopy in which the physician uses a hollow lighted tube inserted into the colon to follow the disease's progression. Since most colorectal cancers seem to be shifting higher in the colon, the physician may use a long flexible instrument as well as a rigid scope. If more extensive studies are required, a barium enema or colonoscopy can be used.¹² The effect of diet on the chemistry in the lumen of colon; the chemistry's effect on the cellular biology of the epithelial cells; and the role that the cellular changes play in the development of colon cancer can all be observed by these techniques. Since epidemiological studies require long periods of time, these are all valuable tools which can be used to rapidly test colorectal cancer theories and hypotheses.

1.2 Possible Causes of Colon Cancer.

Recently several hypotheses on the origin of colon cancer have been developed and tested against epidemiological and clinical studies.¹³ These hypotheses include the effects of calcium deficiency, a high fecal pH, and the presence of cytotoxic and genotoxic steroids.

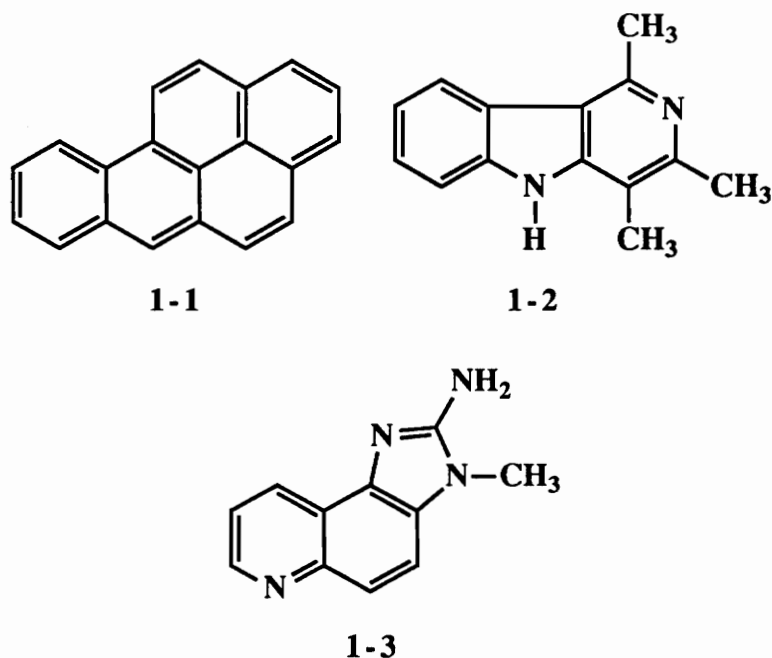
It has been shown that a deficiency of calcium leads to the presence of free bile and fatty acids in the lumen.¹⁴ These compounds are known to be toxic to the colonic epithelium. If the colonic epithelial cells are damaged or destroyed, the result is cell proliferation. Once proliferation occurs then the lumen becomes much more sensitive to carcinogenesis.

Fecal pH may also play a role in the origin of colon cancer.¹⁵ At a high fecal pH caused by a high fat-low fiber diet bile, acids in the colon become more soluble. This

enables cells to proliferate and creates the potential for tumor promotion. Since bile acids are toxic to the colonic epithelium, then there exists the potential for tumor promotion.

It has been proposed that genotoxic and cytotoxic steroids such as 3-ketosteroids act as initiators or promoters for tumor growth on the colonic epithelium.¹⁶ The concentration of these toxic compounds varies depending on the amount of cholesterol found in food or the lumen. These compounds may interfere in the cells' regulatory pathways, but their importance in the origin of human colon cancer is not fully understood.

Pyrolysis products from cooked meats contain known carcinogens and mutagens. Proteinaceous foods that are fried contain various genotoxic compounds such as benzo(a)pyrene **1-1**, 3-amino-1,4-dimethyl-5H-pyrido-(4,3-b)-indole (Trp-P-1, **1-2**), and 2-amino-3-methylimidazo[4,5-f]quinoline (I(Q), **1-3**).^{17,18} These compounds produce elevations in nuclear aberrations in the colon and are considered initiators for colonic epithelial cells. In fact one pyrolysis product **1-3** has been found to induce colon tumors in animal models.¹⁹



Naturally occurring bioactive compounds found in the colon such as the fecapentaenes are also hypothesized to play a role in the cause of colon cancer. These compounds are produced from precursor compounds by certain strains of anaerobes called *Bacteroides* and are the most abundant mutagens in the colon.²⁰ The following section contains a more thorough discussion of the biological activity of the fecapentaenes and how they relate to colon cancer.

1.3 Isolation and Structural Determination of Fecapentaene-12.

In 1977, Dr. Robert Bruce reported finding mutagenicity in ether extracts of human feces.²¹ It was shown that these extracts were active on *Salmonella* tester strains TA-98 and TA-100.^{22,23} Initially, the isolation and chemical characterization of these mutagens proved difficult due to their acid, light, and air sensitivity plus low fecal concentration. It was found, however, that incubation of feces under anaerobic conditions in the presence of bile dramatically increased their concentrations by 20 to 50 fold.²⁴ The problem of air oxidation was circumvented by the discovery that the pure mutagen could be stabilized by the addition of mild base and the antioxidant BHT (butylated hydroxy toluene).²² These two discoveries thus provided a means for sufficient quantities of mutagen to be isolated and purified for spectral analysis. Chromatographic (HPLC) techniques then revealed a mixture of closely related stereoisomers which were observed in most fecal samples (Figure 1-1).²⁵

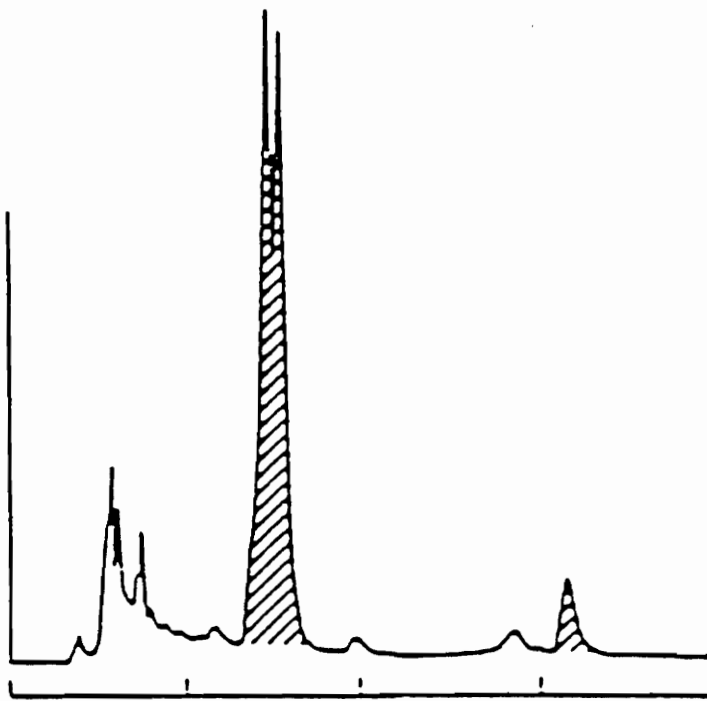


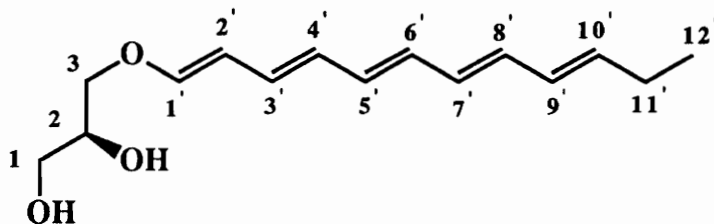
Figure 1-1. HPLC Chromatogram of Natural FP-12.

Spectroscopic techniques were the main tool used for the elucidation of the mutagen's structure. The UV spectrum showed three peaks at 320, 340, and 360 nm. This was consistent with that of a pentaene.^{26,27} Mass spectral analysis showed a molecular ion at m/z 250.²⁸

The actual structure of the mutagen was determined by its ^1H NMR spectrum and by degradative ozonolysis studies. The ^1H NMR spectrum indicated the presence of a vinylic ethyl group.^{29,30} This was confirmed by the formation of propanal upon ozonolysis. The stereochemistry of the 2° hydroxyl group of the glycerol was determined as *S* by HPLC comparison of (+)- α -methoxy- α -(trifluoromethyl)- α -phenylacetyl diester (MTPA diester) of hydrogenated mutagen with the MTPA diester of synthetic (*S*)-3-(dodecyloxy)-1,2-propanediol.

^1H NMR studies showed that the stereochemistry of the double bonds contains the all-*E* configuration in approximately 90% of the natural material isolated. Some *Z* configuration may exist at the 1', 3', and 5' carbons.^{31,32}

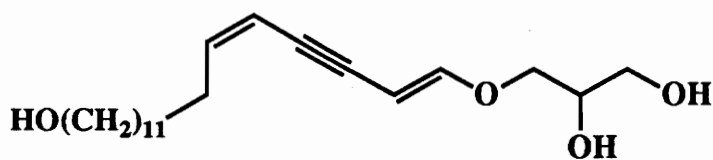
Based on these studies, the mutagen was assigned the structure **1-4**, (*S*)-3-(1,3,5,7,9-dodecapentaenyloxy)-1,2-propanediol; the trivial name fecapentaene-12 (FP-12) was coined.^{33,34}



1-4

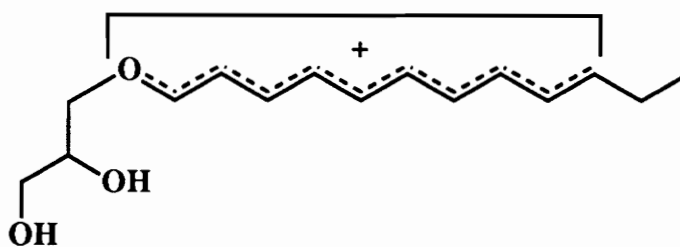
There is only one other molecule which bears any resemblance to the unique structure of FP-12. (+)- Raspailyne **1-5**, which was isolated from the marine sponge

Rasapailia pumila,³⁵ contains a glycerol backbone with a conjugated enol ether, but unlike FP-12 respalyne is not mutagenic and it does not contain a pentaenyl ether.



1-5

It is the pentaenyl ether system of FP-12 that is responsible for its high reactivity to air, light, and acid. In the presence of acid, FP-12 would become an unstable carbocation 1-6 which could undergo rearrangement or undergo attack by one of the hydroxyl groups. It is interesting that the lability does not increase with increasing numbers of double bonds. The enol ether also renders FP-12 susceptible to oxygen. It has been shown that a crystalline sample of FP-12 completely decomposed in 30 minutes on standing in air.³⁶ FP-12 is also unstable in light. Complete decomposition has been observed in as little as 30 seconds when exposed to UV light. This occurs presumably through free radical mechanisms and, although the products have not been characterized, it is believed that a polymerization occurs.³⁷



1-6

1.4 Fecapentaene-12 Syntheses.

Since FP-12 is isolated in very small quantities and it is highly unstable, biological studies needed to determine its role in colon cancer were virtually impossible to conduct. Because of this, suitable quantities of FP-12 were needed from a synthetic source. FP-12 proved to be a challenging synthetic target due to the highly labile pentaenyl ether. The air, light and acid instability made handling of the intermediates extremely difficult. However, many of these problems were overcome and several syntheses were developed which provided the quantities needed for the biological evaluation and final structure confirmation.

The first synthesis of racemic FP-12 was made by Gunatilaka *et al.* in 1983.³⁸ The key step used Wittig chemistry to create the 5'-6' double bond in the product as shown in Figure 1-2. The intermediate **1-7** was made in a stepwise fashion from 1-benzylglycerol. The aldehyde functionality in **1-7** was then treated with the ylide of (E,E)-2,4-heptadienyltriphenylphosphonium bromide **1-8**. This gave a mixture of the Z and E isomers (**1-9** and **1-10**). The final transformation to FP-12 was made by deprotecting the silyl ethers (*t*-butyldimethyl silyl ethers) using fluoride ion. This synthesis provided independent evidence of the structure of fecapentaene-12.

In 1984, Nicolaou provided a second synthesis.³⁹ This synthesis proceeded using an analogous methodology. In this pathway, the dienol **1-11**, in which the silyl protecting groups are *t*-butyl diphenylsilyl ethers, is reacted with diphenylhepta-1,4-dienyl phosphine oxide **1-12**. In this methodology, a "Wittig Adduct" **1-13** can be isolated as a mixture of diastereomers. The adduct can undergo elimination using potassium *t*-butoxide and deprotection with fluoride to give FP-12 as a mixture of 5-E and 5-Z isomers (Figure 1-3).

A third synthesis developed in 1984 by Van der Gen and co-workers also used Wittig chemistry to generate the 1'-2' double bond.⁴⁰ Starting from diphenylchlorophosphine, the Wittig reagent **1-14** was made in four steps. The LDA

generated anion of **1-14** is condensed with 2,4,6,8-undecatetraenal to produce **1-15**. Treatment with potassium *t*-butoxide gave protected FP-12 which can be deprotected to FP-12 using tetrabutylammonium fluoride (Figure 1-4).

A fourth synthesis developed by Pfaendler in 1988 avoided the use of the Wittig or Horner-Wittig reactions.⁴¹ The protected tosyl glycerol **1-17** was converted to the propenal **1-18**. This was reacted with the anion of alkyne **1-19** to give the key intermediate **1-20**. Reduction of **1-20** with lithium aluminium hydride then give all E protected FP-12. Deprotection then affords FP-12 in high yield (Figure 1-5). With these syntheses available, it was now possible to evaluate fecapentaene-12's biological properties.

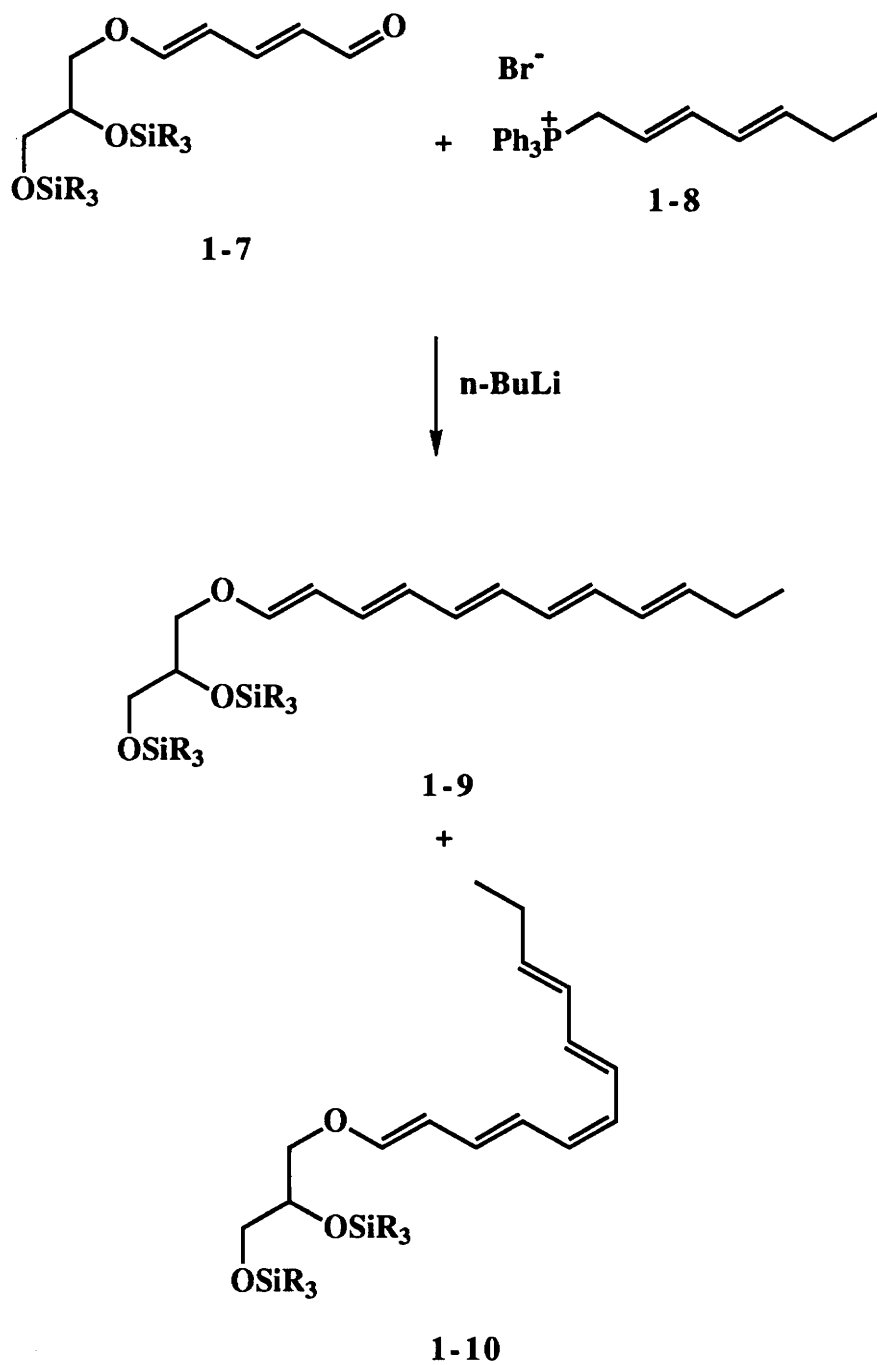


Figure 1-2. Gunatilaka and Kingston's Synthesis of FP-12.

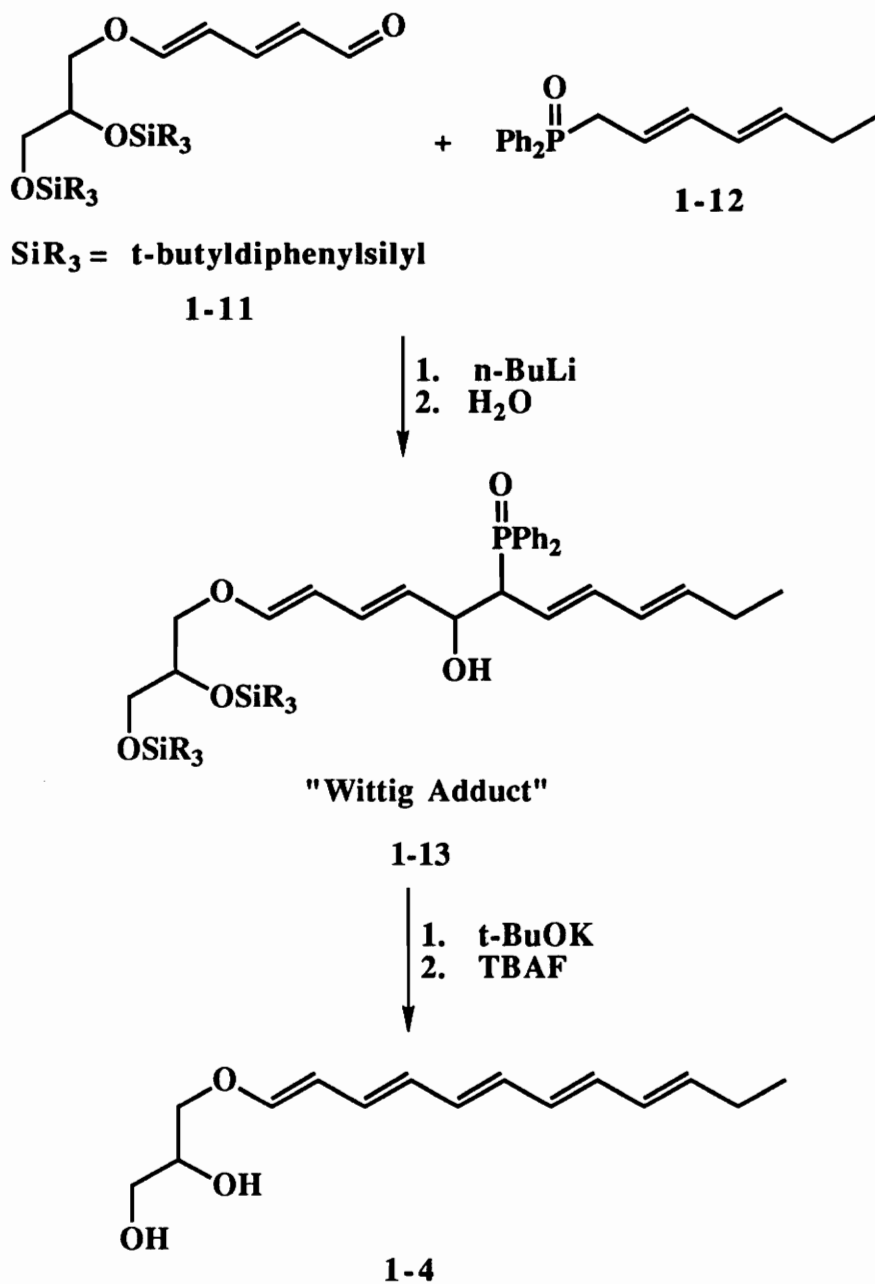


Figure 1-3. Nicolaou's Synthesis of FP-12.

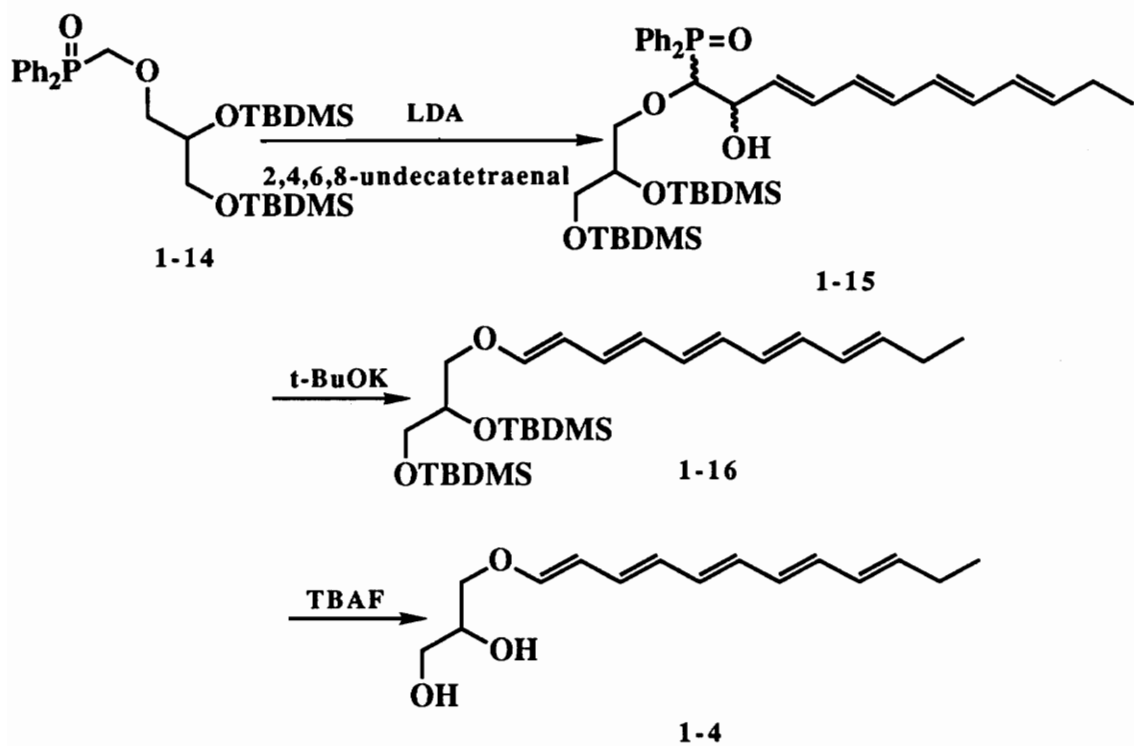


Figure 1-4. Van der Gen's Synthesis of FP-12.

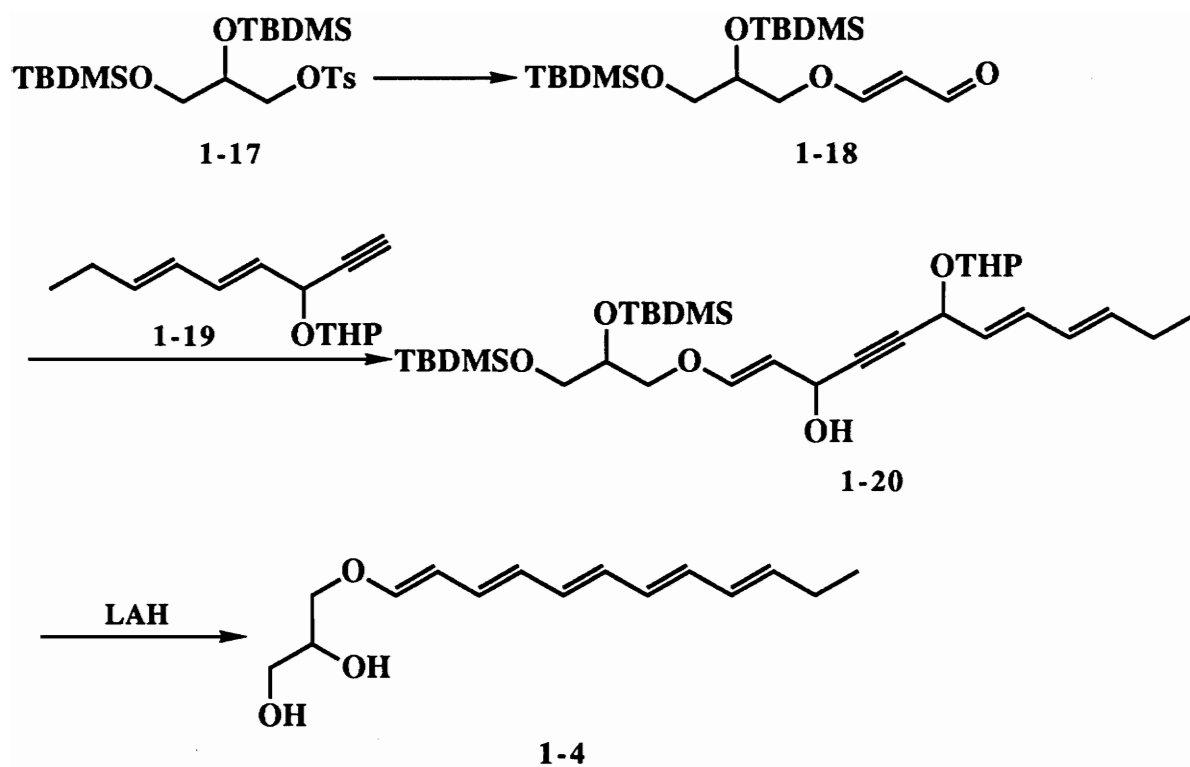
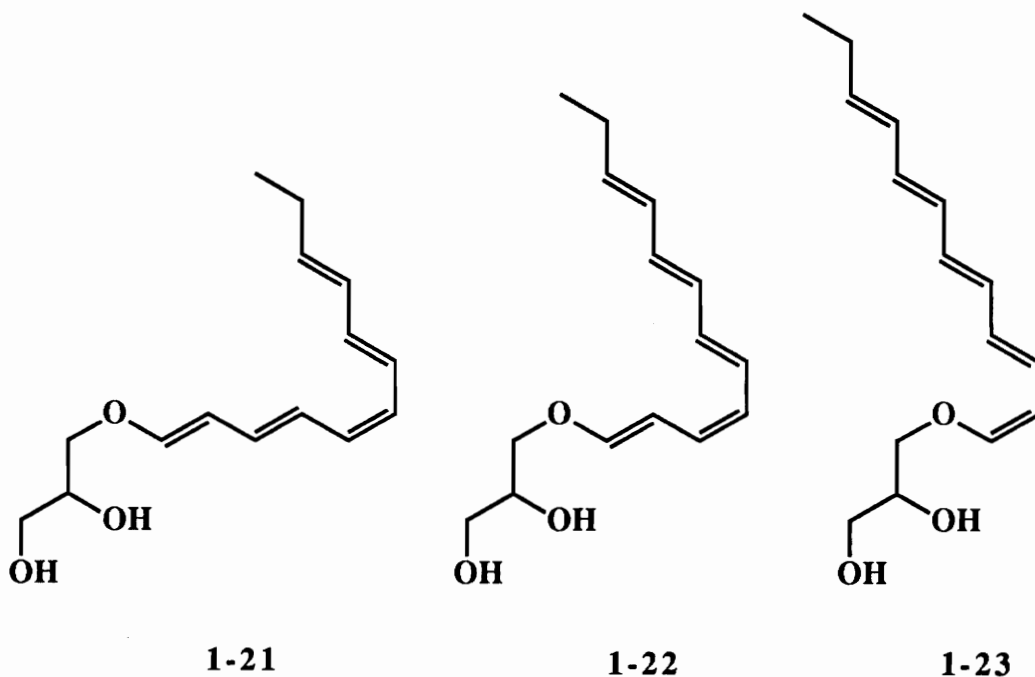


Figure 1-5. Pfaendler's Synthesis of FP-12.

1.5 Fecapentaene-12 Distribution.

HPLC studies on the fecal extract, which contained mutagens, showed the presence of a minor component (Figure 1-1). It was determined that this minor component contained a tetradecapentaene side chain instead of the dodecapentaene side chain as observed in FP-12. This new component was later determined to be fecapentaene-14 (FP-14).³² The other components appearing in the chromatogram were assumed to be geometric isomers of FP-12. If these were indeed geometric isomers there would, in theory, be thirty-two possible cis-trans isomers of FP-12. The question then arose concerning the distribution of these isomers in nature. It was found by comparative HPLC studies that the ratio of FP-12 to FP-14 varies from individual to individual and that varying amounts of geometrical isomers are also excreted. It was shown that all trans FP-12 is the major isomer with 5'-cis FP-12 **1-21** existing as a minor component. (In some cases 5'-cis FP-12 is the major component.) In some samples the distribution of cis-trans isomers is 10% 5'-cis **1-21**, 45% 3'-cis **1-22**, 35% all trans and 10% 1'-cis **1-23**.⁴²



These results indicate that there is not a set percentage of isomeric distribution. The number of isomers varies with the excreter. This undoubtedly plays a role in the difficulty of isolation of the fecapentaenes.

In addition to the molecular distribution of the fecapentaenes, there is also the question of the distribution of the fecapentaenes in human populations. Colon cancer has a higher incidence in developed nations such as North American, New Zealand, and Western Europe. The lowest rates are found in Africa, Asia, and Latin America i.e. underdeveloped countries.^{43,44,45} Comparative studies of religious groups have allowed researchers to look for differences in lifestyle. Seventh-Day Adventists consume less meat and follow a lacto-ovovegetarian diet. These people show a 60% reduction in the incidence of colon cancer.^{46,47,48} The incidence of colon cancer is also lower in the Mormon population who eat more whole-grain breads and cereals.^{49,50} These results show that certain food preferences are associated with either a high or low incidence of colon cancer. It is speculated that a high fat and low fiber diet increase the levels of bile acids which, in turn, facilitate the generation of endogenous genotoxins. Thus a worldwide correlation between the incidence of colon cancer and total fat consumption has been established.⁵¹

This correlation was further supported by Reddy and co-workers.⁵² They examined high risk and low risk populations for the production of fecal mutagens. The low risk population typically consumed more grains, cereals, and fruits. Their fecal extracts showed no mutagenicity on strains TA-98 or TA-100. Twenty-two percent of the fecal extracts from the high risk population, i.e. people who consumed more meat and fat, showed activity on TA-98 and 11% was active on TA-100. These studies further support the hypothesis of the high-fat low-fiber diet correlation with increased colorectal cancer incidence.

1.6 Biological Activity of Fecapentaene-12.

Fecapentaene-12 has been shown to be a potent mutagen by the Ames test using *Salmonella* strains TA-98 and TA-100 with over 2000 revertant colonies per microgram.^{53,54,55} FP-12 displays a variety of genotoxic effects in mammalian systems such as single strand DNA breakage;^{56,57} nuclear aberrations and mitotic figures;^{58,59} unscheduled DNA synthesis and cellular transformations;⁶⁰ and mutations and sister chromosome exchanges.^{56,61}

Although FP-12 has been shown to be mutagenic by the Ames test, this does not necessarily mean FP-12 is carcinogenic. There have been several studies aimed at determining whether or not FP-12 is a carcinogen. Initially, it was shown that FP-12 did not cause tumor formation in rats,⁶² but subsequent studies have shown that FP-12 does have weak carcinogenic activity in newborn mice.⁶³ Specifically liver, lung and stomach tumors and sarcomas were found in newborn mice when intraperitoneal injections of FP-12 were made. Shamsuddin and Ullah⁶⁴ have also shown that FP-12 promoted tumor formation in 30% of male F-344 rats. These studies indicate that FP-12 is definitely carcinogenic. It may be that in the first carcinogenicity study was negative because the FP-12 did not reach its cellular target in a biologically active form. This may be rationalized by making allowances for the instability of FP-12. It may also be the case that very young or very old mice (or humans for that matter) lack the protective mechanisms needed for protection against colonic mutagens. Thus conditions such as polyposis, G-I disorders, colitis and inflammatory bowel diseases may decrease natural cellular defenses and allow cell proliferation to occur.

It is known that FP-12 is a potent genotoxin. No metabolic activation by microsomal enzymes was required to observe the mutagenicity when assayed by the Ames test. This indicates that the mutagen is direct-acting. This observation has led to several

structure activity relationships. The results of these tests^{53,54,65,66,67} indicate that the mutagenicity of fecapentaene depends on the number of conjugated double bonds i.e. the greater the extent of conjugation the more mutagenic fecapentaene becomes.^{53,65} There was no observed activity in the sulfur analog of the enol ether linkage thus displaying the importance of the oxygen atom of the enol ether.⁶⁷ Additional studies have also indicated that the length of the carbon tail,⁵³ the stereochemistry of the double bonds,^{54,66} and the glycerol unit⁵³ are not important to mutagenicity.

Intestinal carcinogens originate from three sources. These include the microflora, the host, or the diet. Wilkins *et al.*⁶⁸ have shown that incubation of fresh feces dramatically increases the levels of fecapentaene. This was not the case if the feces were first sterilized or supplemented with antibiotics prior to incubation.⁶⁹ In addition, it was also shown that incubation of fecal extracts with various strains of the intestinal anaerobe *Bacteroides* resulted in production of FP-12.⁶⁸ Finally, it was observed that the addition of bile greatly increased the production of FP-12.⁶⁸ Since bile would solubilize any reactants, reactions leading to FP-12 would be possible.

Van Tassell *et al.*⁶⁸ conducted an elaborate set of experiments in 1982 to determine the proper conditions needed for the production of FP-12. Various strains of the intestinal anaerobe *Bacteroides* were screened for FP-12 production. *Bacteroides thetaiotaomicron* was found to give the best results. This species was incubated with various sources of feces and subjected to different experimental conditions (Table 1-3). Incubating fecal samples from excretors and nonexcretors with the *Bacteroides* anaerobe showed a large difference between these two sources. When autoclaved feces from a nonexcretor was inoculated with feces from a nonexcretor, the production of FP-12 greatly increased. This indicates the presence of fecapentaene producing bacteria in nonexcretors. Furthermore, and more importantly, this indicates the possibility of the presence of a precursor which

could be processed by the *Bacteroides* cell membranes into FP-12. After further investigation, a biological precursor was indeed found and isolated. For a general discussion of fecapentaene-12 and its precursor, see Kingston *et al.*⁷⁰

Table 1-3. *In Vitro* Incubation of Fresh and Autoclaved Feces and Fecal Extract Broth.

Substances Incubated	Source	Inoculum (5%)	HPLC Area	
			0 hr	72 hr
Fresh Feces	Excreter	None	3200	40000
	Nonexcreter	None	<100	<100
Autoclaved Feces	Excreter	Feces (E)	3300	31000
	Nonexcreter	Feces (E)	250	400
	Excreter	Feces (N)	3100	29000
	Nonexcreter	Feces (N)	<100	<100
Fecal Extract	Excreter	Feces (E)	3000	18000
	Nonexcreter	Feces (E)	<100	<100
	Excreter	Feces (N)	2800	2800
	Nonexcreter	Feces (N)	<100	<100

1.7 Statement of Goals.

Experimental evidence has shown that there are three basic requirements for the production of fecapentaene *in vivo* and *in vitro*. The first, provided by bile, is an environment in which all the reactants are soluble. The second is the presence of bacterial membranes which provide the necessary enzymes needed for the conversion of any precursors to FP-12. Finally, the third requirement is the presence of precursors which are transformed by *Bacteroides* into FP-12. Thus far most studies have attempted to elucidate how the fecapentaenes are formed. Through these studies certain correlations have been established as to the role FP-12 plays in the etiology of colon cancer. Research in this area is still being actively pursued. Additional studies involving DNA interactions, biosynthesis and epidemiology are needed to help solve this biological question.

A complex scenario seems to exist which describes the biological activity of mutagens such as the fecapentaenes in the colonic milieu. One possible scenario has been described by Wilkins and co-workers⁷¹ (Figure 1-6). The liver has been included as a means of activating indirect genotoxins. In the case of the fecapentaenes, the precursors may be products of mammalian metabolism. This theory is supported by the evidence that autopsy samples of humans and pigs (the only other animal known to produce fecapentaenes⁷²) showed uniform concentrations in the colon.⁷³ No precursors were found in sterilized pig feed, therefore the precursors probably do not arise from the diet.⁷³ Furthermore, precursors were found in neo-natal germ-free pigs whereas no fecapentaenes were detected since there were no bacterial enzymes present.⁷⁴ Since precursors do not originate from diet or intestinal flora, it seems likely that the precursors originate from the host organism.

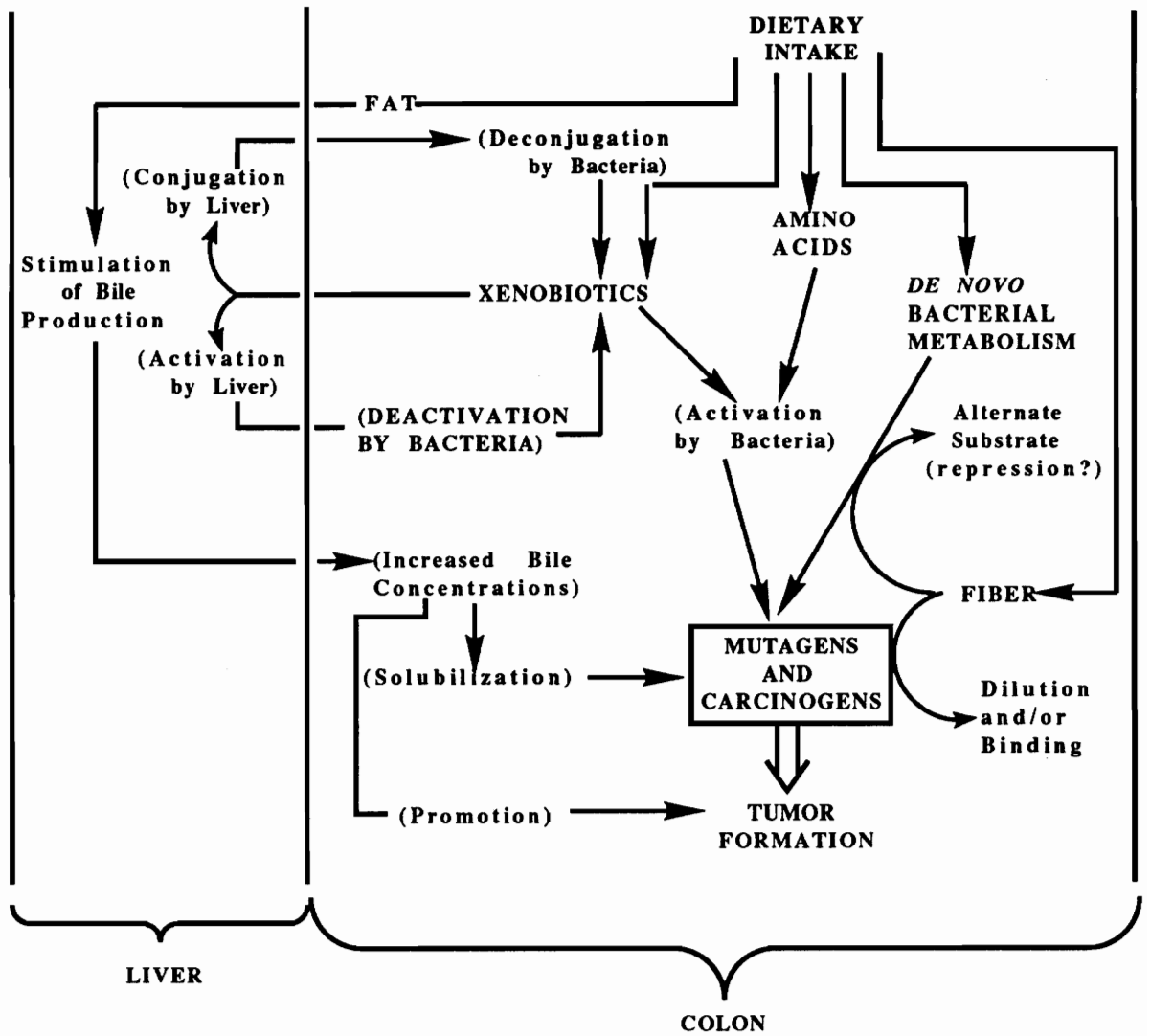


Figure 1-6. Schematic Representation of the Possible Roles of the Human Intestinal Microflora in Colon Cancer.

FP-12 has been shown to be highly mutagenic and its precursor is converted to FP-12 by intestinal anaerobes. It is also known that the precursor is found in large amounts only in FP-12 excreters. The relationship between FP-12 and the precursor is not known. The biological activity of the precursor is also not understood. It has been proposed that the precursors are a protective agent produced by mammalian cells to guard against colonic oxidation. If the precursor is indeed of a plasmalogen origin as hypothesized, then it would provide an ideal trap for toxic radicals. Because the precursor displays the same instabilities as FP-12 and plasmalogens are notoriously difficult to isolate, these questions and theories cannot be fully addressed until the structure of the precursor becomes known and it becomes available in larger quantities.

Thus far the evidence suggests that the fecapentaenes and their precursors play a role in the etiology of colon cancer. The exact biological activity of the precursor is not completely understood. Since the structure of the precursor is unknown and is isolated in very small quantities, it was elected to pursue two research avenues. First, the structure of the precursor needs to be elucidated using chemical techniques. Secondly, a synthetic route to the precursor is needed which will provide independent structural confirmation and also provide the quantities needed for biological evaluation.

II. Structure Elucidation of Plasmalopentaene-12

2.1 Introduction.

The observation that fecapentaene-12 production was promoted by the intestinal anaerobe *Bacteroides* and that production was enhanced by bile indicates that a precursor must be present in human feces.^{23,69} Purification and structural elucidation of the precursor has been hampered by the same obstacles confronted as those of the fecapentaenes i.e. acid and oxygen liability, molecular heterogeneity, and low concentration in feces. Although a plasmalogen-like structure was suspected, this could not be confirmed until these obstacles could be overcome.

Enough of the precursor has been isolated to conduct preliminary biological activity studies. The precursor was tested against two bacterial assays; the *Salmonella* assay on tester strains TA98 and TA100 without microsomal activation and the SOS chromatest which is a genotoxicity assay designed to detect an induction of DNA repair in *Escherichia coli*. It was shown by these assays that the precursor, unlike fecapentaene-12, was not mutagenic under any test concentrations up to 100 µg/plate.⁷⁵ At this time the exact role the precursor plays in colon cancer is unknown; however, some hypotheses have been presented. It is believed that the precursor is produced by the colonic epithelial cells. The precursor could then serve as a protective agent guarding against the constant oxidation and reduction processes that occur at the oxidized anaerobic interface. If the precursor contains the pentaenyl portion as found in fecapentaene-12 then this would provide an ideal trap for toxic radicals. This is supported by the fact that plasmalogens have been shown to protect cells from the harmful effects of radicals.^{76,77} If this hypothesis is true then the precursor may provide a first line of cellular defense against colon cancer. This idea or any others

cannot be supported or refuted until the structure of the precursor is confirmed and the material becomes available in larger quantities.

2.2 Precursor Isolation and Purification.

Van Tassell and co-workers⁷⁸ collected and lyophilized fecal samples from a 36-year old nonsmoking Caucasian female in good health. The freeze-dried feces were extracted with a series of three solvents, each stabilized with 100 µg/mL of butylated hydroxy toluene (BHT). First, the feces were extracted with hexanes. The hexanes extracts were then discarded. Second, the feces were extracted with methyl *t*-butyl ether and again the extracts were discarded. Finally, the feces were extracted using methanol.

Liquid-liquid extraction was performed on the methanol extracts using hexanes. The hexane extracts were pooled and concentrated and precipitated with acetone. The precipitate was collected by centrifugation and resuspended in a methanol/chloroform mixture. This was filtered and the sample was stored under argon at -80°C until purification.

Throughout the extraction process, the presence of the precursor was monitored by incubating the extracts with bacterial cell-free lysates of *Bacteroides thetaiotamicron* 5482 and assaying for the production of fecapentaenes.

The precursor extracts were purified by HPLC. The first separation was done on a silica column eluting with chloroform/methanol (44:6 v/v). The eluent was pooled and concentrated for the final purification. This was again performed by HPLC on an amine column eluting with chloroform/methanol/ammonium hydroxide (65:35:1, v/v/v). The purified precursor was stored in sealed containers under argon at -80°C.

The concentration of purified precursor was calculated by absorbance at 338 nm. An extinction co-efficient of 74,000 (that of fecapentaene-12) and an estimated molecular

weight of 700 were used as constants for the estimation. Making these assumptions, it was thus estimated that there were approximately 33 μg of precursor per gram of unincubated freeze-dried feces.

It was noted that when the precursor was stored anaerobically for extended periods the precursor would undergo a rearrangement. The lime-green fluorescence of the precursor would change to royal-blue with an accompanied change from the characteristic 360 nm UV triplet to a broad singlet in the 260 to 290 range. The chromatographic properties changed to that of a less polar compound. It was also found to be stable in the presence of air and light and also found to be nonmutagenic. The structure of this rearranged compound is currently unknown.

2.3 Preliminary Structure Determination Experiments.

The UV spectrum of the purified precursor is essentially the same as that of fecapentaene-12.^{29,30} Absorbances for the precursor were seen at 321, 335, and 353 nm compared to 320, 335, and 352 nm for fecapentaene-12. This suggests the presence of a pentaenyl ether moiety. Although the precursor showed virtually the same UV spectrum it was very different on TLC.⁷⁹ The TLC data show that the precursor is more polar than FP-12 in normal phase systems such as silica gel and less polar on reversal phase systems. This amphipolar character (i.e. displaying polar and non-polar character) indicates the presence of a highly polar substituent possibly a phosphate on at least one of the hydroxyl groups on the glycerol backbone.

The evidence thus far indicates that at least two of the hydroxyl groups of the glycerol backbone are functionalized. It was necessary to determine the extent of functionalization of the precursor versus that of fecapentaene-12. Both compounds were treated with Sylon BTZ, a potent silylating reagent. Sylon BTZ is the commercial name for

a mixture of *N,O-bis*(trimethylsilyl)acetamide, trimethylchlorosilane, and trimethylsilylimidazole available from Supelco, Inc. This reagent will silylate any hydroxyl group in any position. The products were analyzed by TLC analysis (see Table 2-1 for results) and no silylation occurred, indicating that the precursor had no free hydroxyl groups.

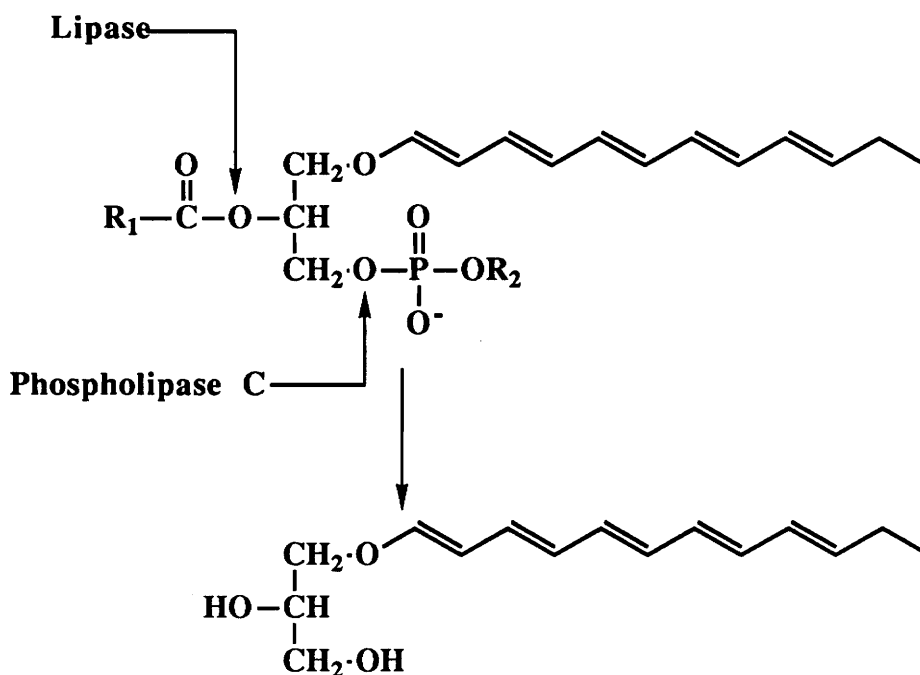
MS/MS data indicated the presence of long chain fatty acids. These fatty acids may arise from an acyl linkage on one of the previously discussed hydroxyl groups.

Table 2-1. Treatment of Fecapentaene-12 and Precursor with BTZ.

Sample	Rf (C ₁₈)	Rf (Silica)
Fecapentaene-12	0.86	0.60
Fecapentaene-12 + BTZ	0.86	0.88
Precursor	0.64	0.22
Precursor + BTZ	0.64	0.22

[C₁₈-methanol (100), Silica- Chloroform: methanol (92:8)]

Wilkins and co-workers conducted hydrolysis studies on the purified precursor.⁷⁸ Initial hydrolytic studies using lipases and phospholipases were unsuccessful. It was later found that incorporation of the precursor into liposomes or micelles allowed hydrolysis by a mixture of *Candida* lipase and *Bacillus cereus* phospholipases C to occur, which in turn yielded fecapentaene-12. Based on this information, it is possible to speculate on how the precursor is hydrolyzed to FP-12 (Scheme 2-1).

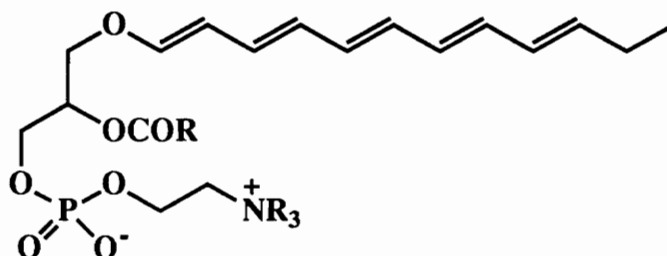


The phospholipase C first removes the phosphate group from the *sn*-3 position, then the lipase hydrolyses the group at the *sn*-2 position. The pentaenyl ether moiety remains at the *sn*-1 position because of the inability of the lipase to hydrolyze enol ethers. The hydrolysis of the precursor to FP-12 not only gives an indication as to the nature of the substituents other than the pentaenyl group, but it also gives some indirect indication of the regiochemistry of the precursor.

From the available experimental evidence, it can be concluded that:

1. The UV data indicate the presence of a pentaenyl ether moiety identical to that of fecapentaene-12.
2. The MS/MS data, hydrolysis data, and amphipolar nature indicate the presence of a long chain fatty acid and a phosphate group.

As a result of these data, the precursor can be classified as a plasmalogen with extended conjugation. It is known that plasmalogens are more abundant in anaerobic bacteria than in mammalian tissue.^{80,81} In anaerobic bacteria phosphatidylethanolamine and phosphatidylcholine are the most prevalent.⁸² In mammalian tissue, only the ethanolamine and choline derivatives are found.^{81,82} (For the percent plasmalogen composition in mammalian tissue, see Table 2-2.) Since it is believed that the precursor may originate from mammalian cells or from bacterial cells, it can be concluded that possibly the phosphate group will be that of phosphorylethanolamine or phosphorylcholine. A general structure of the precursor was then proposed as that shown below.



The exact nature of the acyl group and the phosphate group were not known. We thus began a chemical approach to the determination of the groups at the *sn*-2 and *sn*-3 positions i.e. the nature of the acyl and phosphate groups.⁸³

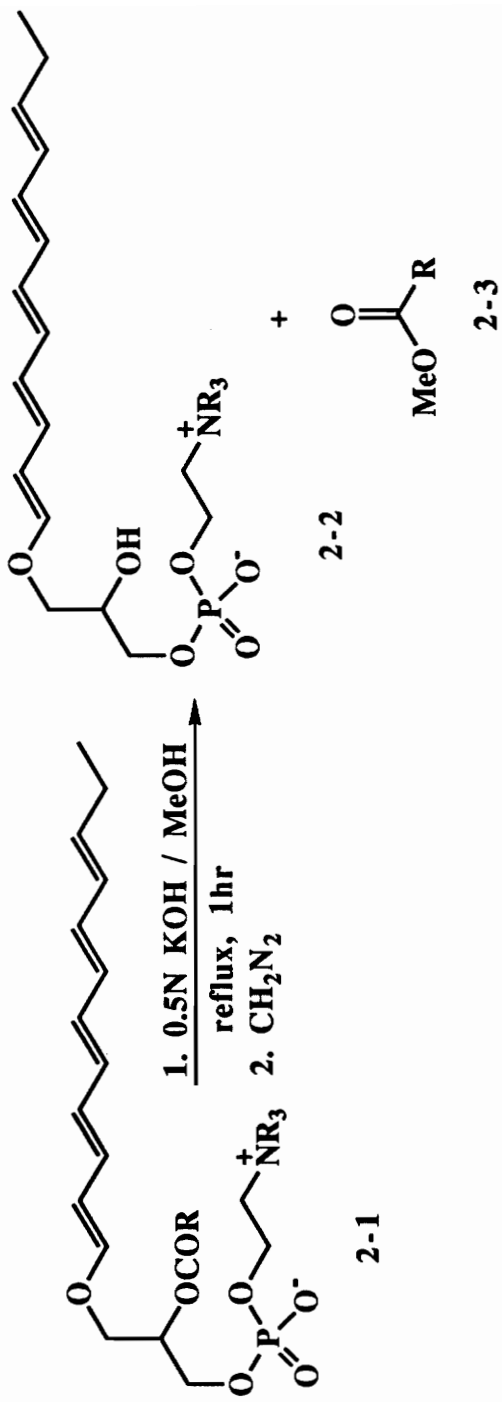
Table 2-2. Plasmalogen Composition in Mammalian Tissue.

Source	% of Total Phospholipid	
	Choline	Ethanolamine
Man, kidney	2.3	6.6
Rat, intestinal smooth muscle	2.5	8.2
Rat, intestinal mucosa	1.1	2.9
Sheep, liver	0.8	3.6
Fetal Lamb, liver	0.4	2.0
Bovine, liver	1.5	3.6
Sheep, kidney	3.2	7.4
Lamb, kidney	1.1	10.6
Sheep, spleen	3.0	9.8

2.4 Elucidation of the Functional Groups at the *sn*-2 and *sn*-3 Positions.

2.4.1 Determination of the Acyl Group at the *sn*-2 Position.

The functional group at the *sn*-2 position of the precursor was, as previously indicated, believed to be a long chain fatty acid. This was confirmed by hydrolysis/methylation analysis (Scheme 2-2).



Scheme 2-2

Deacylation of the precursor was accomplished by treating the precursor **2-1** with 0.5N alcoholic potassium hydroxide. The fatty acid product of the hydrolysis was then converted to its methyl ester derivative by treatment with diazomethane.⁸⁴ The resulting methyl ester mixture was subjected to GC-MS analysis (Figures 2-2 and 2-3). The analysis showed the presence of two major and two minor esters. The major esters were identified as methyl hexadecanoate (Figure 2-4) and methyl octadecanoate (Figure 2-5). Their identity was confirmed by capillary GC comparison with authentic samples. The minor esters were identified as methyl 9-octadecenoate and 9,12-octadecadienoate. Once again the identity of these esters were confirmed by cochromatography with authentic samples.

The identity of the minor esters was further confirmed by hydrogenation followed by methylation analysis. If the minor esters do indeed contain double bonds as suspected, then the GC-MS analysis of the hydrogenated product would show the presence of just two esters, as the minor esters would become methyl octadecanoate. Hydrogenation of the precursor (Scheme 2-3) with Adams catalyst (PtO₂) for 24 hours gave the fully hydrogenated product. This hydrogenated precursor **2-4** was then subjected to the same hydrolytic conditions as previously described, namely 0.5N alcoholic KOH followed by treatment with diazomethane, to give a methyl ester mixture **2-3** which was subjected to GC-MS analysis. The presence of the minor esters was not observed, and only the major esters were evident (Scheme 2-3), thus confirming the identity of the minor esters as unsaturated derivatives of methyl octadecanoate.

The identity of the acyl group at the *sn*-2 position was now known. The data and experimental evidence clearly indicate the presence of methyl esters of four major long chain fatty acids. The precursor does appear to be a mixture of the four fatty acid derivatives with octadecanoic acid (stearic acid) being the most predominant.

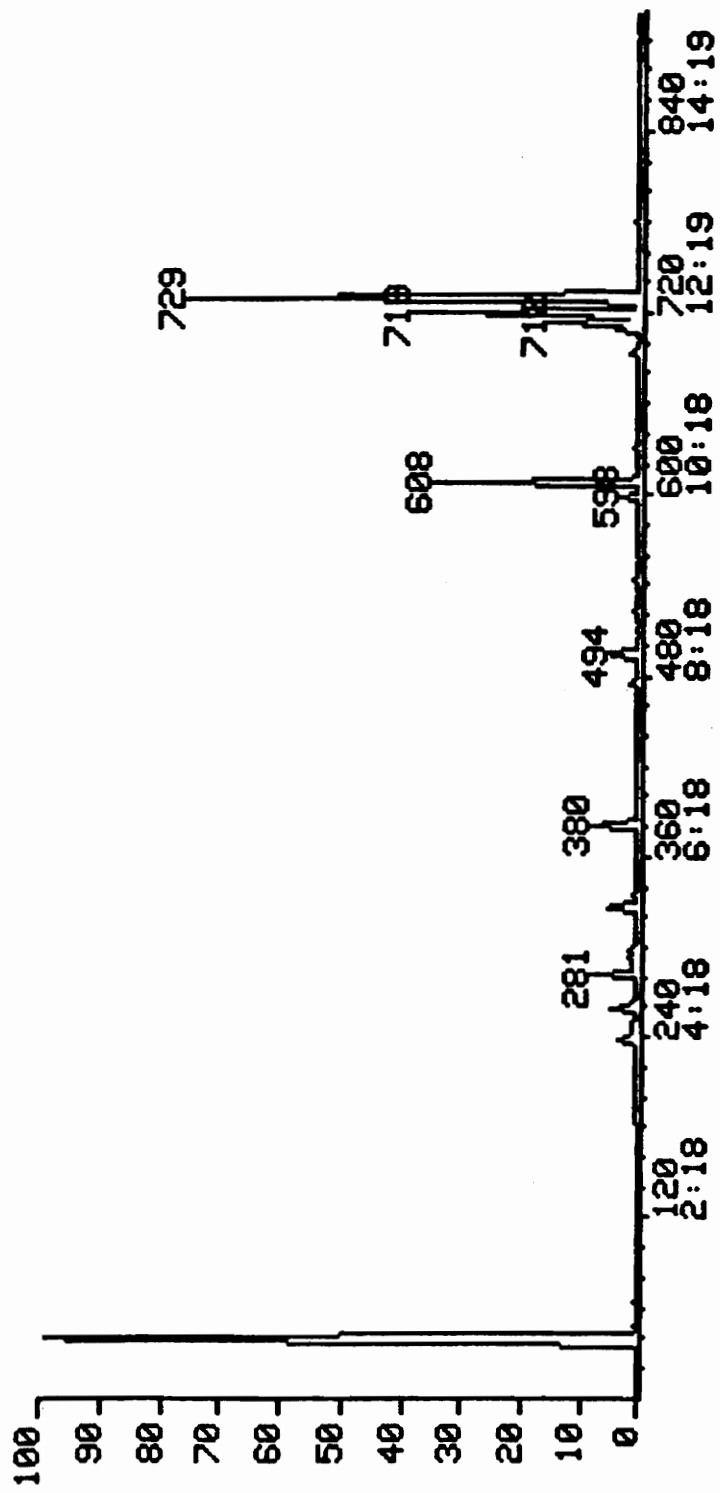


Figure 2-1. GC of the FP-12 Precursor Fatty Acid Methyl Ester

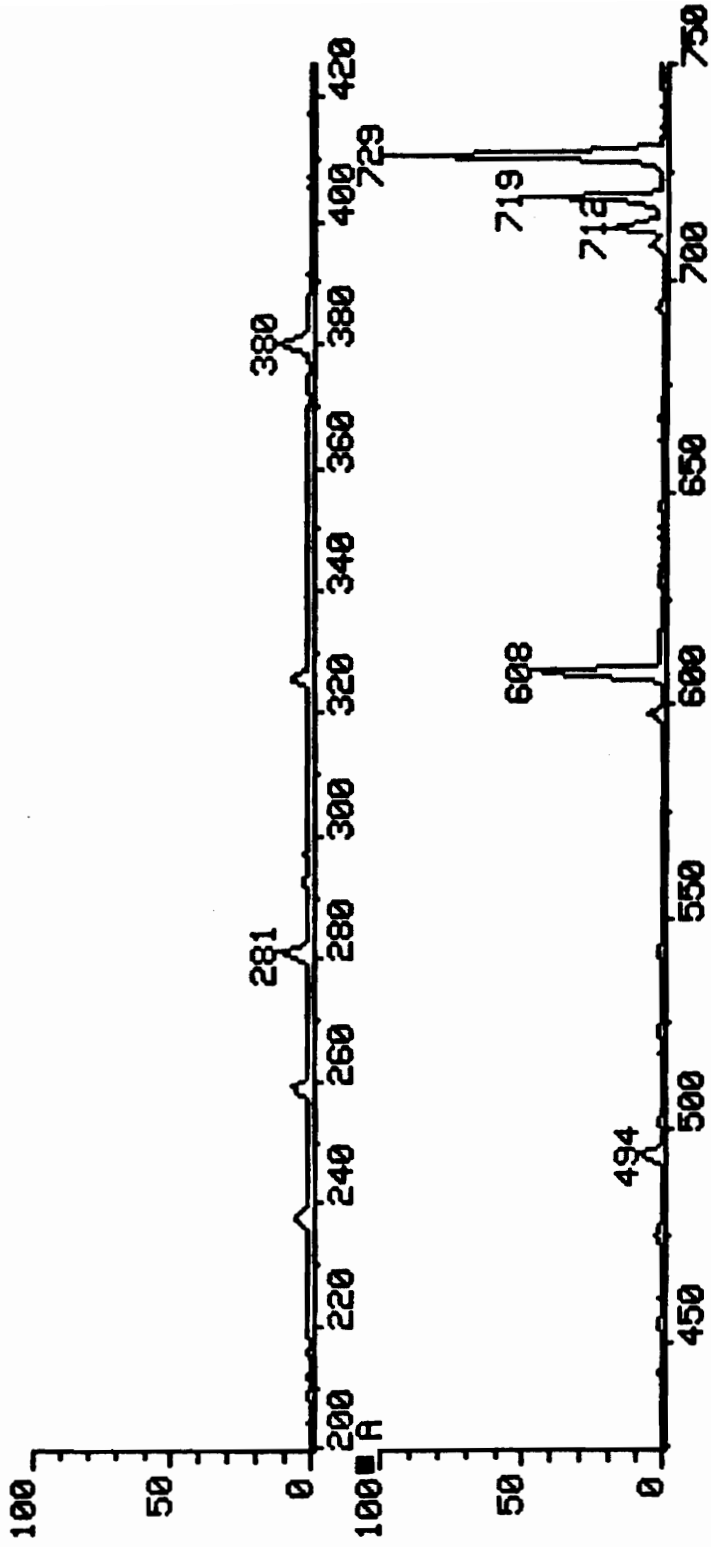


Figure 2-2. Expansion GC of the FP-12 Precursor Fatty Acid Methyl Ester

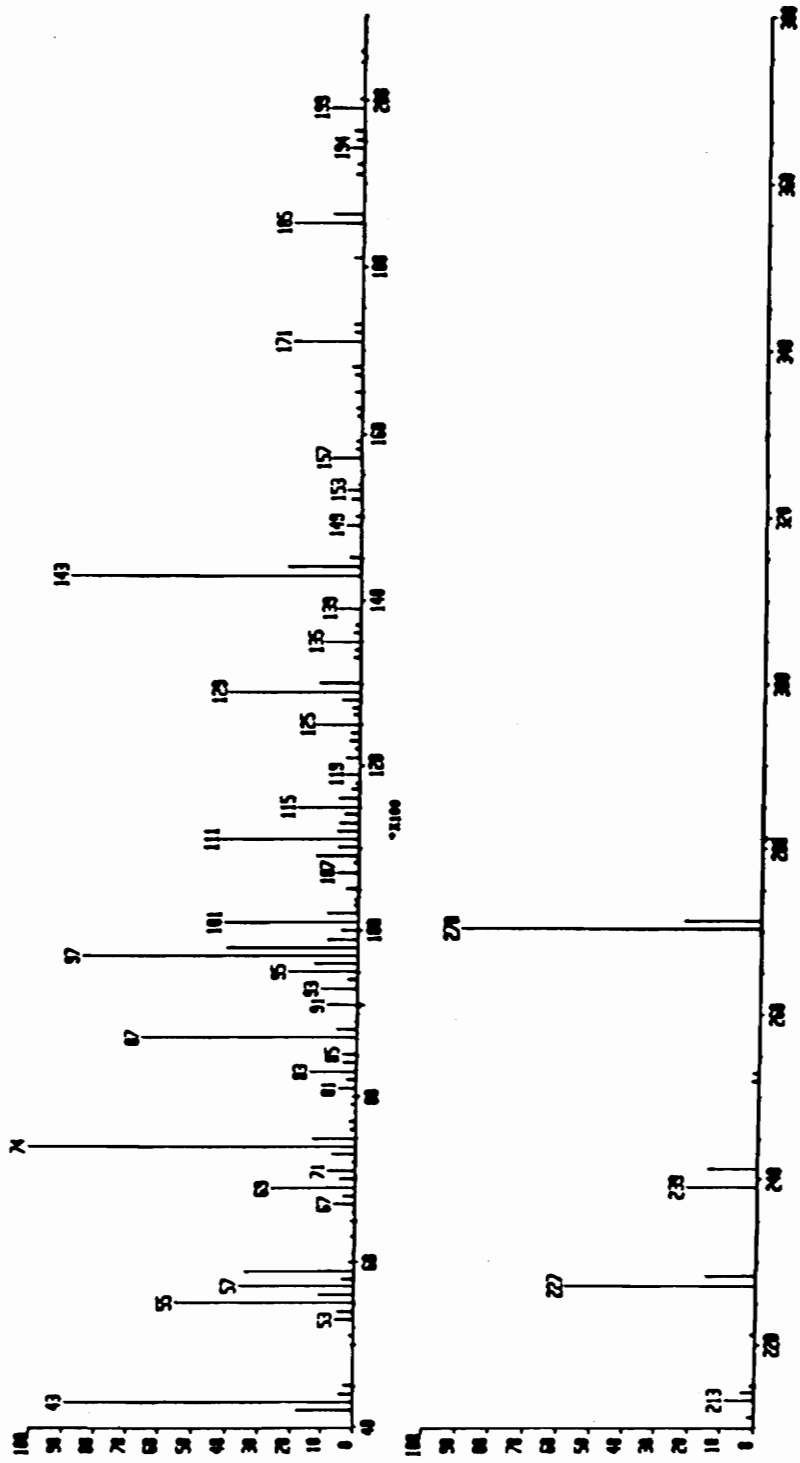


Figure 2-3. MS of Methyl Hexadecanoate.

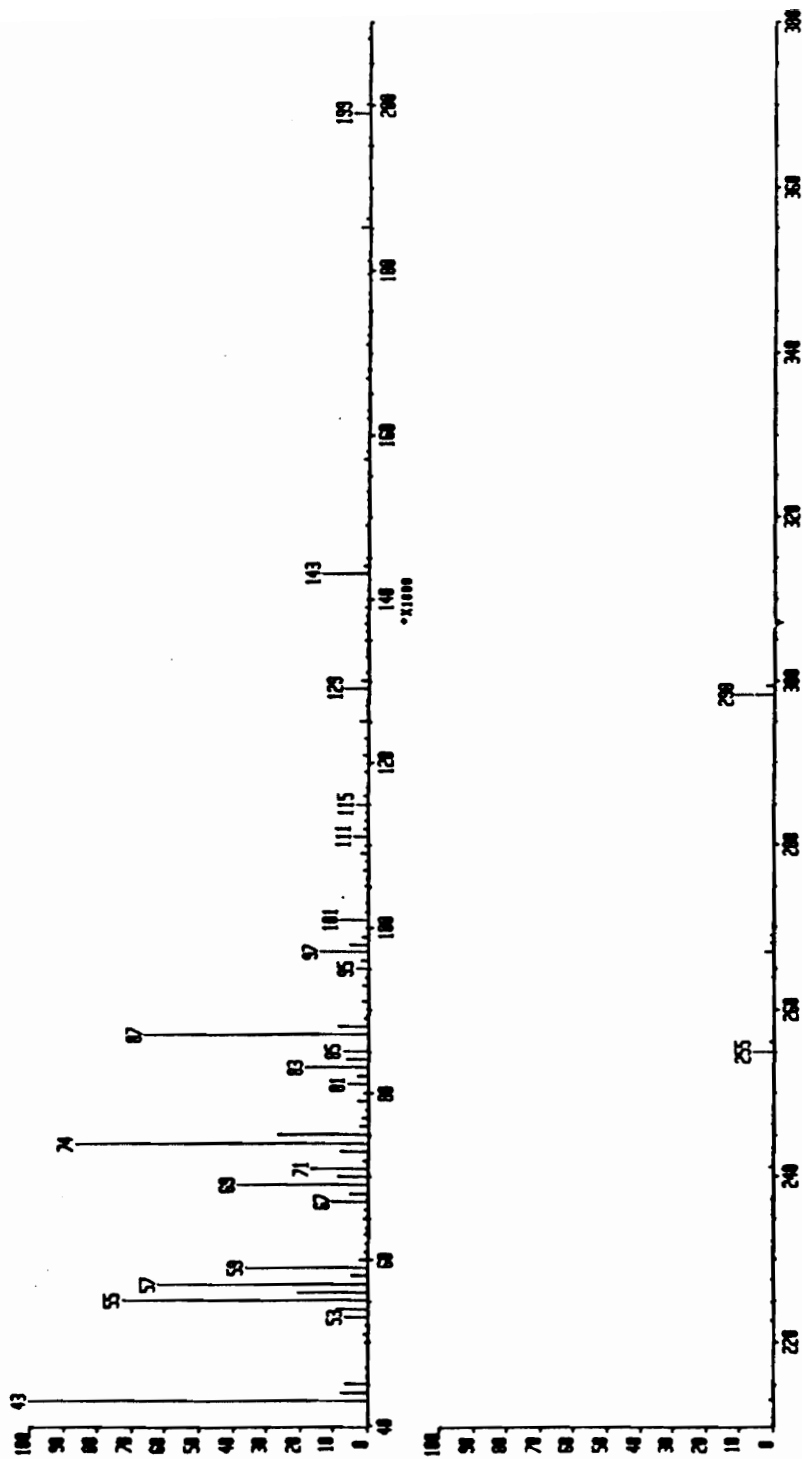
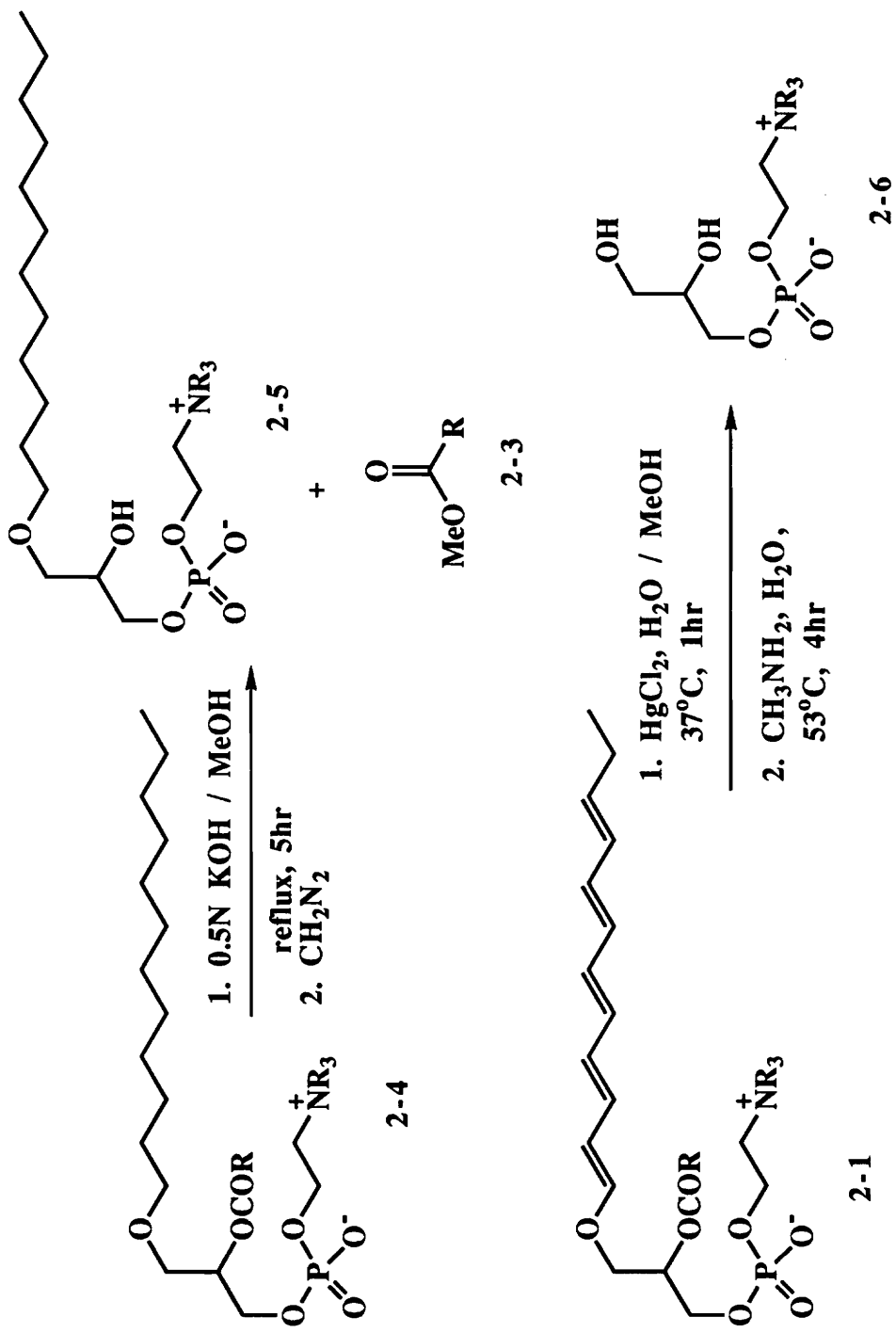


Figure 2-4. MS of Methyl Octadecanoate.

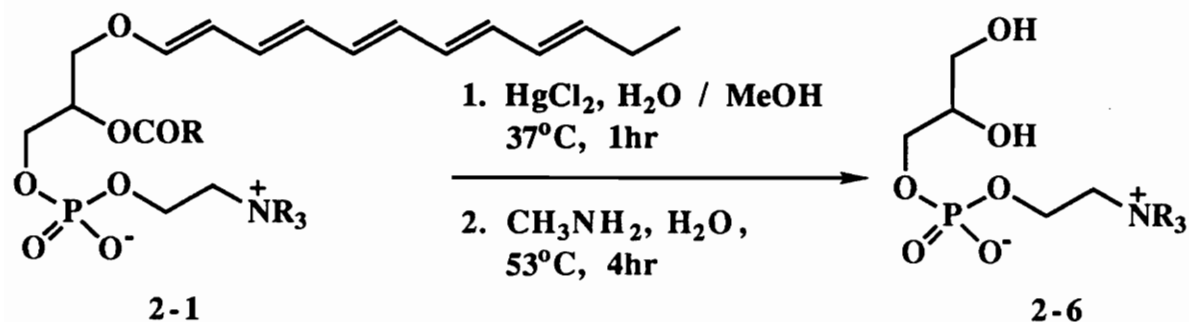


Scheme 2-3

2.4.2 Determination of the Functional Group at the *sn*-3 Position.

Previously it was mentioned that due to the precursor's amphipolar character the functional group at the *sn*-3 position was believed to be a phosphate. It is known that the three most abundant phosphates in nature are the ethanolamine, choline and serine derivatives. The nature of the phosphate group was determined by hydrolytic studies.

The identity of the phosphate group at the *sn*-3 position was determined by a modification of the procedure of Clarke and Dawson.⁸⁵ The pentaenyl ether functionality at the *sn*-1 position of the precursor 2-1 was hydrolyzed to the monoacyl phospholipid using HgCl₂ (Scheme 2-4)



Scheme 2-2

Next the *sn*-2 acyl group was hydrolyzed using methylamine. After extraction with butanol/ light petroleum/ ethyl formate the aqueous residue containing the glycerophosphoryl derivative 2-6 was subjected to descending paper chromatography using phenol saturated with H₂O, acetic acid ethanol (50:5:6 v/v/v) as the eluting solvent.⁸⁶ The glycerophosphoryl derivative 2-6 cochromatographed with authentic glycerophosphoryl ethanolamine which was clearly separated from other possible products, namely glycerophosphoryl choline, and glycerophosphoryl serine (Figure 2-5).

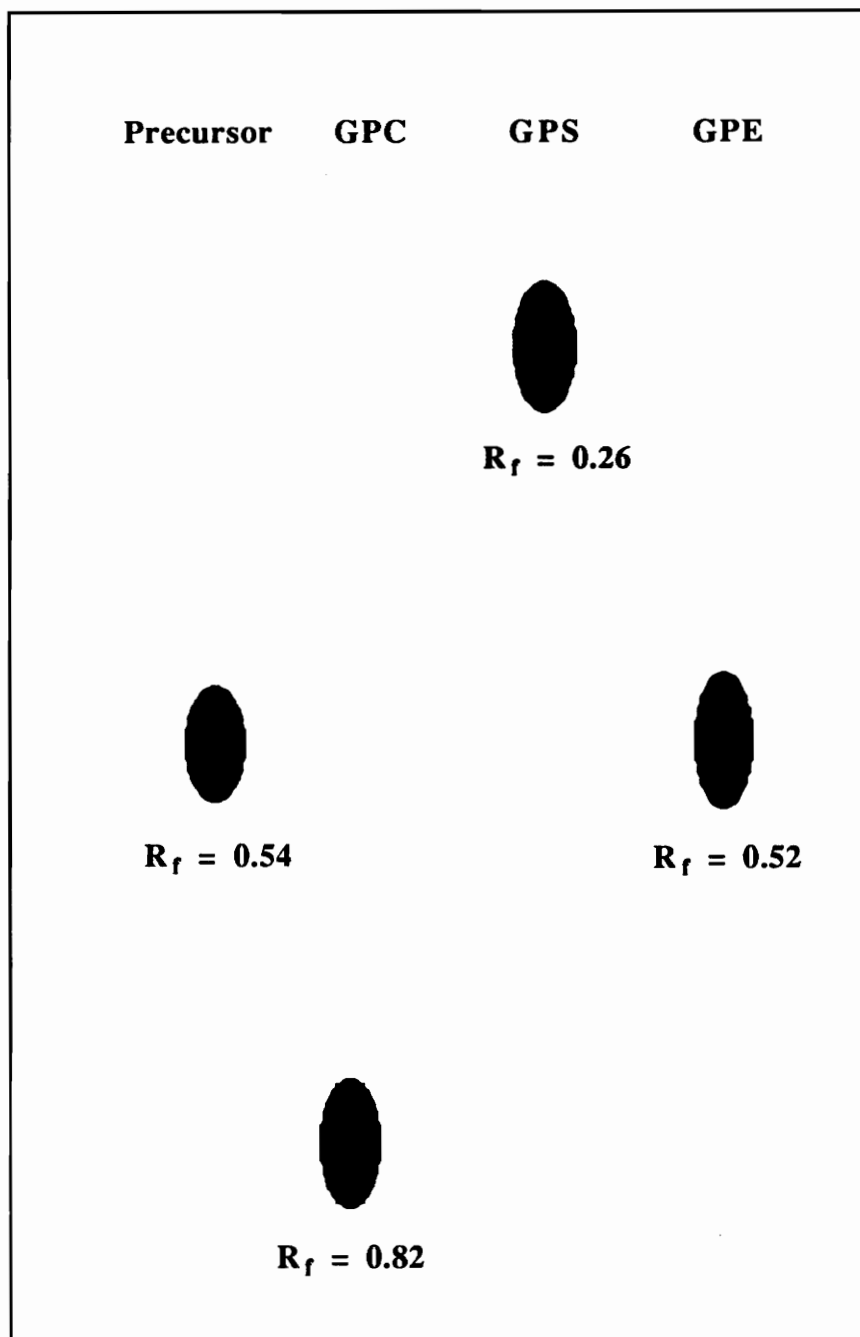


Figure 2-5. Descending Paper Chromatogram of FP-12 Precursor for Phosphate Determination.

Other separation techniques such as paper electrophoresis were also tried, but were unsuccessful.

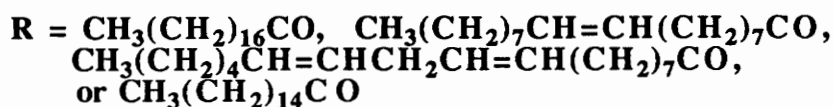
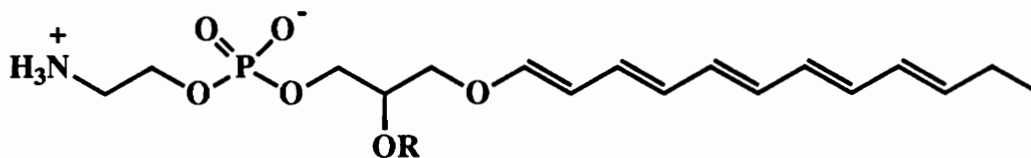
Unlike the *sn*-2 position where a mixture of esters is possible, the evidence clearly shows that the *sn*-3 position of the precursor is exclusively a phosphoryl ethanolamine containing no other phosphates analogs.

2.5 Conclusions.

Since the precursor preparation was based on HPLC isolation with UV detection, it is possible that small amounts of saturated phospholipids may have possibly cochromatographed with the precursor. If the amounts of phospholipids were substantial, then the structural elucidation studies of the precursor may be in question. To circumvent this possible problem, a control experiment was performed. The material eluting from the final HPLC separation immediately before and after the UV-absorbing precursor was collected and subjected to the same analyses as previously described for the precursor. The fraction eluting prior to the precursor showed no indications of any phospholipids, but the fraction eluting after the precursor showed small amounts of plasmalogens (about 10%). Since the precursor is very labile, it has proved impossible to thus far obtain a sample that does not contain any impurities. However, since the amounts of contaminants are small, the results thus far obtained are not invalidated.

Based on the evidence obtained from the various degradation techniques, it was now possible to assign a structure to the precursor of fecapentaene-12. This compound, which was given the name plasmalopentaene-12 (PP-12) (1-*O*-1,3,5,7,9-dodecepenteanyl-2-*O*-stearoyl-3-*sn*-glycero-3-phosphorylethanolamine), contains a mixture of esters at the *sn*-2 position: the major component is the stearate ester. Therefore the stereochemistry at this position is (*R*) based on the conversion of PP-12 into FP-12.^{30,31} The positions of

the acyl group and the phosphoethanolamine group is confirmed by the identification of 3-glycerophosphoryl ethanolamine and by conversion of PP-12 to FP-12 by lipase and phospholipase C⁷⁸ as previously discussed.



2.6 General Experimental Procedures.

GC-MS analysis was performed on a Hewlett-Packard 5740 gas chromatograph equipped with 25 m x 0.32 mm HP5 column using temperature programming at 150-295°C at 10°C/min which was interfaced with a VG Analytical 7070E-HF mass spectrometer operating at 70eV with a 200°C source temperature and a scan m/z 50-450 at 2 sec/decade. GC analysis was done on a Hewlett-Packard 5890A gas chromatograph equipped with a 30 m x 0.25 mm RSL-200 Alltech column and a FID detector using temperature programming 150-300°C at 15°C/min. 0.9 ml/min He flow and a split ration of 50:1. Descending paper chromatography was done on Whatman #1 paper using phenol saturated with H₂O, acetic acid, and ethyl alcohol 50:5:6 (v/v/v). All chemicals were purchased form Aldrich Chemical Company, Milwaukee, Wisconsin. Lipid standards were purchased form Sigma Chemical Company, St. Louis, Missouri. All solvents were distilled before use or were HPLC grade. All reactions using anhydrous conditions were performed under a blanket of argon.

2.7 Experimental.

Preparation of *sn*-2 deacylated plasmalopentaene-12 (2-2)

Plasmalopentaene-12 [50-100 μg ($\approx 1.07 \times 10^{-7}$ mol)], obtained from the Virginia Polytechnic Institute and State University Anaerobic Microbiology Laboratory, was dissolved in 3 ml of anhydrous methyl alcohol. To this solution 5 ml of 0.5N KOH/CH₃OH:H₂O (1:1 v/v) was added. The mixture was refluxed for 1 hour. The reaction mixture was neutralized or made slightly acidic with 6N HCl and extracted with diethyl ether. The extracts were washed with H₂O) followed by brine and dried over Na₂SO₄. The solvents were removed *in vacuo* and the residue was taken up in ethyl acetate. The mixture was methylated by adding CH₂N₂ in diethyl ether until the yellow color persisted.⁸⁵ The solvents and excess CH₂N₂ were removed *in vacuo*. The resulting crude methyl esters were submitted for GC-MS analysis.

Preparation of perhydroplasmalopentaene-12 (2-4)

Plasmalopentaene-12 (50-100 μg , $\approx 1.07 \times 10^{-7}$ mol) was dissolved in 10 ml of anhydrous methyl alcohol. To this mixture PtO₂ (0.04 g, 0.176 mmol) was added. The reaction flask was attached to a hydrogenator with 50 ml hydrogen gas capacity and the system was purged of air and charged with hydrogen gas. The reaction was allowed to proceed for 24 hours at room temperature. The catalyst was filtered off and the solvent was removed *in vacuo* to give perhydroplasmalopentaene-12 2-4 which was used directly in the next step.

Hydrolysis of perhydroplasmalopentaene-12 (2-5)

Crude perhydroplasmalopentaene-12 2-4 was dissolved in 15 ml of anhydrous methyl alcohol and 3 ml of 0.5N KOH/CH₃OH: H₂O 1:1 (v/v) was added. The mixture

was refluxed under argon for 5 hours. The reaction mixture was made acidic with 3N HCl and extracted with diethyl ether. The extracts were washed with water then brine and dried over Na₂SO₄. The solvent was removed *in vacuo* then redissolved in diethyl ether. CH₂N₂ in diethyl ether was added dropwise until the yellow persisted.⁸⁴ The solvents were then removed under a stream of argon. The residue was redissolved in acetone and submitted for GC-MS analysis.

Preparation of monoacylplasmalopentaene-12 (2-6)

Plasmalopentaene-12 ($\approx 300 \mu\text{g}$, 4.0×10^{-7} mol) was dissolved in 1 ml of anhydrous chloroform. A solution of HgCl₂ in H₂O and CH₃OH 1:9 (v/v) (0.04 ml) was added, and the mixture was stirred at 37°C for 1.5 hours. Water (0.5 ml) was added and extracted with an *n*-butanol / light petroleum / ethyl formate 20:4:1 (v/v/v) mixture. The solvents were removed under a stream of argon and then by rotary evaporation, with *n*-propanol added to prevent bumping, to give monoacylplasmalopentaene-12. This was immediately dissolved in 1 ml of *n*-butanol / light petroleum / ethyl formate 20:4:1 (v/v/v) under argon. To this 1 ml of 33% CH₃NH₂ in ethyl alcohol was added and the mixture was stirred at 53°C for 4 hours, following which 0.5 ml of water was added. The solution was extracted with *n*-butanol / light petroleum / ethyl formate 20:4:1 (v/v/v) and washed with water. The water washings were combined, evaporated to dryness, and redissolved in water (40 μl). The solution was spotted along with 100 μg quantities of glycerophosphorylethanolamine, glycerophosphorylcholine, and glycerophosphorylserine on Whatman #1 paper and subjected to descending paper chromatography using phenol saturated with H₂O, acetic acid, ethyl alcohol 50:5:6 (v/v/v) as the eluting solvent. The chromatogram was sprayed with ammonium (molybdate)-perchloric acid (Hanes reagent) and developed with long wave UV light.

III. Synthesis of Precursor Models

3.1 Plasmalopentaene-12 Synthetic Strategy.

Previously, it was stated that plasmalopentaene-12 suffers from the same lability problems as that of fecapentaene-12; specifically its instability towards air, acid, and light. Plasmalopentaene-12 is known to undergo a chemical alteration in which the fluorescence of the purified precursor changed from lime green to royal blue.⁸⁷ This is accompanied by a change in the UV spectrum: the characteristic triplet in the 320-360 nm region changed to a single broad peak in the 260-290 nm region. The structure of the altered precursor is unknown. It is known, however, that it moves with the solvent front on TLC and is non-mutagenic. The pentaenyl ether of fecapentaene-12 and presumably plasmalopentaene-12 is largely responsible for the instability of these molecules. With the exception of the fluoride ion, both electrophiles and nucleophiles react with the enol ether. The fluoride ion plays an important role in the synthesis of fecapentaene-12 and may conceivably be used in a synthesis of plasmalopentaene-12.

The instability of the pentaenyl ether moiety of plasmalopentaene-12 makes its synthesis difficult and challenging. Decreasing the number of conjugated double bonds simplifies the task somewhat. In fact, there are at least two well established methods for the synthesis of ethanolamine plasmalogens. The first route (Figure 3-1) is by Serebrennikova *et al.*^{88,89} Esterification of the secondary alcohol of 1-O-(1'-alkenyl)glycerol with an acyl halide and functionalization of the primary alcohol by iodide displacement with silver dibenzyl phosphate gave a dibenzylphosphate. Conversion to the ethanolamine plasmalogen was then accomplished by a three step sequence. The second route (Figure 3-2) by Vasilenko⁹⁰ involves esterification of 1-O-(1'-alkenyl)glycerol with 2-phthalimidoethyl dichlorophosphate as described by Hirt and Berchtold.⁹¹ The phthalyl

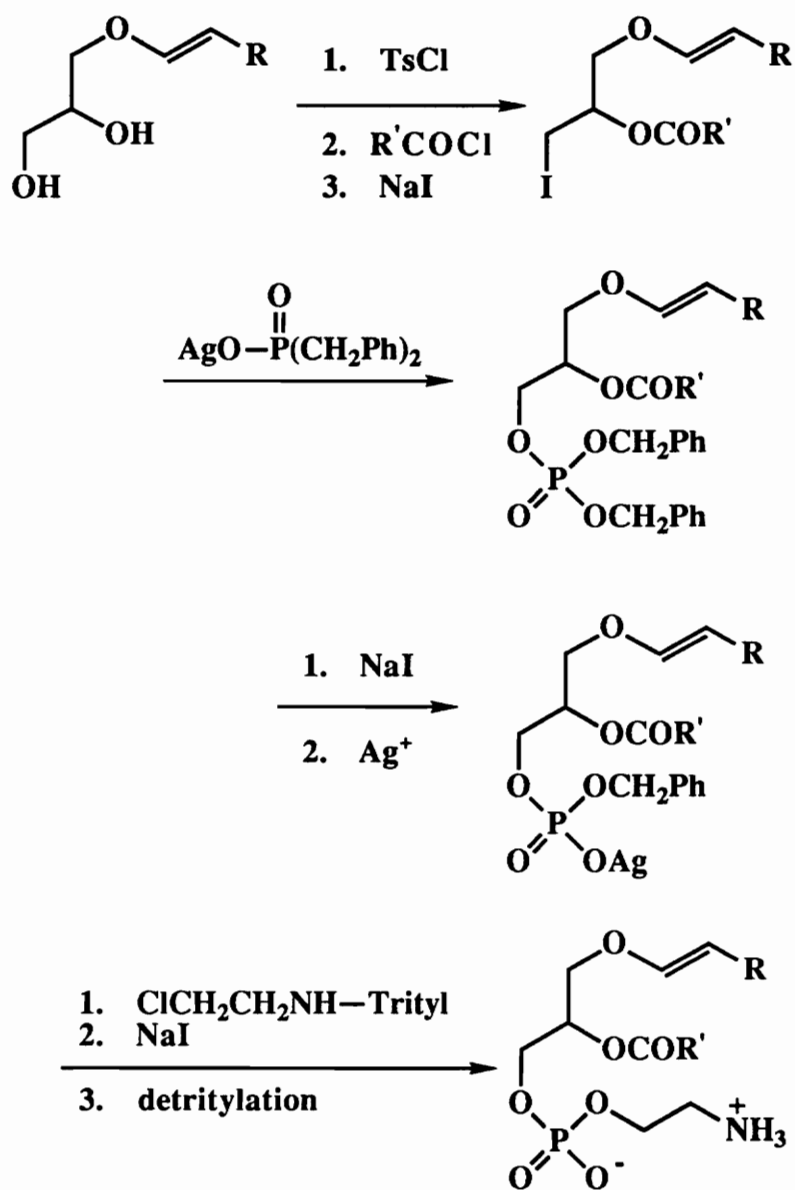


Figure 3-1. Synthesis of Ethanolamine Plasmalogens. Method A.

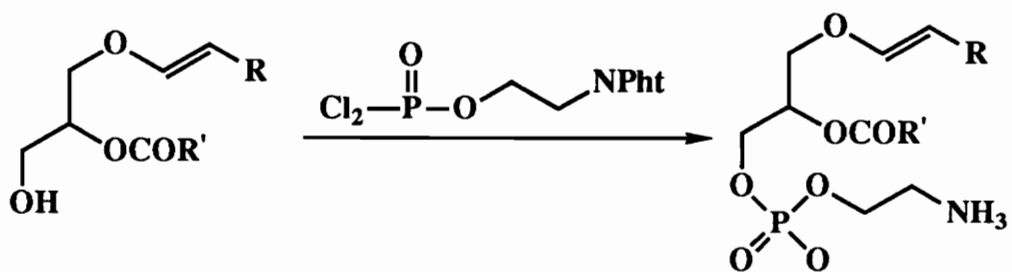
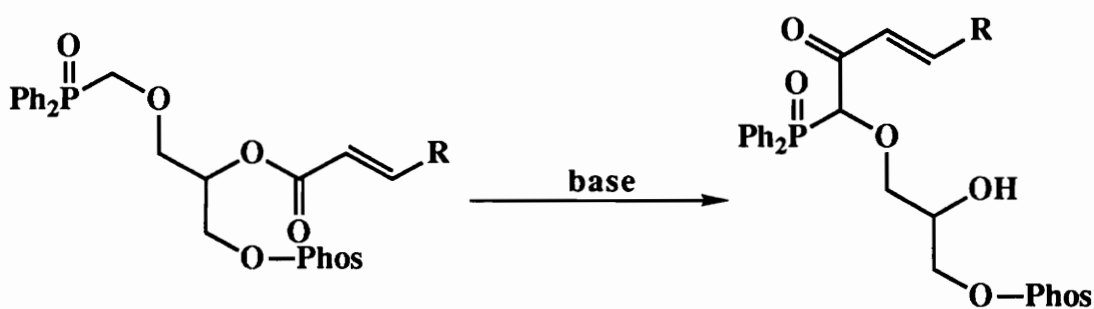


Figure 3-2. Synthesis of Ethanolamine Plasmalogens. Method B.

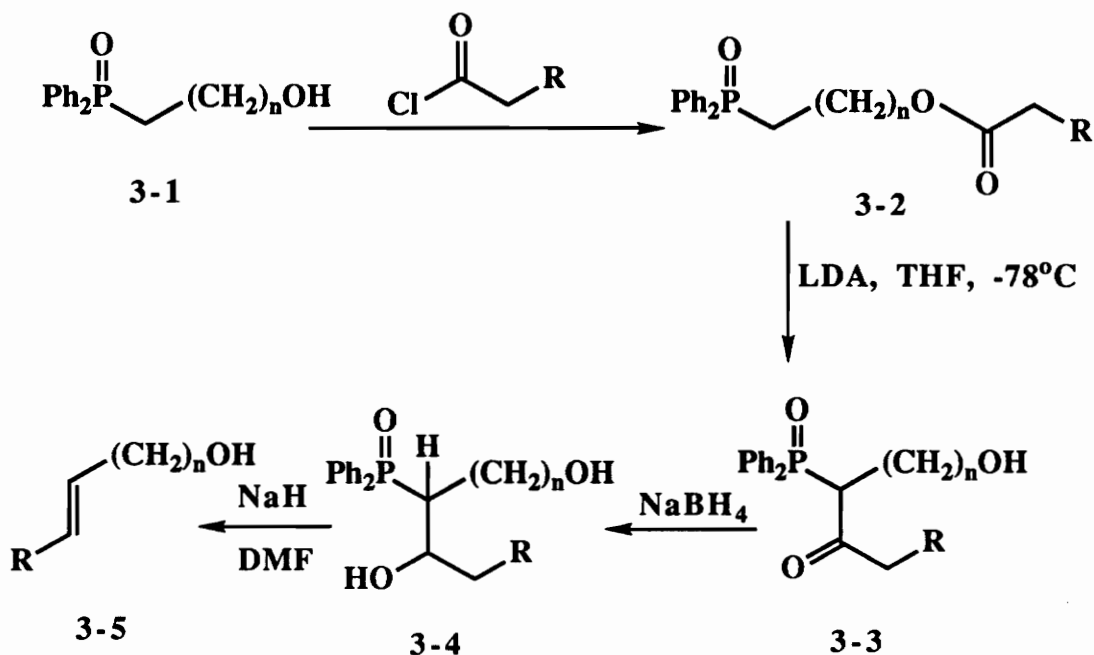
group of the diacyl analog is deprotected by treatment with hydrazine to give the ethanolamine plasmalogen. In each of these syntheses the enol ether was introduced early in the synthesis. This is not desirable with plasmalopentaene-12 since the extended conjugation of the enol ether creates stability problems that are not associated with a normal ethanolamine plasmalogen.

It thus becomes advantageous to develop a synthetic methodology for plasmalopentaene-12 in which the pentaenyl ether functionality is introduced late in the synthesis. This would not only make handling of intermediates easier, but it would also circumvent any problems that may arise due to the instability of the pentaenyl ether until late in the synthesis.

One attractive method for generation of the pentaenyl ether as a latent functionality is by an acyl migration of an unsaturated ester to the α -carbon of an oxygen substituted diphenylphosphine oxide (Scheme 3-1). Once the acyl migration occurs the secondary hydroxyl group can be reacylated and the enol ether can then be generated late in the synthesis by ketone reduction followed by oxyphosphatane elimination. Support for this methodology is provided by Warren^{92,93} (Scheme 3-2).



Scheme 3-1



Scheme 3-2

The anion of the diphenylphosphine oxide **3-2** intramolecularly attacks the ester carbonyl giving the desired acyl migrated product **3-3**. Reduction of the ketone to the hydroxyl and subsequent treatment with sodium hydride gives the desired olefin **3-5**. Looking at Scheme 3-2 reveals how this methodology developed by Warren provides a method for the latent construction of the pentaenyl ether. Thus the proposed retrosynthetic strategy utilizing this methodology is shown in Figure 3-3 where the acyl migration is utilized as the key step.

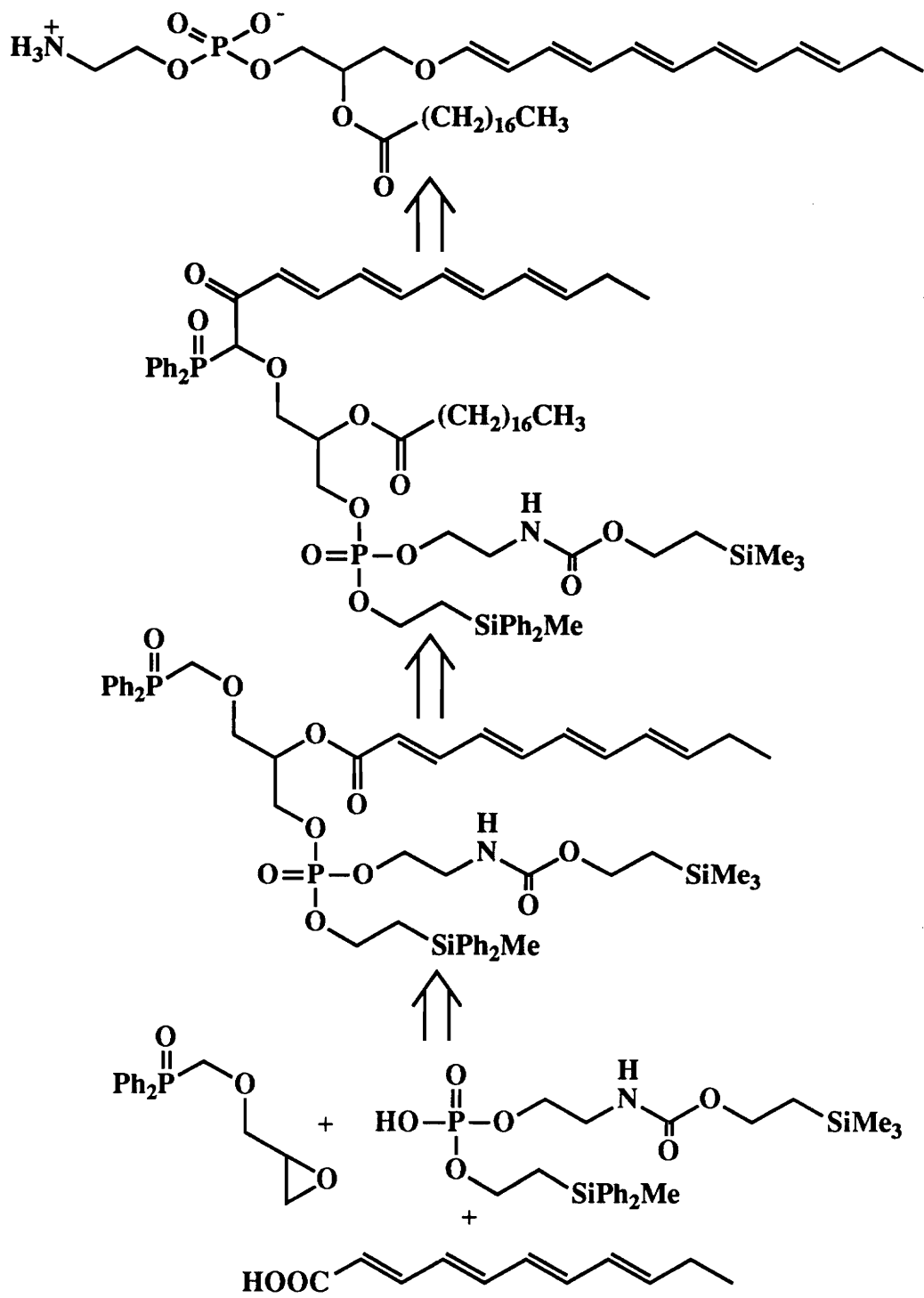
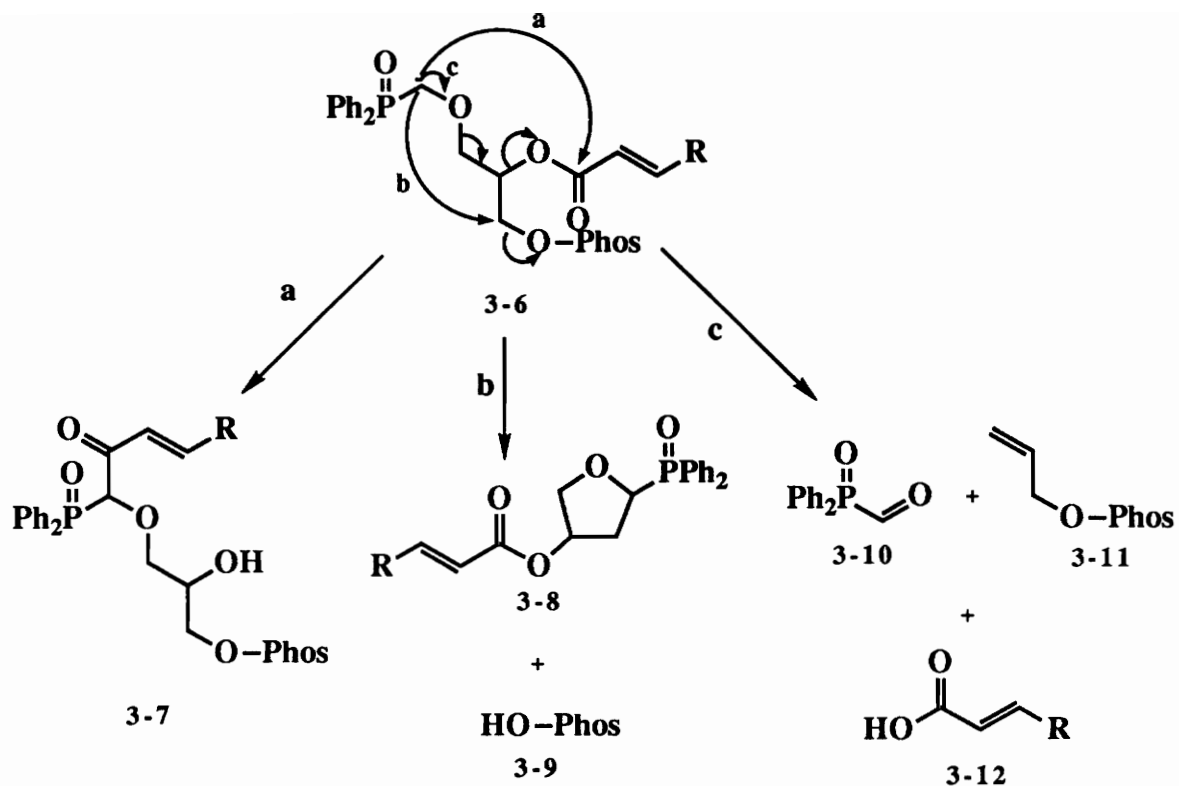


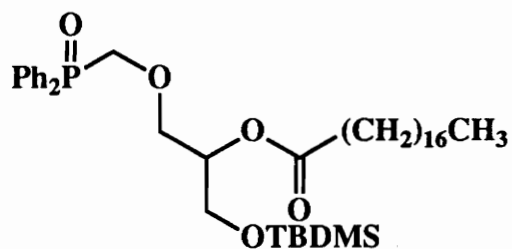
Figure 3-3. Retrosynthetic Analysis for Plasmalopentaene-12.

Several potential problems do, however, exist with the acyl migration methodology as depicted in Scheme 3-3. In pathway "a" the acyl group is successfully migrated by attack of the phosphine oxide anion on the ester carbonyl. This leads to the desired product. A possible side reaction that is of major concern is pathway "b". Since phosphates are good leaving groups, there may be a competition between migration in pathway "a" and cyclization created by pathway "b". It is imaginable that the base generated anion in 3-6 could attack the carbon bearing the phosphate group in an S_N2 fashion. This would give rise to the cyclic five-membered ring product 3-8 and a phosphate. A third possible scenario, although less likely, is the fragmentation of 3-6 as depicted by pathway "c". In this pathway, the anion on 3-6 causes an elimination process to occur which generates an aldehyde, an allylic phosphate and a carboxylic acid. It was believed that pathway "a" would predominate, but pathways "b" and "c" are also possible. The likelihood of these alternate and undesirable pathways occurring, no matter how remote, must be addressed before the acyl migration pathway can be employed as a useful synthetic methodology for the synthesis of plasmalopentaene-12.



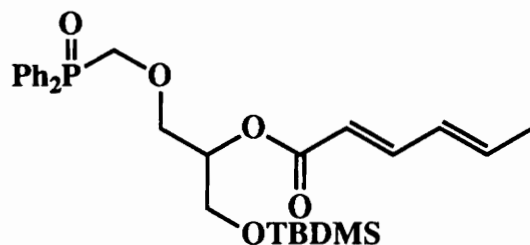
Scheme 3-3

In order to check the viability of the acyl migration strategy, it was necessary to synthesize a series of four model compounds. The first compound 3-13 is necessary to see if the acyl migration will actually occur.



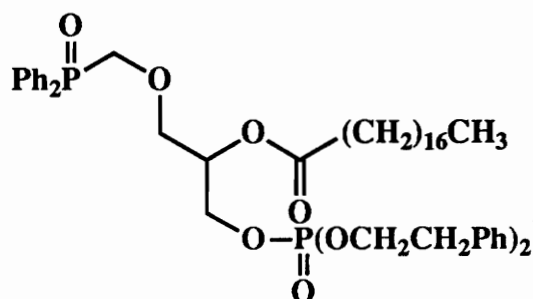
3-13

By using a silyl ether, there will be no possibility of pathway "b" taking place since silyl ethers are poor leaving groups. If the acyl migration does occur, the model can be extended to include an unsaturated acyl group. Compound **3-14** with the unsaturated moiety was used to determine if an unsaturated ester will migrate versus a saturated ester.



3-14

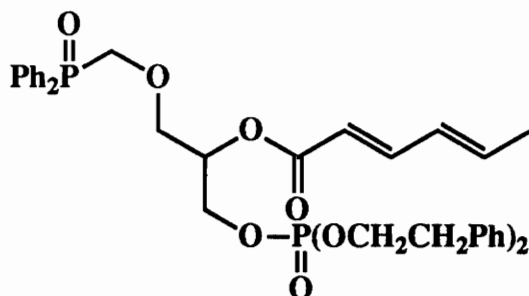
Once again a silyl ether was used in order to avoid any complications from pathway "b". The following two compounds **3-15** and **3-16** were synthesized to determine if pathway "b" will occur in preference to pathway "a".



3-15

The first compound **3-15** uses a saturated acyl group along with diphenethyl phosphate to determine whether the acyl migration will occur in preference to cyclization as depicted by

pathway "b". Finally, compound **3-16** was necessary to see if migration of an unsaturated ester will occur in the presence of the phosphate group.

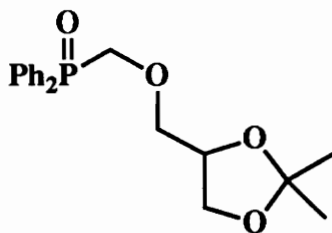


If acyl migration selectively occurs before displacement of the phosphate group in compound **3-16**, then the acyl migration strategy should prove to be a very useful method to generate the pentaenyl ether moiety which will thereby avoid any problems of instability associated with this highly labile functional group.

3.2 Synthesis of the Acyl Migration Models

3.2.1 Synthesis of the Saturated and Unsaturated Silyl Ether Analogs.

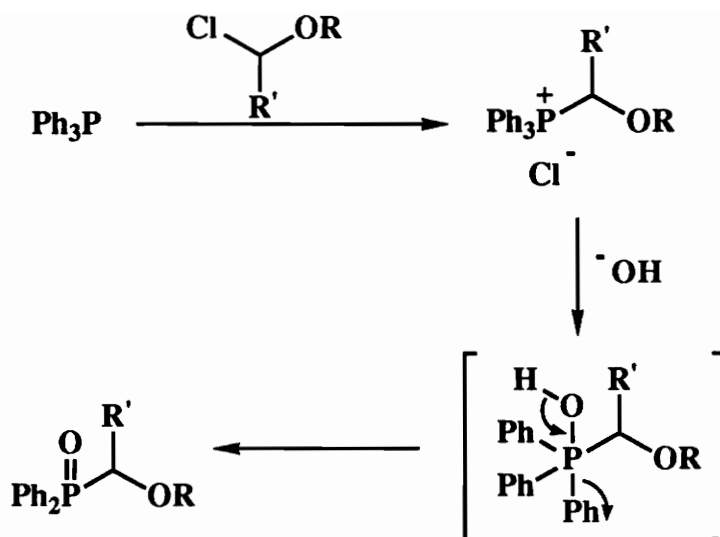
The synthesis of compounds **3-13** and **3-14** proceeded from the common intermediate **3-17**, isopropylidene protected (glyceryloxymethyl)-diphenylphosphine oxide.⁹⁴



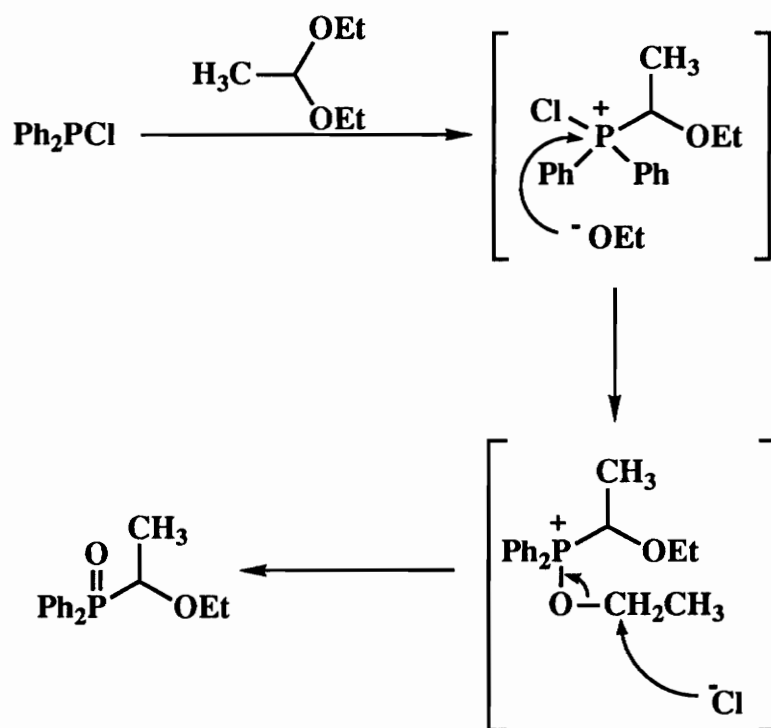
3-17

There have been several reported methods for the synthesis of α -oxygen-substituted diphenylphosphine oxides. The first synthesis of these compounds was accomplished by Trippett⁹⁵ and Hellman⁹⁶ by reacting triphenylphosphine and chloromethyl methyl ether followed by hydrolysis (Scheme 3-4). To date this procedure remains the method of choice for the preparation of most phosphine oxides, but it is not suitable for use in the present case because under some reaction conditions hydrolysis of the product was encountered. Also the use of α -chloroethers is unadvised since these compounds, as exemplified by chloromethyl methyl ether, are suspected carcinogens.

Another method described by Dietscke⁹⁷ reacts chlorodiphenylphosphine with an acetal in an Arbuzov reaction (Scheme 3-5). This method however is not suitable for the synthesis of 3-17 because of the low yields produced. There are other known methods for producing α -oxygen substituted diphenylphosphine oxides, but they suffer shortcomings similar to those previously described.

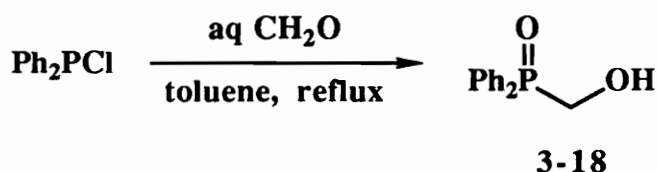


Scheme 3-4



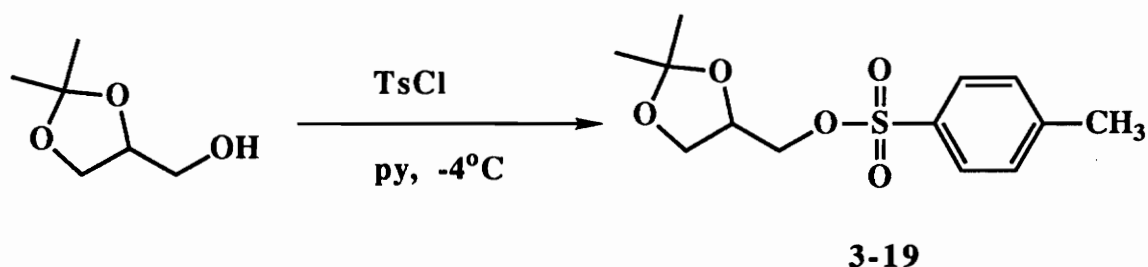
Scheme 3-5

A highly efficient and practical method of synthesizing **3-17** is the method of DeWit.⁹⁵ The starting material for this method was (hydroxymethyl) diphenylphosphine oxide **3-18**. Several syntheses of this molecule are known,^{98,99,100} but the method of choice is reaction of chlorodiphenylphosphine with aqueous formaldehyde. This method avoids the isolation of oxidatively unstable intermediates and avoids the use of any acidic or basic catalysis. Utilizing this procedure, it cleanly, in a one pot reaction, afforded the pure phosphine oxide **3-18**.

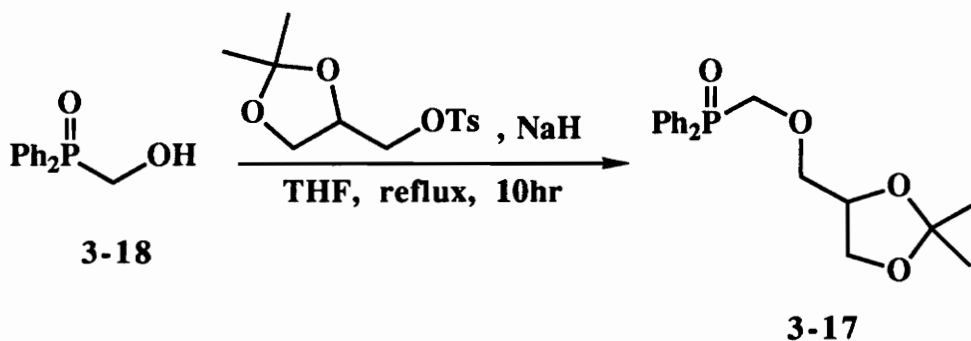


Scheme 3-6

Tosyl solketal **3-19** (Scheme 3-7) which was produced by reacting solketal with *p*-toluenesulfonyl chloride in the presence of pyridine was coupled with **3-18** using the Williamson reaction to give **3-17** in a high yielding reaction.

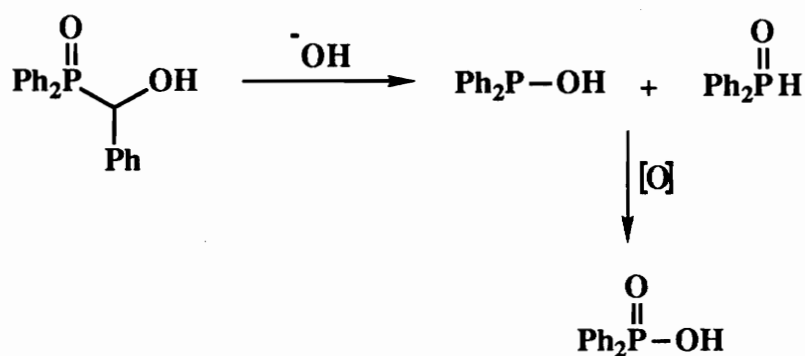


Scheme 3-7



Scheme 3-8

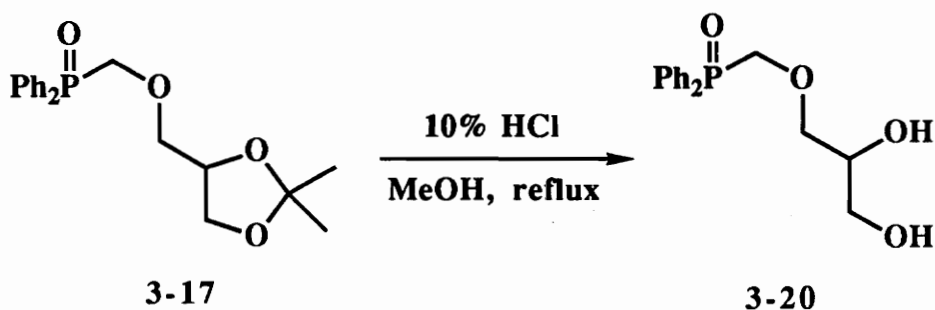
Initially, the reaction was run by adding the tosylate to a DMSO solution of sodium hydride and the phosphine oxide at room temperature then heating to 40°C-45°C. If the temperature was allowed to rise to >50°C some decomposition occurred, presumably from the elimination of formaldehyde. This has been previously reported by Miller *et al.*¹⁰¹ in a similar reaction in which basic treatment of a diphenylphosphine oxide yielded diphenylphosphinic acid (Scheme 3-9).



Scheme 3-9

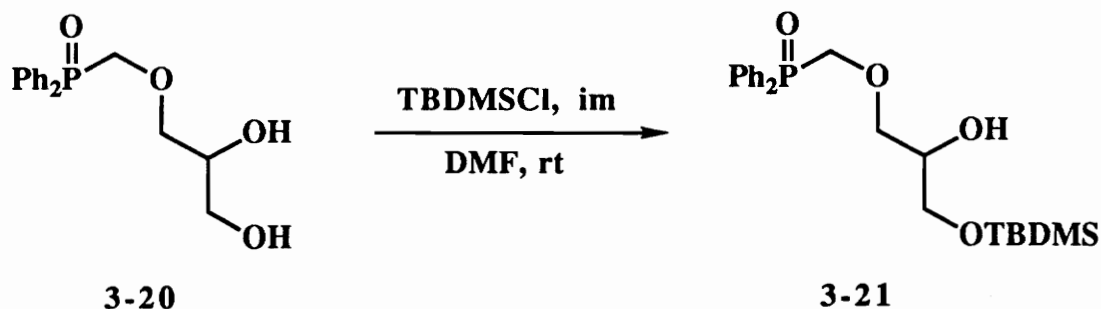
It was thus feared that heating the alkoxide above 50°C to increase the rate of reaction would cause elimination of formaldehyde to predominate. It was found, however, that addition of the phosphine oxide **3-18** to a THF solution of sodium hydride and tosyl

solketal and heating to reflux gave no elimination products. This method also greatly simplified work up and purification. The resulting ketal **3-17** was then deprotected by treatment with dilute acid which then gave the diol **3-20** in high yields (Scheme 3-10).



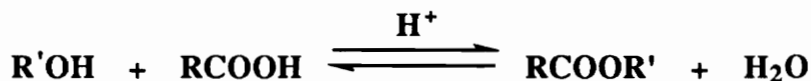
Scheme 3-10

At this point in the synthesis, it now became possible to utilize the differential reactivity of the primary versus the secondary hydroxyl groups. Since the primary hydroxyl group exhibits a higher degree of reactivity, it was possible to selectively protect it versus the secondary hydroxyl. This was especially easy with the protecting group *t*-butyldimethylsilyl chloride (TBDMSCl). The ease of introduction and removal of the TBDMS group are influenced by steric factors that allow for its selective introduction in sterically differentiated polyfunctional molecules. Thus in the case of the diol **3-20** the primary alcohol was selectively protected in the presence of the secondary alcohol using standard conditions of TBDMSCl, imidazole and DMF at room temperature¹⁰² to give **3-21** as shown below (Scheme 3-11). This method worked best when the reaction was run in very concentrated solution.



Scheme 3-11

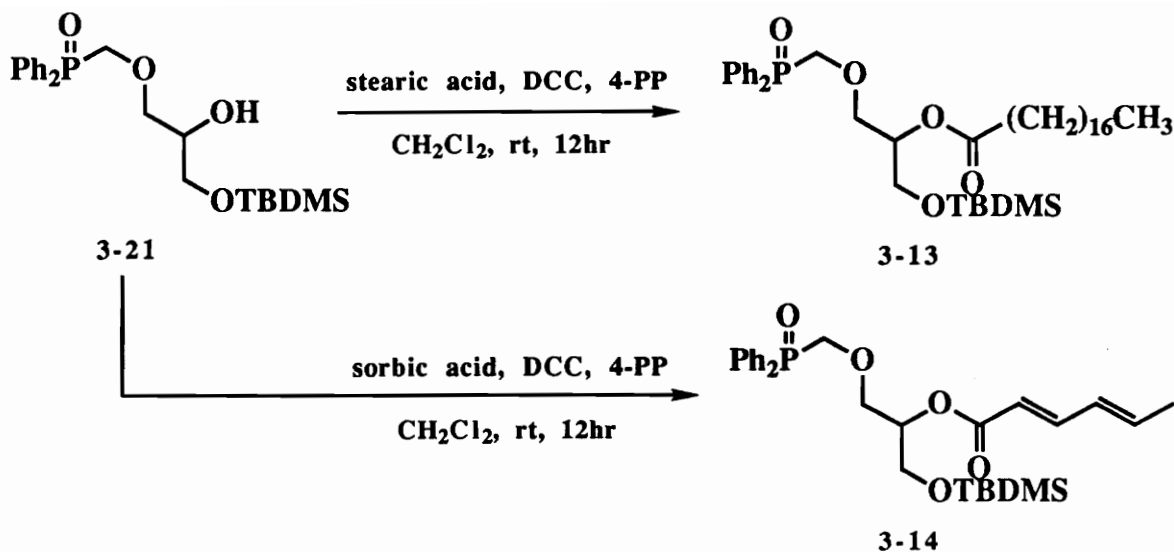
To complete the first two silyl ether analogs, the secondary alcohol must be acylated with a saturated and also an unsaturated acid. There are many methods available to esterify alcohols with an acid.¹⁰³



This reaction can only be accomplished if there is a means of driving the reaction to the right. There are many ways of accomplishing this, some of which are:

1. addition of an excess of one of the reactants,
2. removal of the water by distillation, and
3. removal of the water by use of a dehydrating agent.

It is this latter method which was the method of choice for this synthesis. There are many dehydrating agents available for this purpose. Some of these include *N,N'*-carbonyldiimidazole, chlorosilanes, phenyldichlorophosphate and dicyclohexylcarbodiimide (DCC). DCC¹⁰⁴ is the most popular method and was used in the analog syntheses (Scheme 3-12).

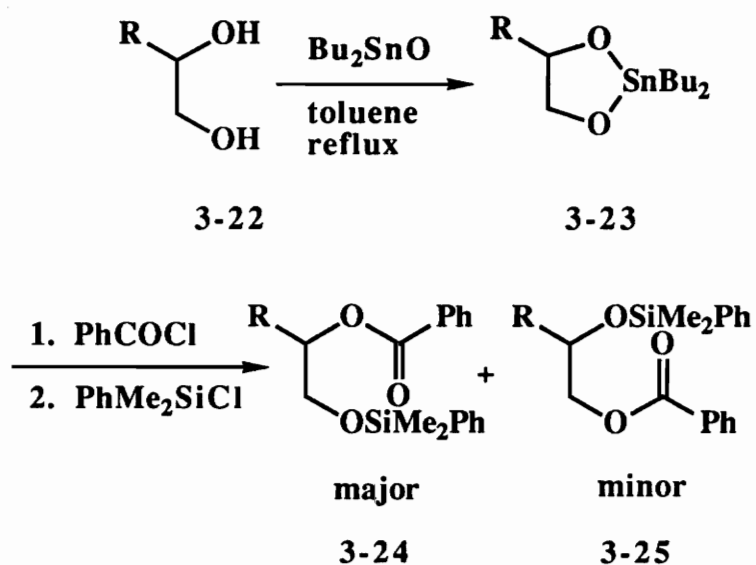


Scheme 3-12

The completion of the synthesis of the saturated and unsaturated silyl ether analogs was accomplished by coupling the alcohol to stearic acid or sorbic acid using DCC and 4-pyrrolidinopyridine (4-PP) as a catalyst. The yields were variable, but usually on the order of 60-80%. This may be attributed to the difficulty in removal of the byproduct, dicyclohexylurea, a known drawback to the reaction. This is especially difficult when acylating with a long chain fatty acid, due to the formation of urea inclusion complexes. These complexes were discovered in 1940 by Bergen,¹⁰⁵ and form when an alkane having seven or more carbons becomes trapped and not bonded in the channel formed by the helical lattice formed from hydrogen-bonded urea molecules. Although the yields were moderate, it was felt that since this was the last step of the model synthesis no optimization was necessary.

An alternate approach that is attractive is conversion of the diol **3-20** to the model compounds **3-13** and **3-14** in one step. Roelens¹⁰⁶ (Scheme 3-13) has shown that diols can be monoacylated with reversed chemoselectivity using organostannane chemistry.

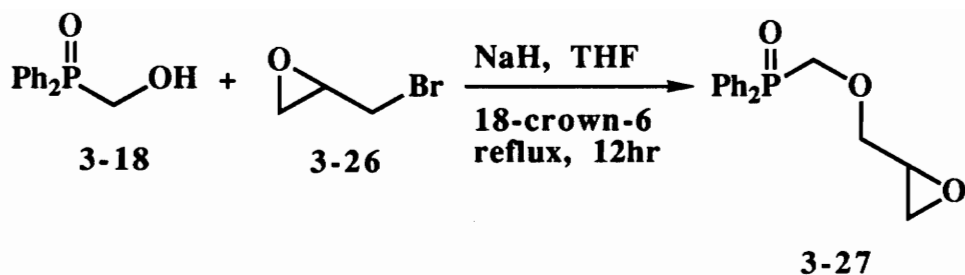
Diols of the general structure **3-22** when treated with dibutyltin oxide gave a dioxastannane **3-23** which can be isolated and purified. When reacted with benzoyl chloride and quenched the desired acyl silane **3-24** is obtained as the major product. This reaction was tried on diol **3-20** and a multitude of decomposition products were detected so the reaction was not pursued any further.



Scheme 3-13

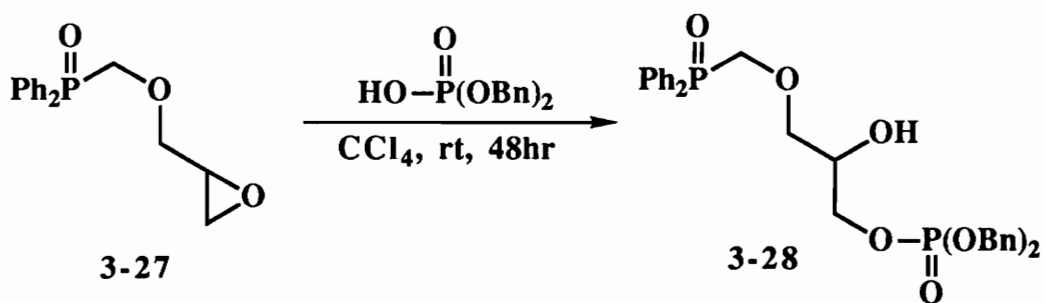
3.2.2 Synthesis of the Saturated and Unsaturated Phosphate Analogs.

The final two compounds **3-15** and **3-16** needed to complete the model studies were synthesized in a manner analogous to the previously described models. (Hydroxymethyl)diphenylphosphine oxide **3-18**, the synthesis of which was previously described, was condensed with epibromohydrin **3-26** in a Williamson synthesis using sodium hydride with a catalytic amount of 18-crown-6. This cleanly gave the diphenylphosphine oxide oxirane **3-27** as a white solid in excellent yields (Scheme 3-14).



Scheme 3-14

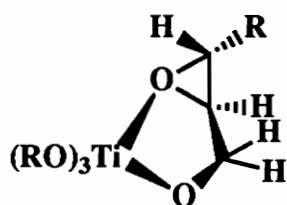
This oxirane can then be opened using a suitable phosphate. It has been shown that phosphoric acid will add to epoxides.¹⁰⁷ Potassium hydrogen phosphate¹⁰⁸ and phosphoric mono¹⁰⁹ and diesters¹¹⁰ have also been added to epoxides. Julia¹¹¹ has recently opened an epoxide using phosphorylcholine in refluxing methanol using potassium acetate as a catalyst. Product was obtained only in a moderate yield (on the order of 40-50%). Having this information, it was deemed possible to open the epoxide of 3-27 with dibenzyl phosphate giving 3-28. The most favorable conditions involved simply allowing the phosphate to stir with the oxirane at room temperature in carbon tetrachloride (Scheme 3-15).



Scheme 3-15

It is known that Lewis acids can facilitate nucleophilic ring opening of epoxides. For example, Sharpless¹¹² has shown that even though epoxides are weak Lewis bases,

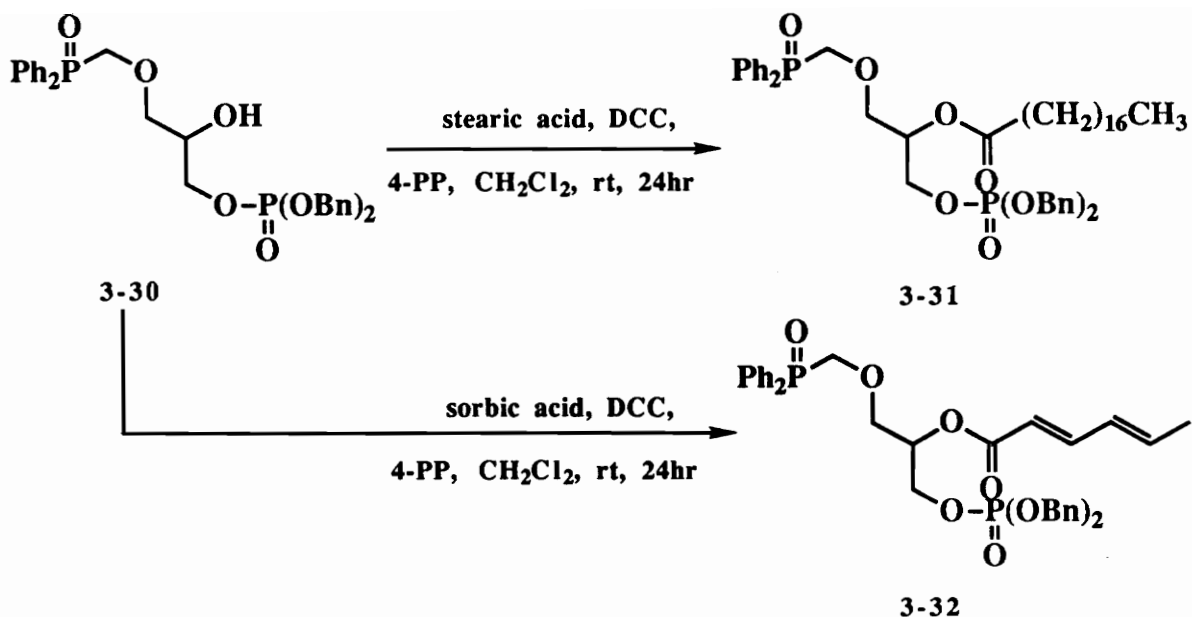
coordination by titanium isopropoxide, a weak Lewis acid, is made possible by the hydroxyl group of any epoxy alcohol **3-29**. This coordination greatly facilitates ring opening reactions with a wide variety of intermolecular nucleophiles. When there is an ether group instead of the hydroxyl group, the reaction does not occur.



3-29

When Lewis acid catalysis using boron trifluoride etherate was tried, the oxirane ring opening reaction by dibenzylphosphate occurred but neither the yield nor the reaction time was significantly improved as compared to the conventional method previously described.

Having obtained the phosphate oxirane ring opening product. It was now possible to complete the synthesis of the last two model compounds. This was accomplished in exactly the same fashion as with compound **3-21**. Compound **3-30** was acylated with stearic acid or sorbic acid using dicyclohexylcarbodiimide and 4-pyrrolidinopyridine as a catalyst (Scheme 3-16). Once again the yields are moderate, on the order of 65-75%. This may also be due to urea inclusion complexes. With the four model compounds readily available, it was now possible to determine which pathway in Scheme 3-3 would predominate.

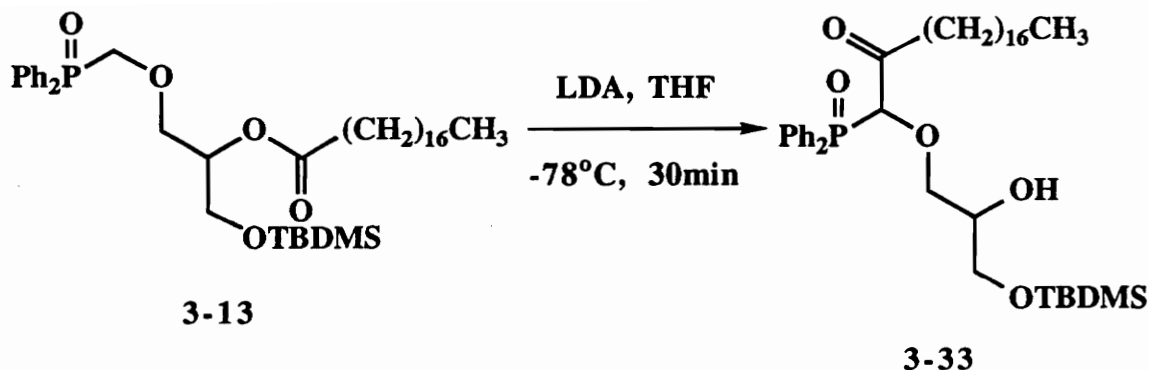


Scheme 3-16

3.3 Acyl Migration Studies.

Previously it was mentioned that there are possible complications with the proposed acyl migration pathway for the synthesis of plasmalopentaene-12 (Scheme 3-3). The synthesis of the four model compounds made it possible to test the viability of the proposed synthetic strategy for plasmalopentaene-12.

To test if the migration would occur on both a saturated and unsaturated analog, compounds **3-13** and **3-14** were both subjected to the migrating conditions. The stearate ester **3-13** was lithiated by treatment with lithium diisopropylamide at -78°C (Scheme 3-17). After 30 minutes the disappearance of **3-13** and the emergence of a less polar spot on TLC was noticed. After work up and purification, the product was identified as the expected migrated product **3-33**. This proves that the migration does occur with saturated systems, however, it was not known how an unsaturated ester would behave under identical migrating conditions.

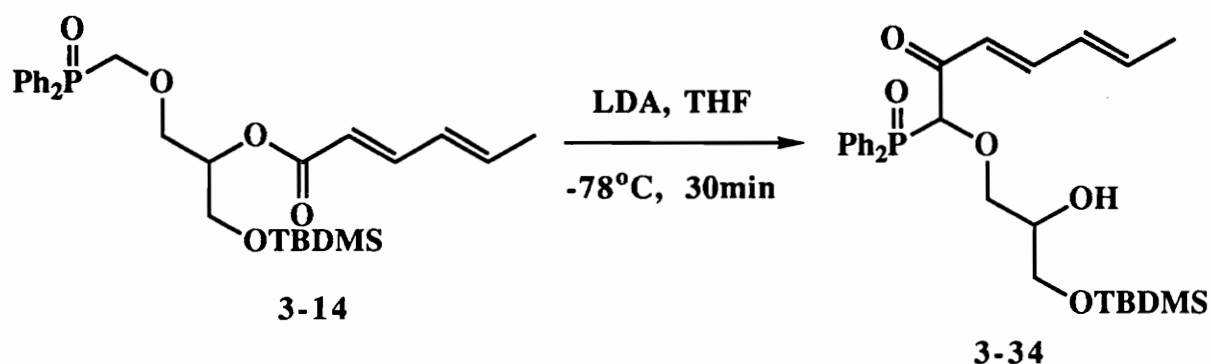


Scheme 3-17

The sorbate ester analog **3-14** was subjected to the same conditions as above (Scheme 3-18). Lithiation of **3-14** alpha to the phosphorus with LDA at -78°C for 30 minutes gave what appeared to be starting material based on TLC. After double elution, the compounds still had the same R_f . This was cause for some concern, but after work up and structure identification compound **3-34** was identified as the only product obtained. It is thus possible to successfully migrate an unsaturated moiety in addition to a saturated functionality.

This transformation was supported by two observations in the ^1H NMR spectrum. First, with the unmigrated material **3-13**, the protons adjacent to the diphenylphosphine oxide appear as a multiplet at 4.25 ppm (Figure 3-3). When the acyl group was migrated to give **3-33**, a new resonance appeared as a pair of doublets at 4.87 and 4.77 ppm (Figure 3-4). This apparent multiplicity presumably arises from coupling of the proton by the phosphorus and from the existence of two signals due to two diastereotopic forms of the proton. The second observation is the change in chemical shift of the glycerol methine proton in compound **3-13** from 4.46 ppm to a downfield shift to approximately 3.5 ppm. These changes in chemical shift are typical for these functional groups (Figure 3-5).

Similarly, the unsaturated and unmigrated material **3-14** shows the methylene protons adjacent to the phosphine oxide at 4.25 ppm and the methine proton on the glycerol moiety at 5.00 ppm. Its migrated counterpart **3-34** shows two overlapping doublets resonating at 4.91 ppm for the proton adjacent to the phosphine oxide and approximately 3.48 ppm for the glycerol methine (Figure 3-6).



Scheme 3-18

The potential exists for enolization to occur in all the migrated products. Fortunately, this was not found to be a problem of significance for the more simple systems such as the migration models. For example, the spectroscopic data support the existence of the keto form for **3-33**. A carbonyl carbon was seen at 206.06 ppm in the ^{13}C NMR spectrum. Also a carbonyl absorption was seen at 1718 cm^{-1} in the IR spectrum. The chemical shift of the methylene protons α to the carbonyl was found as two multiplets shifted slightly downfield from the normal 2.3 ppm to 2.8 and 2.6 ppm respectively. This apparent deshielding was attributed to the possible existence of the methylene protons inside the deshielding cone of the phenyl rings.

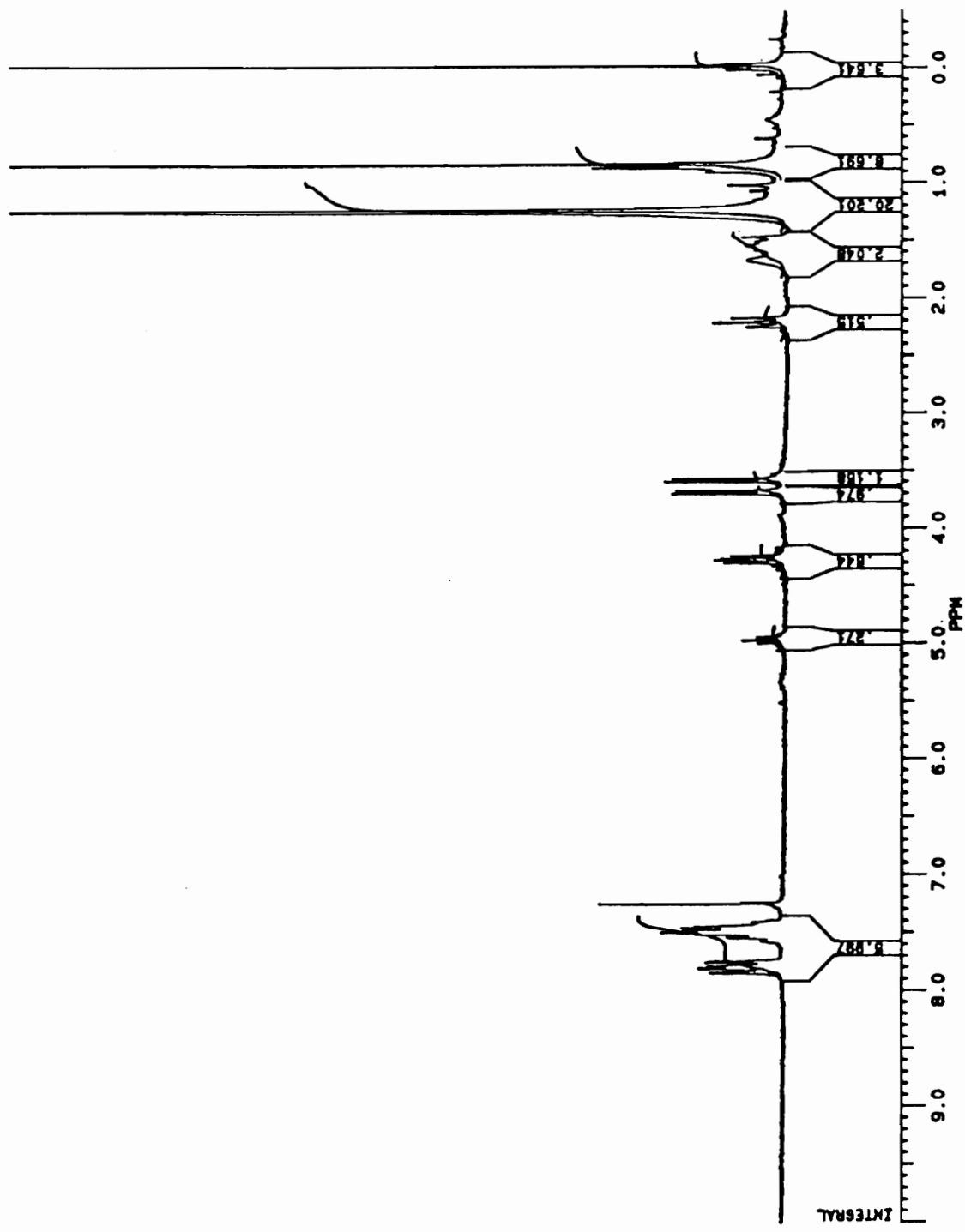


Figure 3-4. 270 MHz NMR of the Unmigrated Saturated Silyl Ether, 3-13.

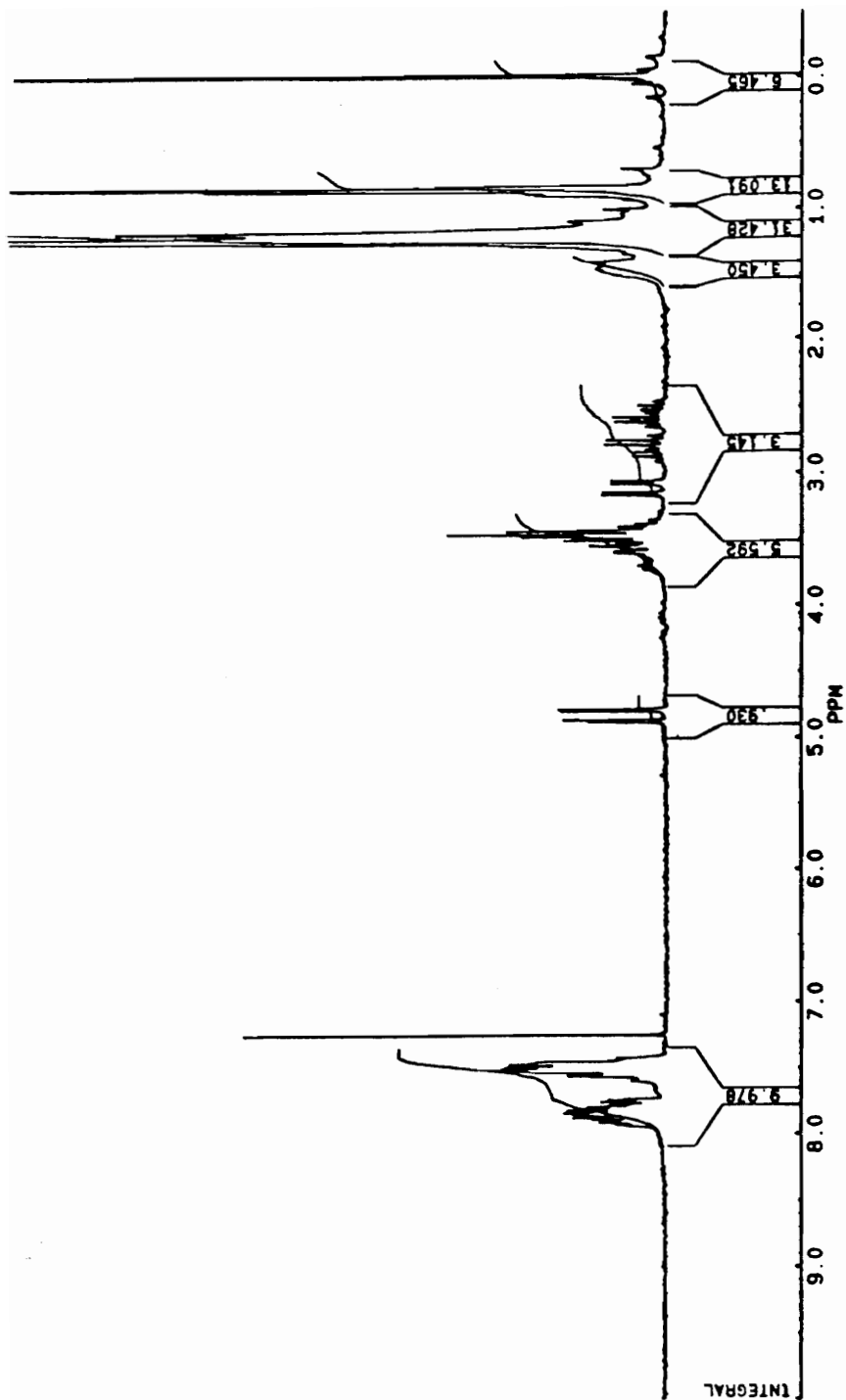


Figure 3-5. 270 MHz NMR of the Migrated Saturated Silyl Ether, 3-33.

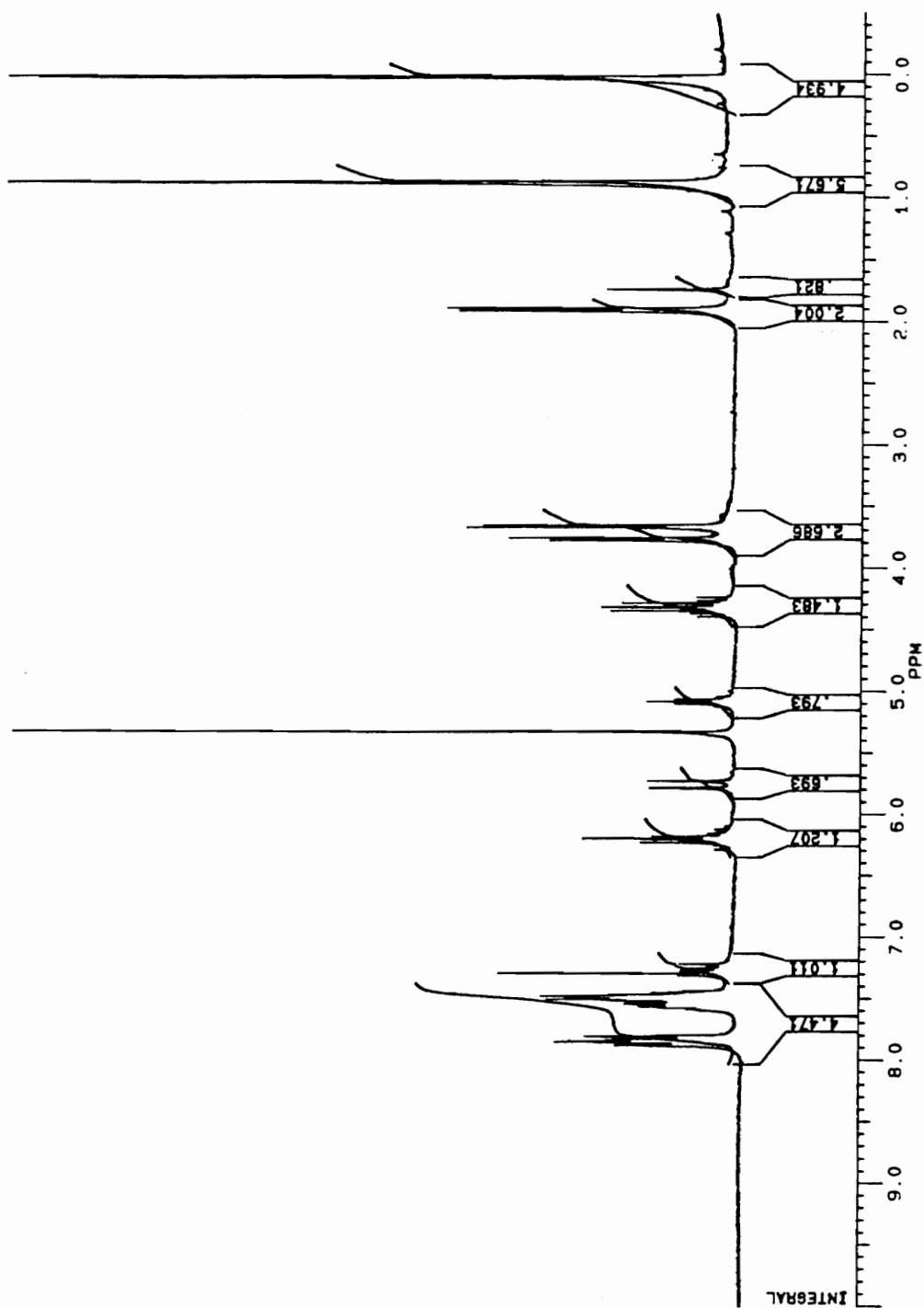


Figure 3-6. 270 MHz NMR of the Unmigrated Unsaturated Silyl Ether, 3-14.

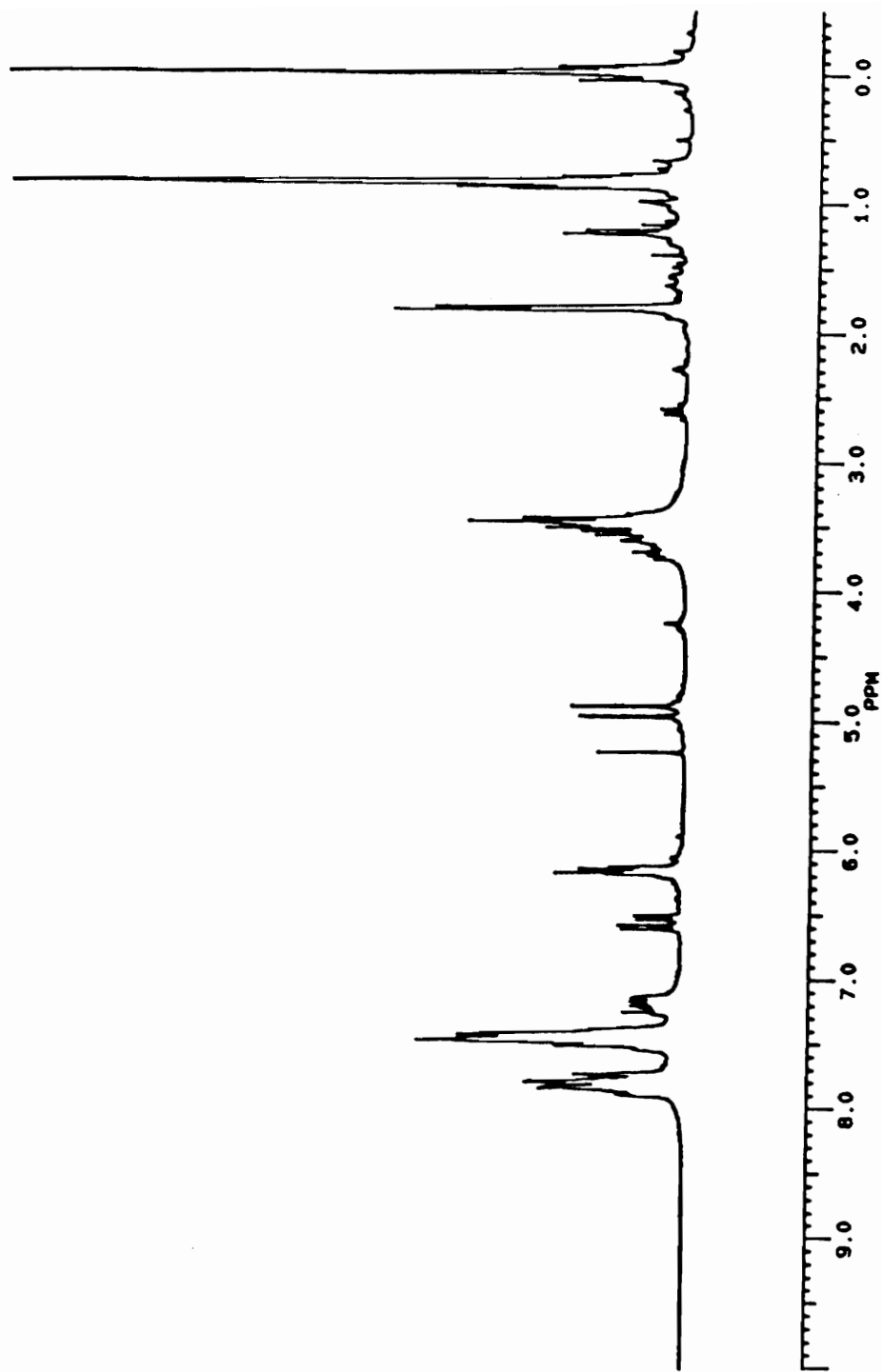
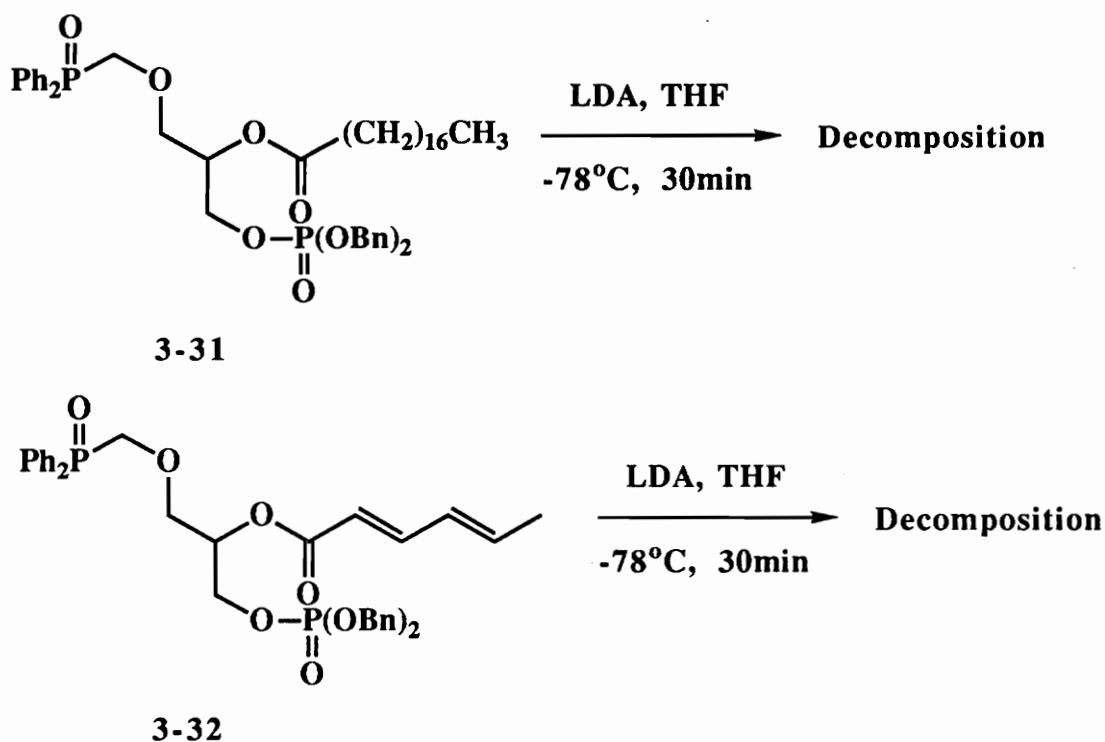


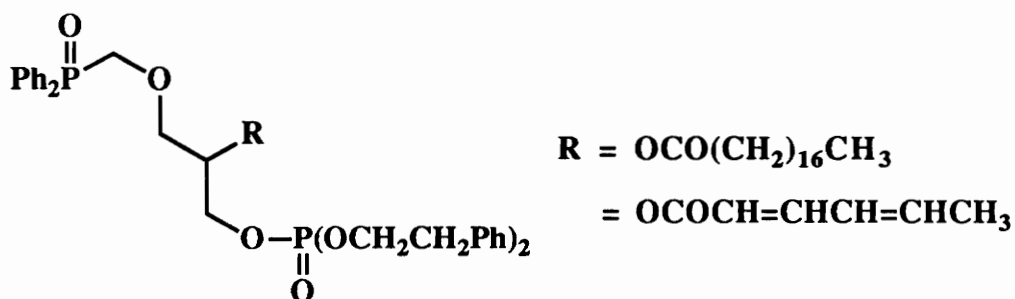
Figure 3-7. 270 MHz NMR of the Migrated Unsaturated Silyl Ether, 3-34.

In order to synthesize plasmalopentaene-12, a phosphate will be required in the place of the silyl ether. This may present a problem with the acyl migration strategy. Earlier it was mentioned that the anion generated with LDA may attack at the phosphate ester carbon giving rise to a cyclized product. To test this, the previously synthesized dibenzylphosphate analogs **3-31** and **3-32** were treated with LDA at -78°C (Scheme 3-19). After 30 minutes, a large number of unidentified products was obtained. It was thus clear that various reactions instead of the desired acyl migration were taking place.



Scheme 3-19

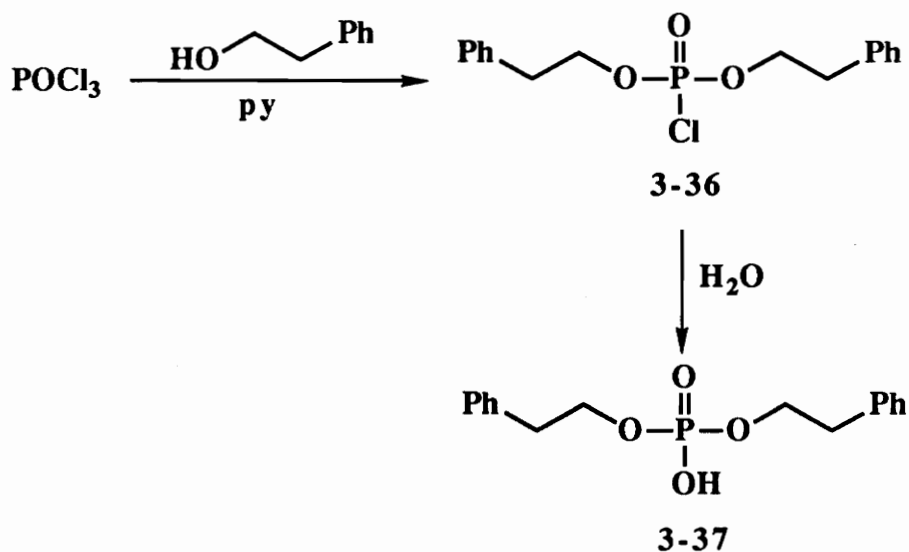
Compounds **3-31** and **3-32** were however more reactive than necessary, due to the presence of reactive benzyl ester functionalities. A less reactive model compound, but one still retaining the desirable UV-absorbing phenyl groups, would be the diphenethylphosphate analog **3-35**.



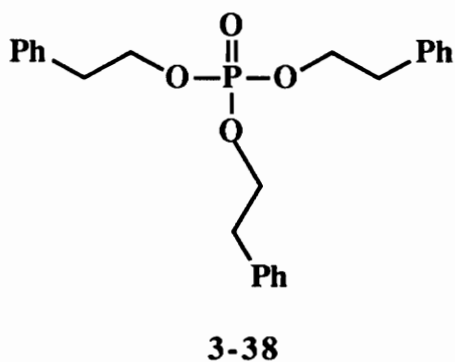
3-35

Compound **3-35** was prepared easily in the same fashion as the dibenzylphosphate analogs **3-31** and **3-32** by using diphenethylphosphate in place of dibenzylphosphate. In order to synthesize **3-35**, diphenethylphosphate must be synthesized since, unlike dibenzylphosphate, it is not commercially available.

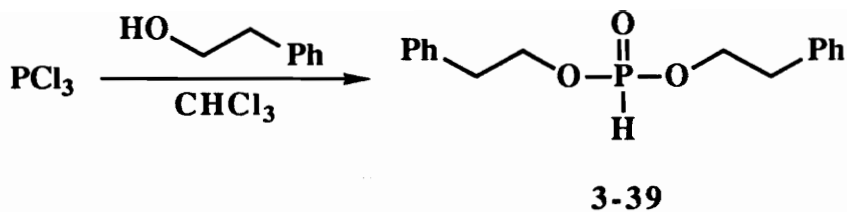
Initially, the reaction of phosphorus oxychloride with phenethyl alcohol presumably gave the phosphorochloridate **3-36**. This was then hydrolyzed in a one pot reaction to diphenethyl phosphate **3-37** (Scheme 3-20). This reaction met with only modest success, and the product was formed in only a low yield. It was difficult to control the rate of addition of phenethyl alcohol such that only dialkylation instead of trialkylation occurred. Compound **3-38** was a major byproduct. GC-MS analysis revealed some product formation, but the reaction mixture was inseparable by standard chromatographic techniques.



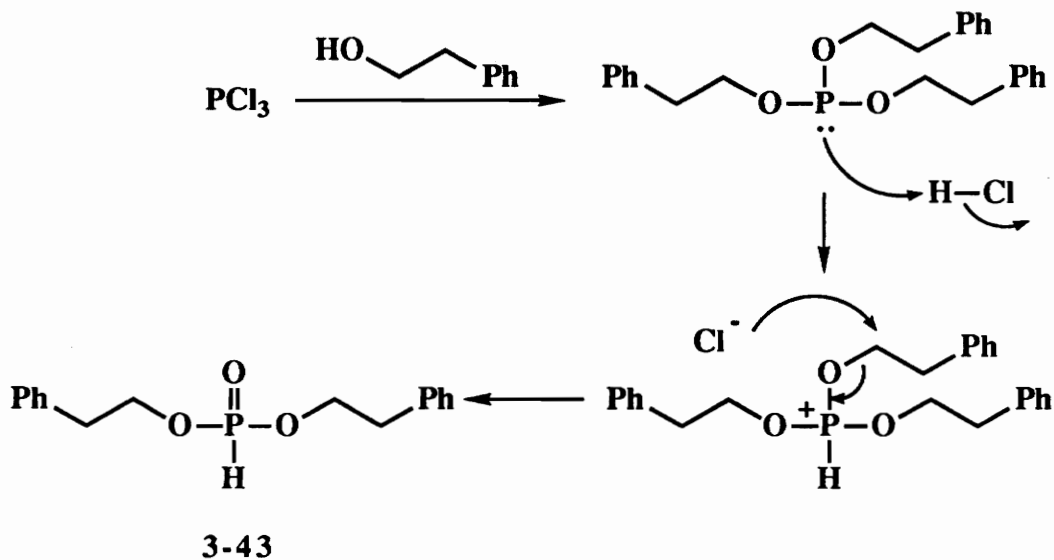
Scheme 3-20



A thorough examination of the chemical literature revealed an alternate and better route to the synthesis of diphenethyl phosphate¹¹³ (Scheme 3-21). The simultaneous addition of phosphorus trichloride and phenethyl alcohol to chloroform while blowing off the HCl by a stream of argon gave in high yield diphenethyl hydrogen phosphite **3-39** presumably via an Arbuzov reaction (Scheme 3-22).

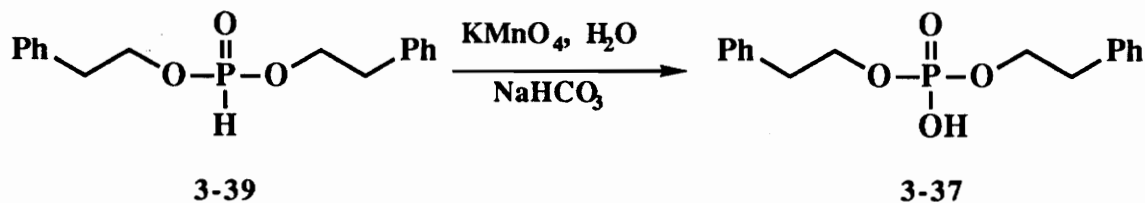


Scheme 3-21



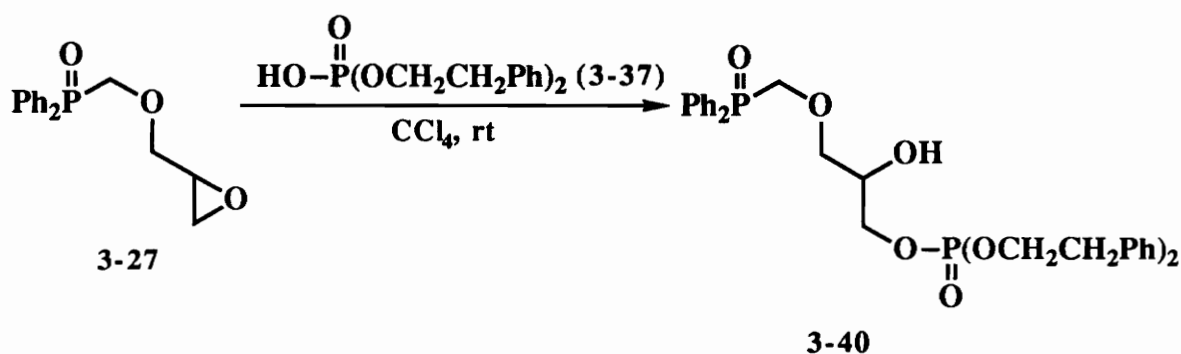
Scheme 3-22

The diphenethyl hydrogen phosphite 3-39 was then readily oxidized in a high yield to the phosphate 3-37 using potassium permanganate (Scheme 3-23). After work up it was found that no further purification was necessary and the diphenethyl phosphate could be used directly in the next step.



Scheme 3-23

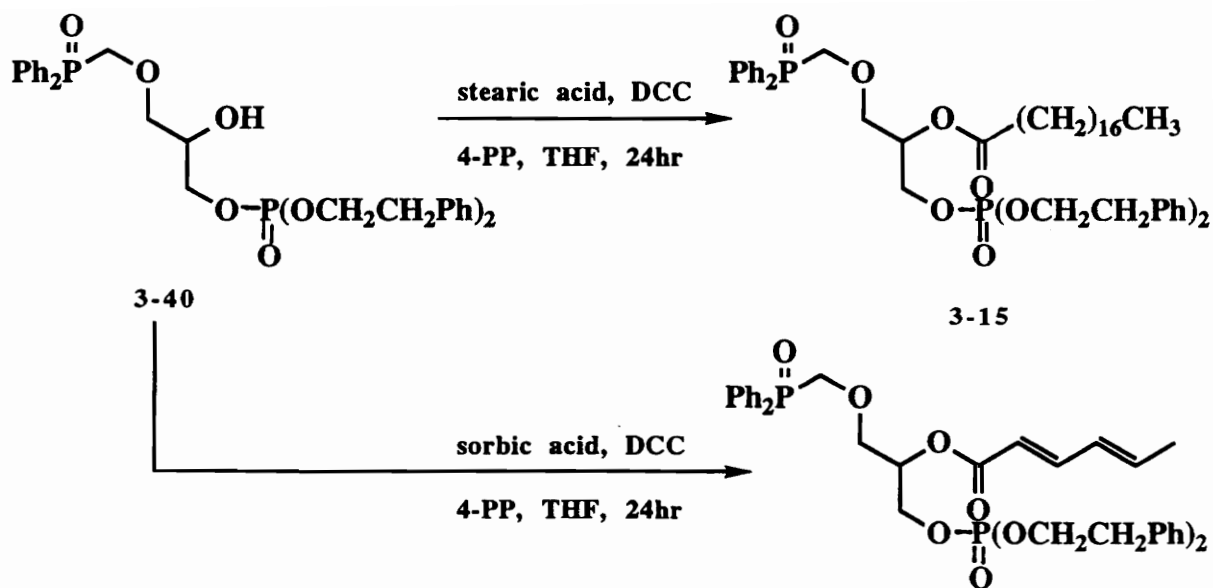
The synthesis of the diphenethyl phosphate analogs proceeded in exactly the same fashion as that of the dibenzyl phosphate analogs. Epoxide **3-27** was ring-opened using diphenethyl phosphate in carbon tetrachloride at room temperature to give the phosphate **3-40** (Scheme 3-24).



Scheme 3-24

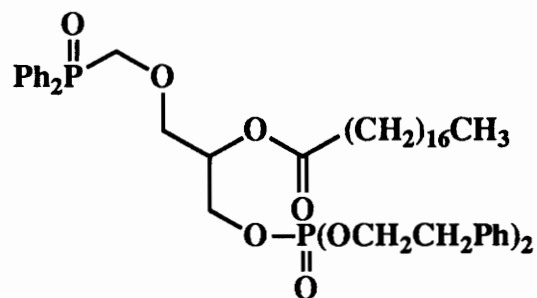
At this stage, the secondary alcohol of **3-40** was acylated with either stearic acid or sorbic acid using dicyclohexylcarbodiimide as the acylation agent (Scheme 3-25). The yields of **3-15** and **3-16** were once again only moderate. This was likely again due to the formation of urea inclusion complexes.¹⁰⁵

Acyl migration studies using these new model compounds **3-15** and **3-16** provided the desired migrated products **3-41** and **3-42**. When **3-15** and **3-16** were lithiated using LDA, the acyl group in each case migrated to give ketones **3-41** and **3-42** in very high yield with no indication of any cyclization or decomposition (Scheme 3-26).

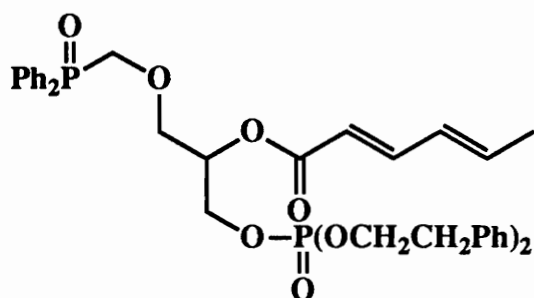


Scheme 3-25

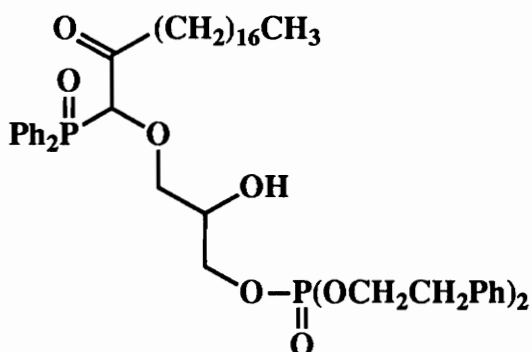
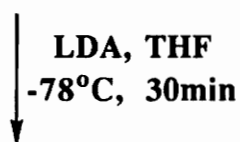
Evidence that the migration took place was obtained from the ^1H NMR spectrum. As in the case of the saturated **3-13** and unsaturated **3-14** silyl ether analogs, the methylene protons adjacent to the phosphine oxide in compounds **3-15** and **3-16** were observed as multiplets at 4.31 ppm and 4.32 ppm respectively. The methine hydrogen on the glycerol backbone of the saturated analog **3-13** and the unsaturated analog **3-14** gave a signal at 5.12 ppm and 5.18 ppm respectively (Figures 3-7 and 3-8). There was a marked change in the splitting pattern and chemical shift of the migrated material. For instance, in the case of the saturated analog, **3-41** the proton adjacent to the phosphine oxide was observed as a pair of doublets and the methine proton on the glycerol backbone was shifted to approximately 3.50 ppm. Similarly in the case of the unsaturated phosphate **3-42**, the signal for the proton adjacent to the phosphine oxide was also observed as a pair of doublets at 4.87 and 4.82 ppm, and the methine proton gave a signal at approximately 3.80 ppm (Figures 3-9 and 3-10).



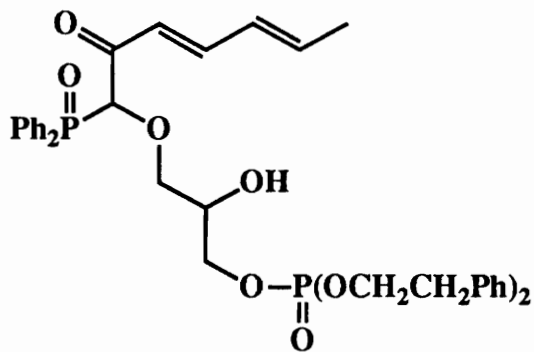
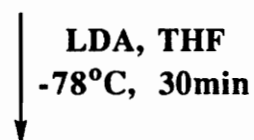
3-15



3-16



3-41



3-42

Scheme 3-26

The results of these acylation migration experiments indicate that this proposed synthetic strategy may prove to be a useful method for the construction of plasmalogens, particularly plasmalopentaene-12. This method offers two major advantages. First, the hydroxyl at the *sn*-2 position can easily be functionalized with a wide variety of acyl groups which thereby make it a relatively simple task to make a wide variety of analogs of plasmalopentaene-12. This would be crucial to study the structure activity relationships of plasmalopentaene-12. Second, and most important, the acyl migration method offers the

advantage of generating the highly unstable pentaenyl ether moiety of plasmalopentaene-12 in a late stage of the synthesis. This would make the handling of intermediates much less complicated than it otherwise could be.

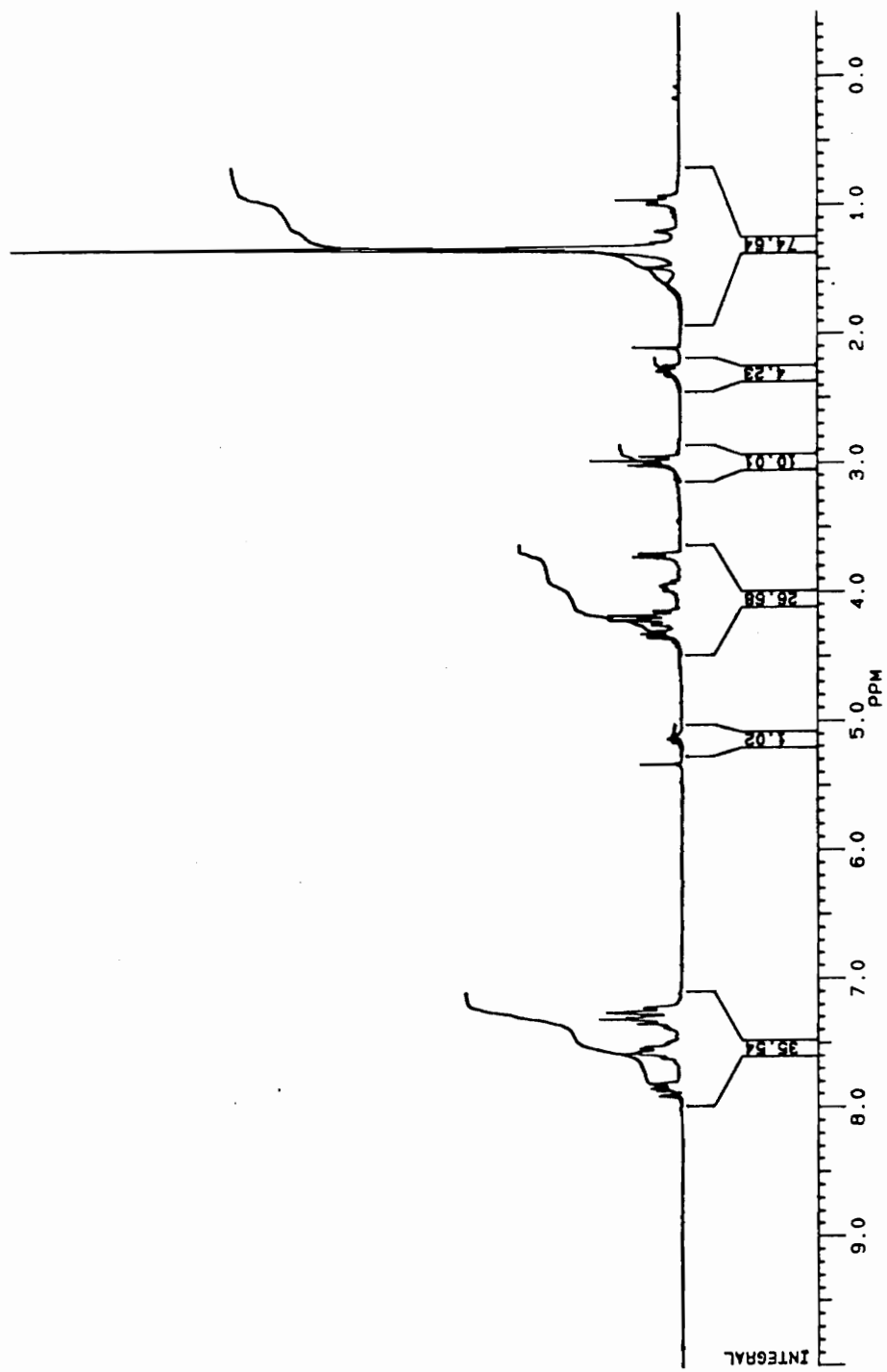


Figure 3-8. 270 MHz NMR of the Unmigrated Saturated Phosphate, 3-15.

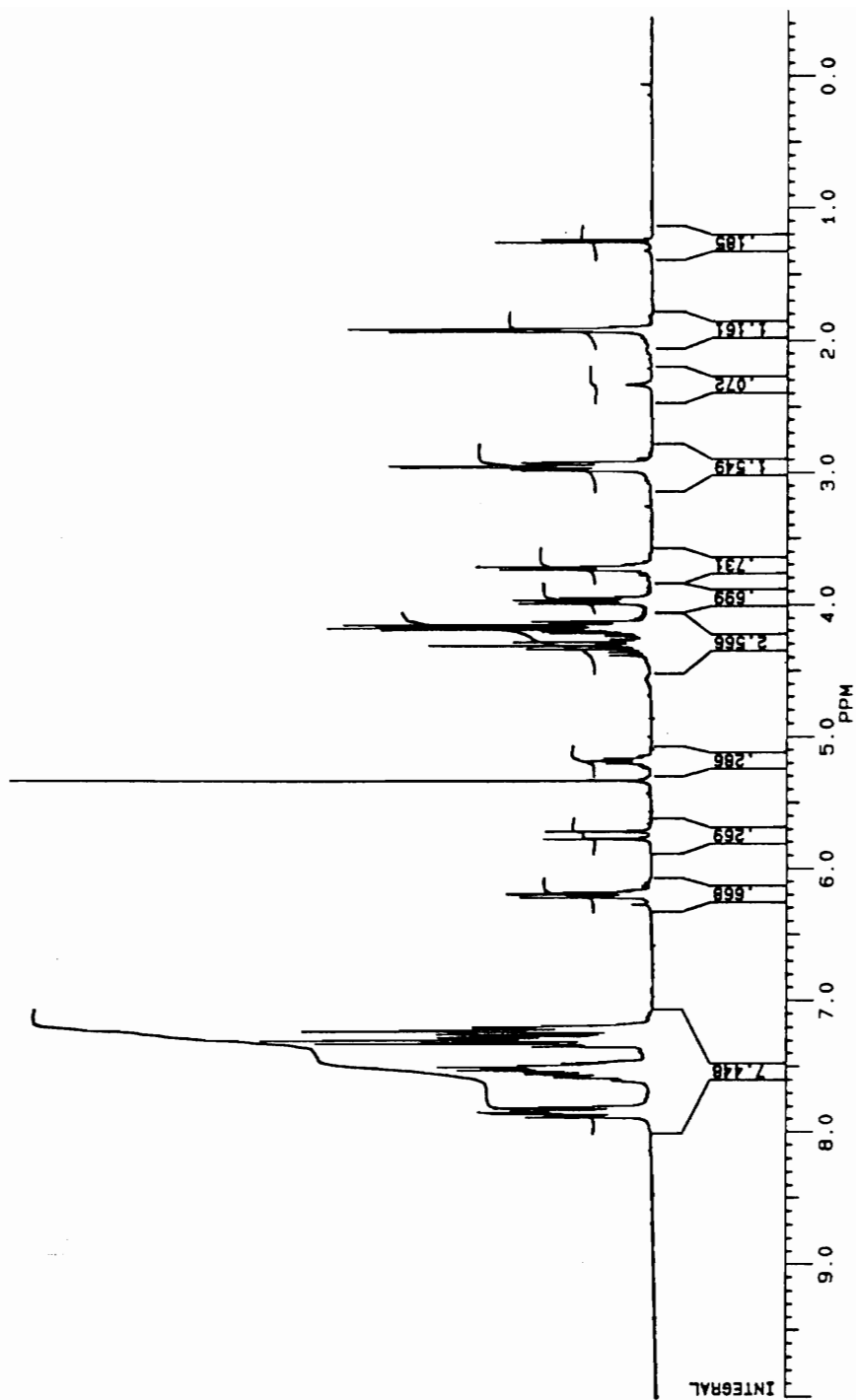


Figure 3-9. 270 MHz NMR of the Unmigrated Unsaturated Phosphate, 3-16.

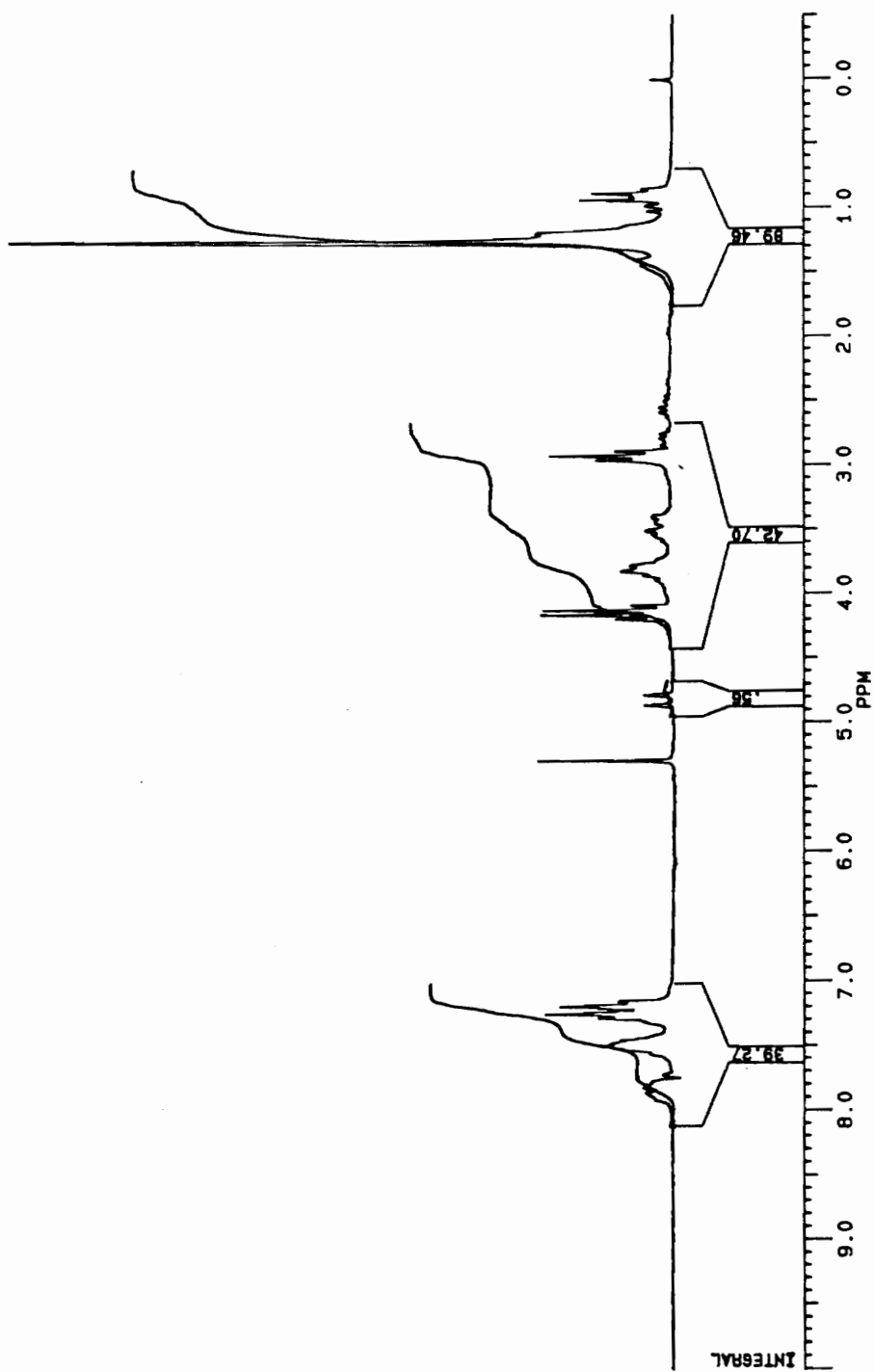


Figure 3-10. 270 MHz NMR of the Migrated Saturated Phosphate, 3-41.

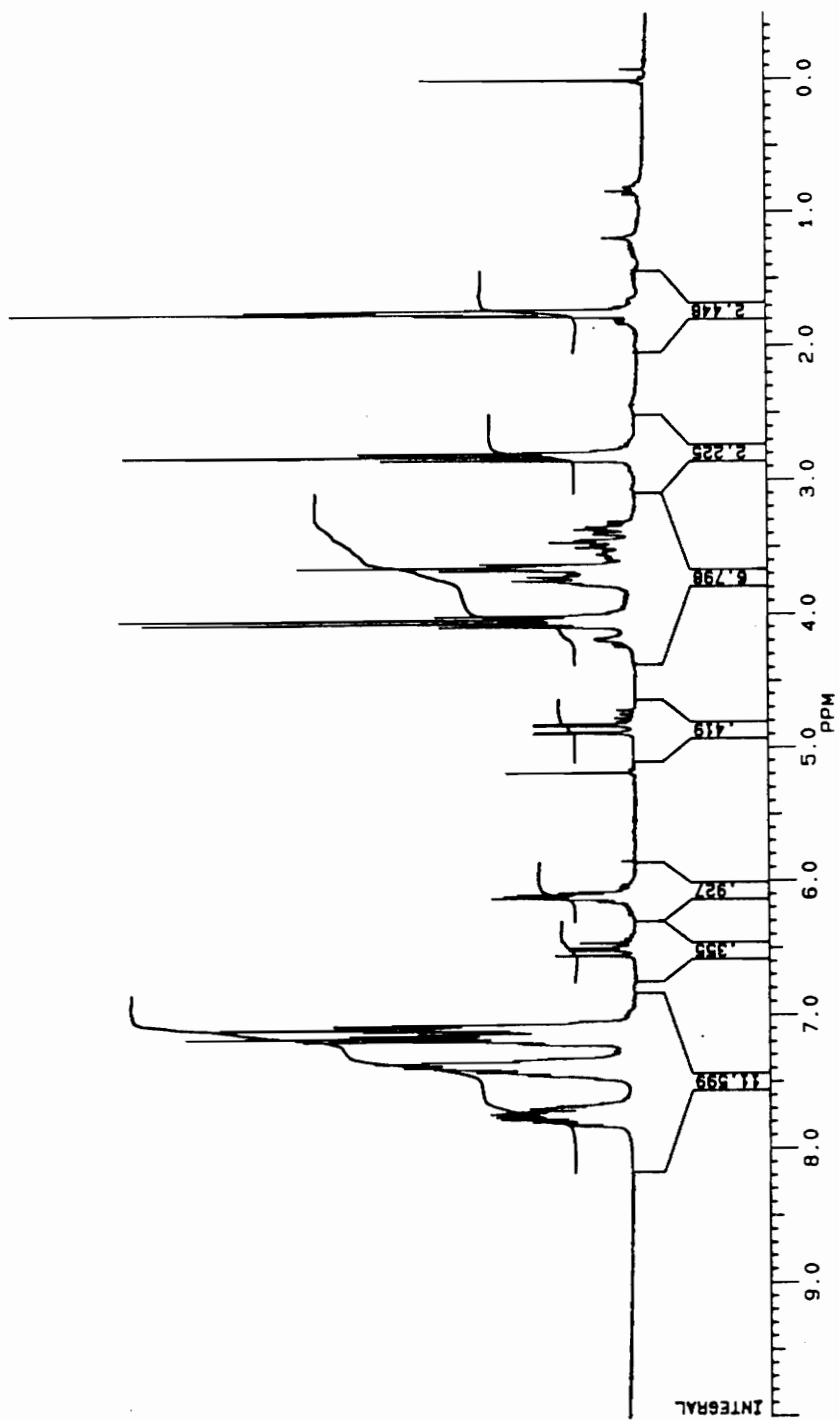


Figure 3-11. 270 MHz NMR of the Migrated Unsaturated Phosphate, 3-42.

3.4 General Experimental Procedures.

Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra (neat or KBr disc) were recorded on a Perkin Elmer 710B spectrophotometer.

^1H and ^{13}C nuclear magnetic resonance spectra were recorded on Bruker WP200, WP270SY, or Varian Unity 400 MHz spectrometers. ^1H and ^{13}C chemical shifts are given in ppm (δ) relative to tetramethylsilane. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constants (J) are given in Hertz (Hz). All spectra were recorded in deuteriochloroform (CDCl_3) at room temperature except where indicated.

Mass spectral data were obtained with a VG Analytical 7070E-HF mass spectrometer. GC-MS data were obtained in a Hewlett-Packard 5890 gas chromatograph linked to Hewlett-Packard 5970B quadrupole mass spectrometer. UV absorptions were recorded on a Beckman DU-50 spectrophotometer using HPLC grade methyl alcohol as a solvent.

Analytical thin layer chromatography was carried out on E. Merck aluminum supported silica gel 60 (0.2 mm, F₂₅₄) plates. Preparative thin layer chromatography was performed on 20 cm x 20 cm GF₂₅₄ (0.5 mm) glass supported plates. Silica gel for flash chromatography was E. Merck 230-400 mesh. Pretreating silica gel with triethylamine involved stirring 2 kg of E. Merck 230-400 mesh silica gel in 2 l of 10% triethylamine in hexanes overnight. The mixture was filtered on a Büchner funnel and traces of triethylamine were removed under high vacuum.

All reactions utilizing anhydrous solvents were conducted under an argon atmosphere. All solvents were purified and dried according to standard procedures. All

reagents purchased from chemical companies were used directly, with the exception of methoxy but-2-en-3-yne which was purchased from Fluka.

Many of the compounds prepared were mixtures of diastereomers. No attempts were made at separating these mixtures, therefore some NMR spectra show mixtures of peaks. These peaks were designated as mixtures of diastereomers in the NMR experimental data.

3.5 Experimental.

Preparation of (hydroxymethyl)diphenylphosphine oxide (3-18)

Chlorodiphenylphosphine (16.88 g, 77.0 mmol) was dissolved in 100 ml of toluene and 40% aqueous formaldehyde (5.3 g, 177 mmol) was added. The solution was heated to reflux for 24 hours. The solvents were removed *in vacuo* to give a colorless oil. The oil was dissolved in 150 ml of CH₂Cl₂. This was washed with 3 x 100 ml H₂O. The H₂O washings were re-extracted with 3 x 150 ml CH₂Cl₂. The extracts were combined and washed with 2 x 100 ml H₂O and 1 x 100 ml brine. The extracts were dried over MgSO₄, filtered and the CH₂Cl₂ removed *in vacuo* to give a white solid which appeared as a single spot on TLC. Yield 23.8 g (88%), mp 130-133°C (lit⁹⁴ 136-137°C). ¹H NMR (CDCl₃) δ 7.81-7.69 (m, 4H, Ph₂PO), 7.55-7.36 (m, 6H, Ph₂PO), 6.30 (t, 1H, J = 6.5 Hz, OH), 4.4 (dd, 2H, J = 6.5 Hz and J = 1.5 Hz, PCH₂). MS (CI) *m/z* (relative intensity) 233 (MH⁺, 100), 219 (25), 203 (70). The spectral properties are identical to those previously described.⁹⁴

Preparation of 2,2-Dimethyl-1,3-dioxolane-4-ylmethyl *p*-toluenesulfonate (3-19)

Solketal (10.63 g, 80.0 mmol) was dissolved in 50 ml of dry pyridine and cooled to -10°C. To this solution *p*-toluenesulfonylchloride (15.33 g, 80.0 mmol) was added

portion-wise. The mixture was stirred at 4°C for 18 hours. Water (200 ml) was added and the mixture was extracted with 3 x 200 ml ether. The extracts were washed with 5 x 200 ml of 10% HCl followed by 2 x 200 ml H₂O and 1 x 200 ml brine. The extracts were dried over MgSO₄ and the solvents were removed *in vacuo* to give a colorless oil. The oil was crystallized from ether/hexanes to give white crystals. Yield 16.06 g (70%), mp 40-42°C. ¹H NMR (CDCl₃) δ 7.80 (d, 2H, J=8.3 Hz, Ar), 7.36 (d, 2H, J = 8.1 Hz, Ar), 4.27 (m, 1H, CH) 4.0 (m, 3H, CH and CH₂) 3.77 (m, 1H, CH₂), 2.46 (s, 3H, ArCH₃), 1.34 (s, 3H, CH₃), 1.31 (s, 3H, CH₃). MS (CI) *m/z* (relative intensity) 287 (MH⁺, 100), 271 (5), 229 (50), 133 (35), 115 (48). The spectral properties are identical to those previously described.¹¹⁴

Preparation of [(2,2-dimethyl-1,3-dioxolane-4-yl-methoxy)methyl]-diphenylphosphine oxide (3-17)

Sodium hydride (97%, 1.16 g, 47.0 mmol) was suspended in 60 ml of dry THF. Tosylsolketal (10.0 g, 29.0 mmol) was then added to the suspension along with a catalytic amount of 18-crown-6, followed by 3-18 (10 g, 43.0 mmol) in portions over a 1 hour period at room temperature. The mixture was then refluxed for 10 hours, cooled and 100 ml of distilled water added. This was extracted with 3 x 100 ml dichloromethane. The extracts were washed with 2 x 100 ml H₂O and 1 x 100 ml brine. They were dried over MgSO₄, filtered and the solvent removed *in vacuo*. Purification by flash chromatography eluting with 94% CH₂Cl₂/ 6% MeOH gave 10.75 g (79%) of 3-17 as a white solid, mp 83-86°C. ¹H NMR (CDCl₃) δ 7.87-7.76 (m, 4H, Ph₂PO), 7.57-7.44 (m, 6H, Ph₂PO), 4.34 (dd, 2H, J = 3.0 Hz and 8.0 Hz, Ph₂POCH₂), 4.18 (m, 1H, CH), 3.91 (m, 1H, CH₂), 3.67 (m, 3H, 2 x CH₂), 1.35 (s, 3H, CH₃), 1.32 (s, 3H, CH₃). ¹³C NMR (CDCl₃) δ 132.1-128.4 (Ph₂PO), 109.4 (C(CH₃)₂), 74.3 (CH), 74.1 (CH₂), 69.0 (Ph₂POCH₂), 66.3 (CH₂), 26.6 (CH₃), 25.3 (CH₃). IR 3058, 2985, 1437, 1183, 1121,

1098, 1050, 842, 747, 717, 695 cm^{-1} . MS (CI) m/z (relative intensity) 347 (MH^+ , 100), 321 (10), 289 (35), 233 (20), 216 (30), 203 (20). The spectral properties are identical to those previously described.⁹⁴

Preparation of [[(2,3-dihydroxy]propoxy)methyl]diphenylphosphine oxide (3-20)

Compound 3-17 (11.38 g, 32.9 mmol) was dissolved in 350 ml of methyl alcohol and 10% HCl (330 ml) was added. The solution was refluxed for 30 minutes and the solvents were removed *in vacuo*. 300 ml of distilled water was added and this was made basic with 58% NH_4OH . This mixture was extracted with 3 x 250 ml CH_2Cl_2 . The extracts were washed with 3 x 250 ml brine and dried over MgSO_4 . The combined extracts were filtered and the solvent removed under reduced pressure. Purification by flash chromatography (90% CH_2Cl_2 / 10% MeOH as eluent) gave 9.46 g (94%) of 3-20 as a white solid, mp 91-94°C. ^1H NMR (CDCl_3) δ 7.79-7.71 (m, 4H, Ph_2PO), 7.60-7.46 (m, 6H, Ph_2PO), 4.34 (d, 2H, $J = 3.4$ Hz, Ph_2POCH_2), 3.82 (m, 1H, CH), 3.72 (m, 2H, CH_2), 3.59 (m, 2H, CH_2). ^{13}C NMR (CDCl_3) δ 132.33-128.55 (Ph_2PO), 75.18 (CH), 70.65-70.35 (CH_2), 68.63 (PCH_2), 63.29 (CH_2). IR 3360, 3058, 2922, 2876, 1483, 1171, 1723, 1096, 744, 720, 695 cm^{-1} . The spectral properties are identical to those previously described.⁹⁴

Preparation of 1-[[2-hydroxy-3-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]propoxy)methyl]diphenylphosphine oxide (3-21)

Compound 3-20 (2.45 g, 8.0 mmol) was dissolved in 20 ml of anhydrous dimethylformamide. To this solution imidazole (1.14 g, 16.8 mmol) followed by *t*-butyldimethylchlorosilane (1.33 g, 8.8 mmol) was added. The mixture was stirred at room temperature for 12 hours after which 50 ml of distilled water was added. This was extracted with 3 x 50 ml ether. The ether extracts were washed with 5 x 50 ml H_2O

followed by 1 x 50 ml brine. The extracts were dried over MgSO₄ filtered, and the solvents removed *in vacuo*. Purification by flash chromatography and eluting with 95.5% CH₂Cl₂ / 4.5% MeOH gave 2.06 g (77%) of **3-21** as a colorless oil. ¹H NMR (CDCl₃) δ 7.83-7.71 (m, 4H, Ph₂PO), 7.58-7.39 (m, 6H, Ph₂PO), 4.31 (d, 2H, J = 3.4 Hz, Ph₂POCH₂), 3.76 (m, 1H, CH), 3.62 (m, 2H, CH₂), 3.51 (d, 2H, J = 5.4 Hz, CH₂), 2.51 (s, 1H, OH), 1.86 (s, 9H, Si(CH₃)₃), 0.0 (s, 6H, Si(CH₃)₂). ¹³C NMR (CDCl₃) δ 132.17-128.43 (Ph₂PO), 75.11 (CH₂), 70.59 (CH), 69.05 (Ph₂POCH₂), 25.81 (C(CH₃)₃), -5.52 (CH₃). IR 3420, 3015, 2943, 2910, 1522, 1450, 1286, 1210, 1151, 1110, 911, 804, 762, 729, 708, 683 cm⁻¹. MS (CI) *m/z* (relative intensity) 421 (MH⁺, 83), 405(5) 363 (45), 345 (18), 215 (100), 203 (85), 183 (15), 115 (20), 89 (35).

Preparation of 1-[[2-octadecyloxycarbonyl-3-[[[(1,1-dimethylethyl)-dimethylsilyl]oxy]propoxy]methyl]diphenylphosphine oxide (3-13)

Compound **3-21** (1.07 g, 2.6 mmol) was dissolved in 7 ml of anhydrous CH₂Cl₂ and stearic acid (1.11 g, 3.9 mmol) was added to this solution. Next dicyclohexylcarbodiimide (0.805 g, 3.9 mmol) and a catalytic amount of 4-pyrrolidinopyridine were added. The solution was stirred at room temperature for 20 hours. The solvent was removed *in vacuo* to leave a white residue. The residue was suspended in ethyl acetate and filtered. The solvent was removed to leave a colorless oil. Purification by flash chromatography using 98% CH₂Cl₂ / 2 % MeOH as the eluting solvent gave 1.76 g (98.4%) of a colorless oil. ¹H NMR (CDCl₃) δ 7.82-7.75 (m, 4H, Ph₂PO), 7.52-7.40 (m, 6H, Ph₂PO), 4.96 (m, 1H, CH), 4.25 (m, 2H, Ph₂POCH₂), 3.67 (d, 2H, J = 5.0 Hz, CH₂), 3.56 (d, 2H, J = 6.4 Hz, CH₂), 2.19 (t, 2H, J = 7.6 Hz, OCOCH₂), 1.53 (m, 2H, COCH₂CH₂), 1.22 (s, 28H, CH₂), 0.82 (s, 12H, Si(CH₃)₃ and CH₃), 0.0 (s, 6H, Si(CH₃)₂). ¹³C NMR (CDCl₃) δ 132.00-128.28 (Ph₂PO), 72.40 (CH₂), 72.0 (CH), 68.0 (Ph₂POCH₂), 61.47 (CH₂), 34.0 (COCH₂), 32.0 (CH₂CH₃),

29.58 (CH₂), 26.69 (C(CH₃)₃), -5.52 (CH₃). IR 3057, 2925, 2853, 1738, 1592, 1464, 1377, 1360, 1252, 1190, 814 cm⁻¹. MS (CI) *m/z* (relative intensity) 687 (MH⁺, 70), 629 (40), 455 (10), 421 (15), 403 (10), 345 (75), 317 (30), 285 (10), 217 (15), 203 (100), 187 (15), 171(10), 141 (15), 127 (20), 115 (20).

Preparation of 1-[[2-(2,4-hexadienylcarbonyloxy)-3-[[[(1,1-dimethylethyl)-dimethylsilyl]oxy]propoxy]methyl]diphenylphosphine oxide (3-14)

Compound 3-21 dissolved in 35 ml of anhydrous CH₂Cl₂ sorbic acid (0.909 g, 8.10 mmol) was added. To this solution dicyclohexylcarbodiimide (1.67 g, 8.10 mmol) and a catalytic amount of 4-pyrrolidinopyridine (0.12 g, 0.81 mmol) was added. The mixture was stirred at room temperature for 22 hours. The solvent was removed under reduced pressure to give a orangish-brown solid. The solid was suspended in ethyl acetate and filtered. The solvent was again removed *in vacuo* to give an orangish-brown oil. Purification by flash chromatography and eluting with 97% CH₂Cl₂ / 3 % MeOH gave 2.26 g (100%) of 3-14 as a orangish-brown highly viscous oil. ¹H NMR (CDCl₃) δ 7.80-7.71 (m, 4H, Ph₂PO), 7.55-7.48 (m, 6H, Ph₂PO), 7.21 (m, 1H, olefinic), 6.13 (m, 2H, olefinic), 5.67 (d, 1H, J = 16.2 Hz, olefinic), 5.01 (m, 1H, CH), 4.33 (m, 2H, Ph₂POCH₂), 3.70 (d, 2H, J = 6.2 Hz, CH₂), 3.59 (d, 2H, J = 8.1 Hz, CH₂), 1.90 (d, 3H, J = 5.4 Hz, CH=CHCH₃), 0.80 (s, 9H, C(CH₃)₃), 0.05 (s, 6H, CH₃). ¹³C NMR (CDCl₃) δ 166.39 (C=O), 145.47 (vinyl), 132.37 (vinyl), 131.99-131.39 (Ph₂PO), 129.76 (vinyl), 128.55-128.31 (Ph₂PO), 118.66 (vinyl), 76.34 (CH₂), 70.811 (CH), 68.06 (CH₂), 61.70 (Ph₂POCH₂), 25.76 (CH=CHCH₃), 18.54 (C(CH₃)₃), 18.18 (C(CH₃)₃), 34.0 (COCH₂), 32.0 (CH₂CH₃), 29.58 (CH₂), 26.69 (C(CH₃)₃), -5.47 (CH₃). IR 3057, 2929, 2856, 1713, 1645, 1438, 1326, 1244, 1188, 1122, 1000, 838 cm⁻¹. MS (CI) *m/z* (relative intensity) 421 (MH⁺, 10), 405 (5), 363 (5), 257 (5), 243 (15), 217 (20), 203 (100), 187 (5), 133 (5), 115 (5).

Preparation of [(2,3-oxido)propoxy]methyl)diphenylphosphine oxide (3-27)

Sodium hydride (95%, 1.36 g, 53.9 mmol) was suspended in 65 ml of anhydrous THF and epibromohydrin (6.97 g, 50.9 mmol) was then added along with a catalytic amount of 18-crown-6. Next (hydroxymethyl)diphenylphosphine oxide **3-18** (10.0 g, 43.1 mmol) was added at room temperature over a 1 hour period. Once the addition was complete the mixture was allowed to reflux for 10 hours. Distilled water (100 ml) was then added and the solution was extracted with 3 x 100 ml CH₂Cl₂. The extracts were washed with 2 x 100 ml H₂O and 1 x 100 ml brine, dried over MgSO₄, filtered, and the solvent removed under reduced pressure. Purification of the residue by flash chromatography using 85% ethyl acetate / 15% isopropyl alcohol gave 9.45 g (76%) of **3-27**, mp 48-51°C. ¹H NMR (CDCl₃) δ 7.84-7.72 (m, 4H, Ph₂PH), 7.52-7.40 (m, 6H, Ph₂PH), 4.30 (m, 2H, Ph₂OCH₂), 3.87 (dd, 1H, J = 15.8 and 3.4 Hz, CH₂), 3.43 (dd, 1H, J = 15.8 and 8.1 Hz, CH₂), 3.04 (m, 1H, CH), 2.69 (overlapping dd, 1H, J = 6.4 Hz, CH₂), 2.46 (dd, 1H, J = 6.3 and 6.7 Hz, CH₂). ¹³C NMR (CDCl₃) δ 132.15-128.43 (Ph₂PO), 74.09 (CH₁), 74.0 (CH), 70.51 (CH₂), 69.10 (Ph₂OCH₂). IR 3055, 2944, 2922, 1590, 1483, 1437, 1266, 1186, 1121, 1096, 863, 734, 696 cm⁻¹. MS (CI) *m/z* (relative intensity) 289 (MH⁺, 100), 243 (15), 2167 (4), 203 (45), 141 (10), 127 (10).

Preparation of 1-[[2-hydroxy-3-[di(benzyloxy)phosphinyloxy]propoxy]-methyl)diphenylphosphine oxide (3-28)

Compound **3-27** (4.87 g, 16.90 mmol) was dissolved in 30 ml of anhydrous carbon tetrachloride. To this solution dibenzylphosphate (4.71 g, 16.90 mmol) was added and the mixture was stirred at room temperature for 48 hours. The solvents were removed *in vacuo* to yield a colorless oil. Purification by flash chromatography and eluting with 93% CH₂Cl₂ / 7% MeOH gave 4.69 g (49%) of **3-28** as a colorless oil. ¹H NMR (CDCl₃) δ 7.77-7.70 (m, 4H, Ph₂PO), 7.52-7.41 (m, 6H, Ph₂PO), 7.41-7.27 (s, 10H,

POCH₂Ph), 5.02 (s, 2H, CH₂Ph), 4.99 (s, 2H, CH₂Ph), 4.26 (s, 2H, J = 3.5 Hz, Ph₂POCH₂), 4.19 (m, 1H, CH), 3.94 (m, 2H, CH₂), 3.59 (m, 2H, CH₂).

Preparation of 1-[[2-octadecylcarbonyloxy-3-[di(benzyloxy)phosphinoyloxy]propoxy]methyl]diphenylphosphine oxide (3-31)

Compound 3-30 (1.73 g, 3.10 mmol) was dissolved in 20 ml of anhydrous dichloromethane and stearic acid (1.31 g, 3.6 mmol) was added to this mixture. Next dicyclohexylcarbodiimide (0.947 g, 4.60 mmol) and a catalytic amount of 4-pyrrolidinopyridine was added. The mixture was stirred at room temperature for 24 hours, then the solvent was removed under reduced pressure leaving a white residue. The residue was suspended in ethyl acetate then filtered. The solvent was removed *in vacuo* to give a colorless oil. Purification by flash chromatography and eluting with 95% CH₂Cl₂ / 5% MeOH gave 2.53 g (98.1%) of 3-31 as a colorless oil. ¹H NMR (CDCl₃) δ 7.81-7.71 (m, 4H, Ph₂PO), 7.57-7.41 (m, 6H, Ph₂PO), 7.34 (s, 10H, CH₂Ph), 5.06 (m, 1H, CH), 5.02 (s, 2H, CH₂Ph), 5.00 (s, 2H, CH₂Ph), 4.23 (m, 2H, Ph₂POCH₂), 4.00 (m, 2H, CH₂), 3.62 (d, 2H, J = 6.7 Hz, CH₂), 2.19 (t, 2H, J = 7.5 Hz, OCOCH₂), 1.50 (m, 2H, COCH₂CH₂), 1.32 (s, 28H, stearic methylenes), 0.90 (t, 3H, J = 8.7 Hz, CH₃). ¹³C NMR (CDCl₃) δ 172.63 (CH), 135.59-131.21 (Ph₂PO), 128.49-127.82 (CH₂Ph), 71.36-69.35 (CH₂CHCH₂), 69.24 (CH₂Ph), 65.35 (Ph₂POCH₂), 32.60 (COCH₂), 29.21 (CH₂)₁₆CH₃, 13.99 (CH₂)₁₆CH₃. MS (CI) *m/z* (relative intensity) 833 (MH⁺, 28), 662 (10), 573 (10), 555 (40), 459 (10), 414 (10), 369 (100), 355 (60), 341 (5), 325 (29), 309 (20).

Preparation of 1-[[2-(2,4-hexadienylcarbonyloxy)-3-[di(benzyloxy)phosphinoyloxy]propoxy]methyl]diphenylphosphine oxide (3-32)

Compound 3-30 (1.79 g, 3.20 mmol) was dissolved in 20 ml of anhydrous dichloromethane. To this solution, sorbic acid (0.532 g, 47 mmol) was added. Finally

dicyclohexylcarbodiimide (0.978 g, 4.7 mmol) and a catalytic amount of 4-pyrrolidinopyridine were added. The reaction mixture was stirred at room temperature for 24 hours, then the solvent was removed *in vacuo* leaving an orangish-brown residue. The residue was suspended in ethyl acetate and filtered. The solvent was again removed *in vacuo* leaving a brownish-orange semisolid. Purification by flash chromatography and eluting with 95% CH₂Cl₂ / 5% MeOH gave 1.79 g (85%) of an brownish-orange oil. ¹H NMR (CDCl₃) δ 7.82-7.72 (m, 4H, Ph₂PO), 7.51-7.39 (m, 6H, Ph₂PO), 7.31 (s, 10H, CH₂Ph), 7.21 (m, 1H, olefinic), 6.18-6.09 (m, 2H, olefinic), 5.67 (d, 1H, J = 16.2 Hz, olefinic), 5.13 (m, 1H, CH), 5.02 (s, 2H, CH₂Ph), 4.98 (s, 2H, CH₂Ph), 4.21 (m, 2H, Ph₂POCH₂), 4.01 (m, 2H, CH₂), 3.64 (d, 2H, J = 6.5 Hz, CH₂), 1.84 (d, 3H, J = 5.4 Hz, CH=CHCH₃). ¹³C NMR (CDCl₃) δ 166.02 (C=O), 146.07 (vinylic), 140.19 (vinylic), 135.70 (vinylic), 132.09-131.28 (Ph₂PO), 129.57 (vinylic), 128.56-127.88 (CH₂Ph), 117.99 (vinylic), 71.54-69.97 (CH₂CHCH₂), 69.30 (CH₂Ph), 65.48 (CH₂P), 18-60 (CH₂)₁₆CH₂. MS (CI) *m/z* (relative intensity) 565 (MH⁺, 70), 459 (100), 387 (25), 369 (65).

Preparation of 1-[2-hydroxy-3-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]-propoxy]-1-(diphenylphosphinyl)-2-nonadecanone (3-33)

Compound 3-13 (948 mg, 1.50 mmol) was dissolved in 15 ml of anhydrous THF and cooled to -78°C. LDA in THF (1.5 M, 1.33 ml, 2.1 mmol) was added to the solution dropwise. The mixture was stirred at -78°C for 30 minutes, then 10 ml of saturated ammonium chloride was added and the temperature was allowed to warm to room temperature. The organic layer was separated and the aqueous layer was extracted with 3 x 25 ml Et₂O. The organic extracts were combined and washed with 2 x 50 ml H₂O and 1 x 50 ml brine. The extracts were dried over MgSO₄ and the solvent removed under reduced pressure. Purification by flash chromatography eluting with 75% CH₂Cl₂ / 25% EtOAc

gave 683 mg (71%) of **3-33** as a colorless oil. $^1\text{H NMR}$ (CDCl_3) δ 7.92-7.73 (m, 4H, Ph_2PO), 7.59-7.42 (m, 6H, Ph_2PO), 4.86 (d, 0.5H, $J = 3.1$ Hz, diastereomer of Ph_2POCH), 4.77 (d, 0.5H, $J = 3.0$, diastereomer of Ph_2POCH), 3.71-3.37 (m, 5H, CH_2CHCH_2), 3.14 (d, 0.5H, $J = 5.8$ Hz, diastereomer of CHOH), 3.05 (d, 0.5H, $J = 5.9$ Hz, diastereomer of CHOH), 2.77 (m, 1H, diastereomer of COCH_2), 2.57 (m, 1H, diastereomer of COCH_2), 1.44-1.39 (m (unresolved), 2H, COCH_2CH_2), 1.23 (s, 28H, stearic methylenes), 0.85 (s, 12H, $\text{C}(\text{CH}_3)_3$ and $\text{C}(\text{CH}_2)_{16}\text{CH}_3$ (overlapping)), 0.00 (s, 6H, $\text{Si}(\text{CH}_3)_2$). $^{13}\text{C NMR}$ (CDCl_3) δ 206.06 ($\text{C}=\text{O}$), 132.57-131.58 (Ph_2PO), 128.63-128.40 (Ph_2PO), 87.03 (Ph_2POCH), 74.94, 70.55, 63.61, (CH_2CHCH_2), 41.37 (COCH_2), 29.41 ($\text{CH}_2)_{16}\text{CH}_3$, 28.98 ($\text{CH}_2)_{16}\text{CH}_3$, 25.87 ($\text{Si}(\text{C}(\text{CH}_3)_3)$), 14.11 ($\text{Si}(\text{C}(\text{CH}_3)_3)$), -5.41 ($\text{Si}(\text{CH}_3)_2$). IR 3400, 2924, 2853, 1718, 1463, 1438, 1360, 1252, 1186, 1119, 939, 837, 778, 747, 722, 694 cm^{-1} .

Preparation of 1-[2-hydroxy-3-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]propoxy]-1-(diphenylphosphinyl)-3,5-heptadien-2-one (3-34)

Compound **3-14** (895.9 mg, 1.70 mmol) was dissolved in 22 ml of anhydrous THF and cooled to -78°C . To this solution freshly prepared 0.5 M LDA (4.18 ml, 2.10 mmol) was added dropwise. The reaction was stirred at -78°C for 30 minutes. At this time 25 ml of saturated ammonium chloride was added, and it was allowed to warm to room temperature. The layers were separated and the aqueous layer was extracted with 3 x 25 ml Et_2O . The extracts were combined and washed with 2 x 50 ml H_2O and 1 x 50 ml brine. The extracts were dried over Na_2SO_4 , filtered, and the solvent removed *in vacuo*. Purification by flash chromatography using 96% CH_2Cl_2 / 4% MeOH as the eluting solvent gave 879.5 mg (98%) as a thick orangish oil. $^1\text{H NMR}$ (CDCl_3) δ 7.85-7.71 (m, 4H, Ph_2PO), 7.50-7.37 (m, 6H, Ph_2PO), 7.26-7.10 (m, 1H, olefinic), 6.57 (d, 0.5H, $J = 17.5$ Hz, diastereomer of $\text{COCH}=\text{C}$), 6.53 (d, 0.5H, $J = 17.9$ Hz, diastereomer of

COCH=C), 6.18-6.12 (m, 2H, olefinic), 4.94 (d, 0.5H, $J = 2.9$ Hz, diastereomer of Ph₂POCH), 4.86 (d, 0.5H, $J = 2.5$ Hz, diastereomer of Ph₂POCH), 3.71-3.38 (m, 5H, CH₂CHCH₂), 1.80-1.78 (d, 3H, $J = 6.8$ Hz, CH=CHCH₃) 0.76 (s, 9H, Si(CH₃)₃), 0.0 (s, 6H, Si(CH₃)₂). ¹³C NMR (CDCl₃) δ 194.3 (C=O), 144.69 (vinylic), 142.71 (vinylic), 132.01 (Ph₂PO), 130.49 (vinylic), 128.50 (Ph₂PO), 123.63 (vinylic), 87.8 (C₄), 85.9 (Ph₂POCH₂), 74.95, 70.40, 63.55 (CH₂CHCH₂), 25.81 (CH₃), 18.84 Si(CH₃)₃, 18.17 Si(CH₃)₃, -5.48 (Si(CH₃)₂). IR: 3360, 3000, 2950, 1650, 1600, 1550, 1450, 1190, 1100, 1050, 850 cm⁻¹. MS (CI) m/z (relative intensity) 515 (MH⁺, 5), 311 (35), 203 (100), 131 (10), 115 (20), 101 (15), 89 (20), 73 (65).

Attempted preparation of diphenethyl phosphate (3-37) from phosphorus oxychloride

Phosphorus oxychloride (3.29 g, 2.20 mmol) was dissolved in 6 ml of anhydrous benzene, cooled to -5°C and anhydrous pyridine (3.39 g, 4.30 mmol) was added. Next phenethyl alcohol (5.25 g, 4.3 mmol) was added at such a rate that the reaction temperature did not go above 5°C. Once the addition was complete, the reaction was refluxed for 30 minutes then stirred at room temperature for 5 hours. The white precipitate was filtered off and the solvent evaporated to give a brown oil. Distilled water (0.193 g, 10.7 mmol) and anhydrous pyridine (846 mg, 10.7 mmol) were added. The mixture was heated to 110°C and left for 12 hours. Water (25 ml) was added and the solution extracted with 3 x 25 ml CH₂Cl₂. The extracts were washed with 2 x 25 ml 10% HCl, 2 x 50 ml H₂O, and 1 x 50 ml brine. The extracts were dried over MgSO₄, filtered, and the solvents removed *in vacuo* to give a brown oil. Analysis GC-MS indicated a very small amount of product among a complex reaction mixture.

Preparation of diphenethyl hydrogen phosphite (3-39)

Phosphorus trichloride (1.37 g, 10.0 mmol) and phenethyl alcohol (3.67 g, 30.0 mmol) were simultaneously added to 3.5 of anhydrous chloroform at 40°C while the HCl gas produced was blown off by bubbling a stream of argon through the reaction mixture. The reaction was run at 40°C for 30 minutes then at room temperature for 1 hour. Finally the residual HCl was removed by heating at 45°C for 6 hours. The excess solvent was removed under reduced pressure. Purification by vacuum distillation at 4 mm Hg gave 2.50 g (86%) of **3-39** as a colorless liquid. ^1H NMR (CDCl_3) δ 7.31-7.17 (m, 4H, Ph), 4.20 (m, 4H, PO- CH_2), 3.92 (t, 4H, $J = 7.3$ Hz, CH_2Ph). ^{13}C NMR (CDCl_3) δ 136.92 (Ph), 128.94 (Ph), 128.55 (Ph), 126.79 (Ph), 65.46 (P-O- CH_2), 36.78 (CH_2Ph). IR 3029, 2960, 2899, 2432, 1605, 1498, 1455, 1388, 1260, 1062, 980, 751, 701 cm^{-1} . MS (CI) m/z (relative intensity) 291 (MH^+ , 50), 147 (10), 105 (100), 91 (40), 85 (62). The properties are identical to those previously described.¹¹³

Preparation of diphenethyl phosphate (3-37) from diphenethyl hydrogen phosphite

Diphenethyl hydrogen phosphite **3-39** (2.50 g, 8.60 mmol) was suspended in 6 ml of distilled water and cooled to 4°C. Next sodium bicarbonate (0.250 g, 3.0 mmol) was added and stirred vigorously. Potassium permanganate (0.65 g, 4.10 mmol) was added portion-wise until the pink color persisted. The reaction mixture was filtered by vacuum filtration through a pad of Celite. The pink filtrate was decolorized by boiling with activated charcoal. The solution was finally acidified with 12 N HCl and extracted with 4 x 150 ml chloroform. The extracts were washed with 3 x 200 ml H_2O and 1 x 200 ml brine and dried over MgSO_4 . The organic extracts were filtered and the solvent removed *in vacuo* to give 1.89 g (72%) of **3-37** as a colorless oil which crystallized slowly upon standing. No further purification was necessary. ^1H NMR (CDCl_3) δ 11.25 (s, 1H,

POH), 7.29-7.11 (m, 10 H, Ph), 4.04 (q, 4H, $J = 5.4$ Hz, POCH₃), 2.90 (t, 4 H, $J = 7.2$ Hz, CH₂Ph). ¹³C NMR (CDCl₃) δ 136.99 (Ph), 128.91 (Ph), 128.37 (Ph), 126.55 (Ph), 67.77 (POCH₂), 36.46 (CH₃Ph). IR: 3408, 3030, 2962, 2150, 1653, 1497, 1455, 1219, 1013, 885, 744, 700 cm⁻¹. MS (CI) m/z (relative intensity) 307 (45), 147 (18), 105 (100). The spectral properties are identical to those previously described.¹¹³

Preparation of 1-[[2-hydroxy-3-[di(phenethyloxy)phosphinoyloxy]propoxy]methyl]diphenylphosphine oxide (3-40)

Compound 3-27 (2.0 g, 6.90 mmol) was dissolved in 12 ml of anhydrous carbon tetrachloride. Diphenethyl phosphate (2.55 g, 8.30 mmol) was added, and the reaction was stirred at room temperature for 48 hours. The solvent was removed under reduced pressure leaving a colorless oil. Purification by flash chromatography using 91% CH₂Cl₂/9% isopropyl alcohol as the eluting solvent gave 1.80 g (44%) of 3-40 as a colorless oil. ¹H NMR (CDCl₃) δ 7.80-7.73 (m, 4H, Ph₂PO), 7.76-7.47 (m, 6H, Ph₂PO), 7.30-7.16 (m, 10H, CH₂Ph), 4.30 (d, 2H, $J = 4.0$ Hz, Ph₂POCH₂), 4.13 (q, 5H, $J = 6.4$ Hz, OCH₂CH₂Ph and CH), 3.82 (m, 2H, CH₂), 3.63 (m, 2H, CH₂), 2.92 (t, 4H, $J = 7.0$ Hz, OCH₂CH₂Ph), 1.67 (s, 1H, OH). ¹³C NMR (CDCl₃) δ 136.92 (Ph₂PO), 132.77 (Ph₂PO), 131.34 (Ph₂PO), 128.91-126.62 (CH₂Ph), 74.64, 72.25, 70.88, (CH₂CHCH₂), 69.06 (C₄), 68.13 (CH₂Ph). IR 3334, 3059, 3025, 2957, 2898, 1604, 1590, 1497, 1453, 1437, 1266, 1177, 1122, 1093, 1016, 734, 697 cm⁻¹. MS (CI) m/z (relative intensity) 595 (MH⁺, 40), 577 (50), 515 (55), 473 (100), 411 (100), 393 (50), 369 (40), 289 (90), 217 (100), 203 (100), 147 (90).

Preparation of 1-[[2-octadecylcarbonyloxy-3-[di(phenethyloxy)phosphinoyloxy]propoxy]methyl]diphenylphosphine oxide (3-15)

Compound 3-40 (462.5 mg, 0.779 mmol) was dissolved in 5 ml of anhydrous dichloromethane and stearic acid (332 mg, 1.17 mmol) was added. Next

dicyclohexylcarbodiimide (241.0 mg, 1.17 mmol) and a catalytic amount of 4-pyrrolidinopyridine (11.0 mg, 0.075 mmol) were added. Then reaction was stirred at room temperature for 3 hours. The solvent was removed *in vacuo* to leave a white residue. The residue was suspended in ethyl acetate and filtered. The solvent was removed under reduced pressure to leave a colorless oil. Purification by flash chromatography eluting with 80% EtOAc / 20% CH₂Cl₂ gave 569 mg (85%) of **3-15** as a colorless oil. ¹H NMR (CDCl₃) δ 7.90-7.80 (m, 4H, Ph₂PO), 7.59-7.48 (m, 6H, Ph₂PO), 7.37-7.20 (m, 10H, CH₂Ph), 5.12 (m, 1H, CH), 4.31 (m, 2H, Ph₂POCH₂), 4.17 (m, 4H, OCH₂CH₂Ph), 3.94 (m, 2H, CH₂), 3.69 (d, 2H, J = 6.9 Hz, CH₂), 2.97 (t, 4H, J = 7.4 Hz, CH₂CH₂Ph), 2.26 (t, 2H, J = 7.6 Hz, COCH₂), 1.46 (m, 2H, COCH₂CH₂), 1.27 (s, 28H, stearic methylenes), 0.97 (t, 3H, J = 9.0 Hz, (CH₂)₁₆CH₃). ¹³C NMR (CDCl₃) δ 172.51 (C=O), 136.70 (Ph₂PO), 132.01-131.09 (Ph₂PO), 128.73-126.49 (CH₂Ph), 71.30-68.65 (CH₂CHCH₂), 67.95 (CH₂Ph), 65.02 (Ph₂POCH₂), 36.42 (PHCH₂), 29.44 (CH₂)₁₆CH₃, 13.88 (CH₂)₁₆CH₃. IR 3448, 3060, 3028, 2923, 2852, 1739, 1497, 1466, 1454, 1437, 1273, 1181, 1121, 1051, 1016, 745, 697 cm⁻¹. MS (CI) *m/z* (relative intensity) 861 (MH⁺, 50), 555 (10), 525 (50), 411 (60), 285 (70), 271 (70), 217 (100), 203 (100), 147 (70).

Preparation of 1-[[2-(2,4-hexadienylcarbonyloxy)-3-[di(phenethyloxy)-phosphinoyloxy]propoxy]methyl]diphenylphosphine oxide (3-16)

Compound **3-40** (942.0 mg, 1.60 mmol) was dissolved in 11.0 ml of anhydrous THF and sorbic acid (267 mg, 2.40 mmol) was added. Next dicyclohexylcarbodiimide (654 mg, 3.20 mmol) and a catalytic amount of 4-pyrrolidinopyridine (23.0 mg, 0.160 mmol) were added. The reaction was stirred at room temperature for 24 hours. The solvent was removed *in vacuo* leaving an orangish-brown residue, which was taken up in ethyl acetate and filtered. The solvent was again removed under reduced pressure to leave a

thick brownish-orange oil. Purification by flash chromatography using 94% CH₂Cl₂ / 6 % isopropyl alcohol as the eluting solvent gave 700 mg (64%) of **3-16** as an orangish oil. ¹H NMR (CDCl₃) δ 7.89-7.73 (m, 4H, Ph₂PO), 7.60-7.47 (m, 6H, Ph₂PO), 7.36-7.20 (m, 11H, CH₂Ph and olefinic), 6.19 (m, 2H, olefinic), 5.75 (d, 1H, J = 20.6 Hz, COCH=C), 5.18 (m, 1H, CH), 4.32 (m, 2H, Ph₂POCH₂), 4.15 (m, 4H, OCH₂CH₂Ph), 3.96 (m, 2H, CH₂), 3.72 (d, 2H, J = 6.8 Hz, CH₂), 2.95 (t, 4H, J = 7.2 Hz, OCH₂CH₂Ph), 1.92 (d, 3H, J = 6.5 Hz, CH=CHCH₃). ¹³C NMR (CDCl₃) δ 165.89 (C=O), 145.94 (vinyl), 140.13 (vinyl), 136.72 (vinyl), 132.02-131.04 (Ph₂PO), 129.39-126.47, (CH₂Ph), 117.75 (vinyl), 72.82-68.64 (CH₂CHCH₂), 67.87 (CH₂Ph), 65.12 (C₄), 36.25 (PHCH₂), 18.43 (CH=CHCH₃). IR 3060, 3028, 2960, 1714, 1643, 1616, 1437, 1242, 1187, 1015, 868, 800, 744, 698 cm⁻¹. MS (CI) *m/z* (relative intensity) 689 (MH⁺, 1), 595 (2), 473 (20), 411 (6), 259 (20), 217 (20), 203 (35), 105 (100), 91 (30).

Preparation of 1-[2-hydroxy-3-[di(phenethyloxy)phosphinoyloxy]propoxy]-1-(diphenylphosphinyl)-2-nonadecanone (3-41)

Compound **3-15** (152 mg, 0.177 mmol) was dissolved in 1.9 ml of anhydrous THF and cooled to -78°C. 1.5 M LDA monotetrahydrofuran (0.170 ml, 0.265 mmol) was added dropwise. It was stirred at -78°C for 30 minutes then 2 ml of saturated NH₄Cl was added, and the reaction was allowed to warm to room temperature. The layers were separated and the aqueous layer was extracted with 3 x 25 ml ether. The organics were combined and washed with 2 x 25 ml H₂O and 2 x 25 ml brine. The extracts were dried over MgSO₄, filtered, and the solvents removed to give a colorless oil. Purification by flash chromatography eluting with 80% EtOAc / 20 % CH₂Cl₂ gave 51 mg (34%) of **3-41** as a colorless oil. ¹H NMR (CDCl₃) δ 7.88-7.80 (m, 4H, Ph₂PO), 7.57-7.41 (m, 6H, Ph₂PO), 7.33-7.15 (m, 10H, CH₂Ph), 4.86 (d, 0.5H, J = 3.2 Hz, diastereomer of Ph₂POCH), 4.78 (d, 0.5H, J = 3.0 Hz, diastereomer of Ph₂POCH), 4.14 (m, 4H,

OCH₂CH₂Ph), 3.82-3.48 (m, 5H, CH₂CHCH₂), 2.92 (t, 4H, J = 6.9 Hz, OCH₂CH₂Ph), 2.75 (m, 1H, diastereomer of COCH₂), 2.56 (m, 1H, diastereomer of COCH₂), 1.42 (m, 2H, COCH₂CH₂), 1.26 (s, 28H, (steric methylenes), 0.88 (m, unresolved, 3H, (CH₂)₁₆CH₃). ¹³C NMR (CDCl₃) δ 206.3 (C=O), 136.92 (vinyl) 132.45-131.51 (vinyl and Ph₂PO), 128.92-118.46 (CH₂Ph), 126.69 (vinyl), 87.3, 86.2, 74.19 (CH₂CHCH₂), 69.05 (Ph₂POCH₂), 68.10 (CH₂Ph), 41.29 (COCH₂), 36.64 (POCH₂), 29.34 ((CH₂)₁₆CH₂), 14.04 (CH₂)₁₆CH₃. IR 3364, 3062, 3029, 2923, 2853, 2238, 1963, 1817, 1715, 1605, 1592, 1497, 1468, 1378, 1120, 909, 732, 697, 645 cm⁻¹. MS (CI) *m/z* (relative intensity) 861 (MH⁺, 2), 843 (4), 739 (16), 643 (10), 587 (20), 555 (100), 483 (100), 219 (100), 203 (100), 147 (60), 122 (50).

Preparation of 1-[2-hydroxy-3-[di(phenethyloxy)phosphinoyloxy]propoxy]-1-(diphenylphosphinyl)-3,5-heptadien-2-one (3-42)

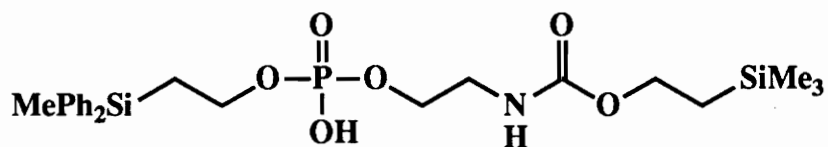
Compound **3-16** (120 mg, 0.174 mmol) was dissolved in 1.9 ml of anhydrous THF and cooled to -78°C. 1.5 M LDA (monotetrahydrofuran) (0.112 ml, 0.174 mmol) was added dropwise and stirred at -78°C for 45 minutes. Saturated NH₄Cl (2 ml) was added, and the reaction was allowed to warm to room temperature. The layers were separated, and the aqueous layer was extracted with 3 x 25 ml ether. The organic layers were combined and washed with 2 x 25 ml H₂O and 1 x 25 ml brine. The extracts were dried over MgSO₄, filtered, and the solvents removed *in vacuo* giving a brownish oil. Purification by PTLC eluting with EtOAc gave 30 mg (25 %) of **3-42** as a brown oil. ¹H NMR (CDCl₃) δ 7.77-7.68 (m, 4H, Ph₂PO), 7.44-7.35 (m, 6H, Ph₂PO), 7.20-7.06 (m, 11H, CH₂Ph and olefinic), 6.61 (d, 0.5 H, J = 15.2 Hz, diastereomer of COCH=C), 6.57 (d, 0.5 H, J = 15.2 Hz, diastereomer of COCH=C), 6.19 (m, 2H, olefinic), 4.87 (d, 0.5 H, J = 3.2, diastereomer of Ph₂POCH), 4.81 (d, 0.5H, J = 3.6 Hz, diastereomer of Ph₂POCH), 4.03 (m, 4H, OCH₂CH₂Ph), 3.74-3.62 (m, 5H, CH₂CHCH₂), 2.92 (t, 4H,

$J = 7.2$ Hz, $\text{OCH}_2\text{CH}_2\text{Ph}$), 1.85 (d, 3H, $J = 5.0$ Hz, $\text{CH}=\text{CHCH}_3$). ^{13}C NMR (CDCl_3) δ 145.05 (vinyl) 143.10 (vinyl), 132.06-131.92 (Ph_2PO), 130.61 (vinyl), 129.05-125.58, (CH_2Ph), 126.78 (vinyl), 83.50, 74.50, 73.47 (CH_2CHCH_2), 68.24 (CH_2Ph), 36.77 (POCH_2), 29.71 ($\text{CH}=\text{CHCH}_3$). The carbonyl signal in the ^{13}C spectrum was not seen due to a poor signal to noise ratio. MS (CI) m/z (relative intensity) 689 (MH^+ , 72), 671 (10) 606 (15), 567 (100), 411 (30), 383 (35), 367 (80), 311 (100), 289 (35), 219 (40), 203 (60), 105 (30).

IV. Synthesis of a Protected Phosphodiester

4.1 Introduction: Explanation of Phosphodiester Choice.

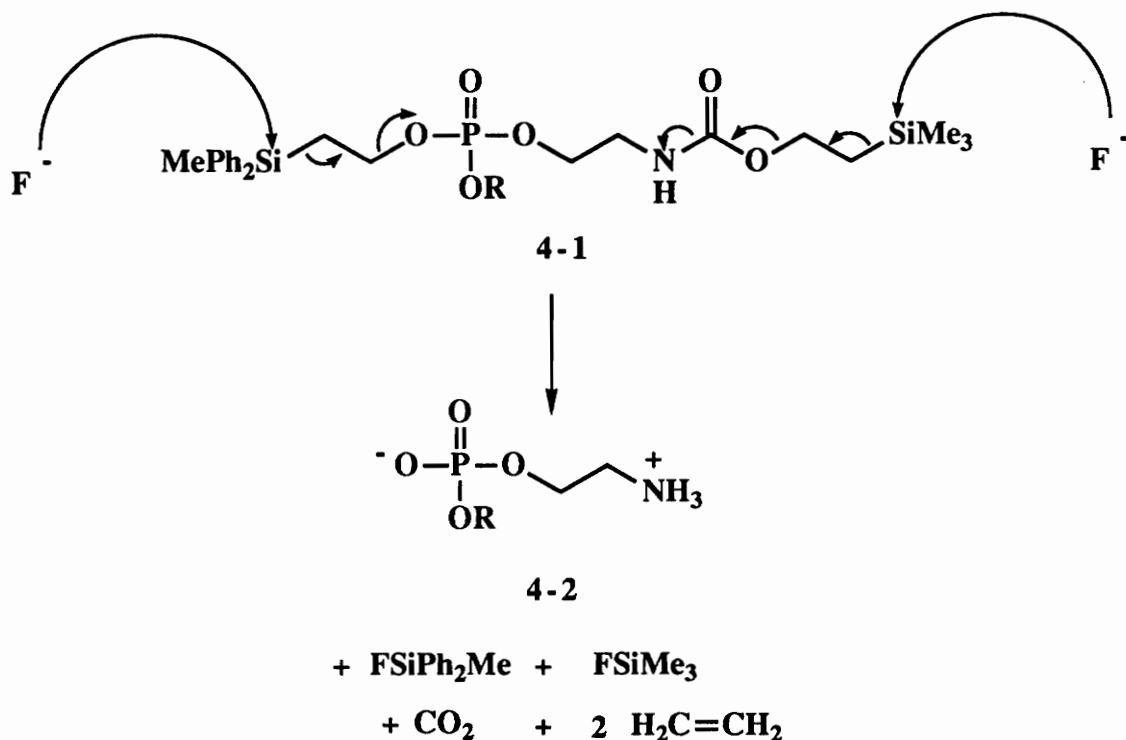
Phosphodiesters occupy an important position among organic derivatives of phosphoric acid because they are essential to two important classes of biochemical compounds: polynucleotides and phospholipids. Plasmalopentaene-12, a phospholipid, is a molecule of particular challenge in regards to the synthesis of its phosphate ester. This is because of the inherent instability of the molecule due to the pentaenyl ether. As previously mentioned, plasmalopentaene-12, is air, acid, base, and light sensitive. The pentaenyl ether is particularly susceptible to attack by nucleophiles. The enol ether functionality of plasmalopentaene-12 is stable however to one nucleophile, namely the fluoride ion. This important piece of information proved to be very useful in the design of a suitably protected phosphodiester which can be used for the synthesis of plasmalopentaene-12. The phosphodiester needed must be protected with groups that will not interfere with the generation of the pentaenyl ether system upon deprotection. The protecting groups must therefore be silyl protecting groups which can easily be deprotected using fluoride ion. With this information in hand the following phosphodiester **4-1** or some analog of it was proposed as the protected phosphate needed for the synthesis of plasmalopentaene-12.



4-1

Attack by fluoride on the silicon atoms of **4-1** would fragment the molecule into two different silyl fluorides, carbon dioxide, two molecules of ethylene, and phosphoryl

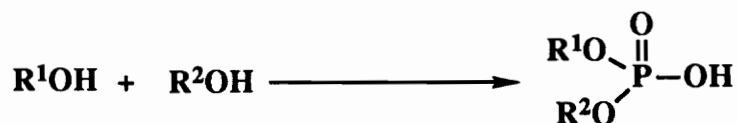
ethanolamine **4-2** (Scheme 4-1). The proposed protected phosphotriester would enable the phosphorylethanolamine to be generated in the last step of the synthesis with the pentaenyl ether remaining intact.



Scheme 4-1

4.2 Methods of Phosphoester Synthesis.

The preparation of simple phosphodiester is generally a simple task, but when the systems become more complex the syntheses are no longer trivial. These problems are further complicated when the phosphodiester are made unsymmetrical by using two different alcohols (Scheme 4-2).

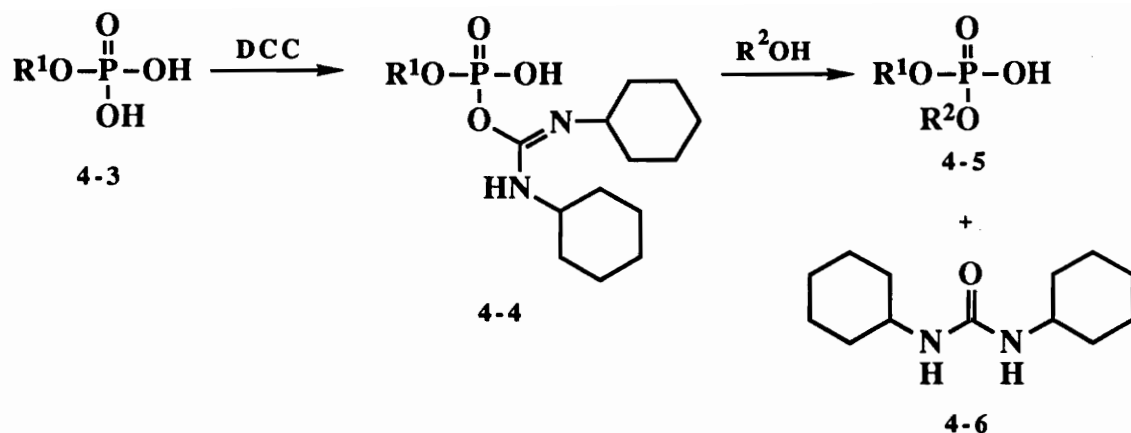


Scheme 4-2

Most phosphodiester syntheses follow either of two methodologies. In the first, no additional activating reagent is needed i.e. the phosphorylating agent already contains leaving groups needed for the generation of the P-OR¹ and P-OR² bonds. In the second, an activating reagent must be introduced to generate the second P-OR bond.

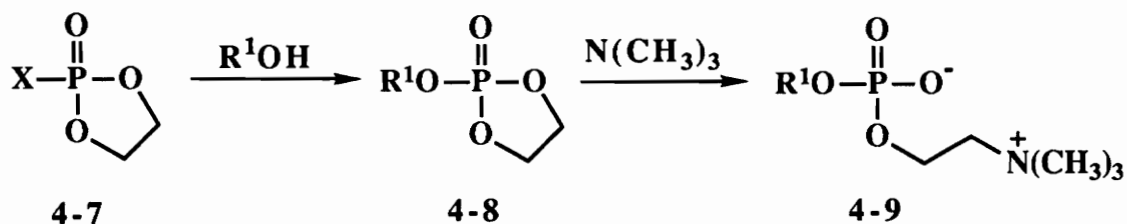
These methodologies can be further subdivided into three categories according to the presence or absence of and nature of the protecting groups present at the phosphorus atom. In the first, no protecting group is used. The second uses a protecting group in which the P-O bond is cleaved upon deprotection, and the third involves a protecting group which is deprotected by O-C bond fission. The third category offers the advantage of preserving the integrity of the P-O bonds of the diester upon C-O cleavage whereas the second category, in which the P-O bond is cleaved, cannot guarantee the integrity of the diester.

There are several strategies for the formation of phosphodiesters. A general example of a useful method of synthesizing phosphodiesters is shown in Scheme 4-3. The phosphomonoester **4-3** is reacted with dicyclohexylcarbodiimide to convert the OH into a good leaving group giving **4-4**. This is then reacted with R²OH giving the phosphoester diester **4-5** and dicyclohexylurea **4-6** as a byproduct.



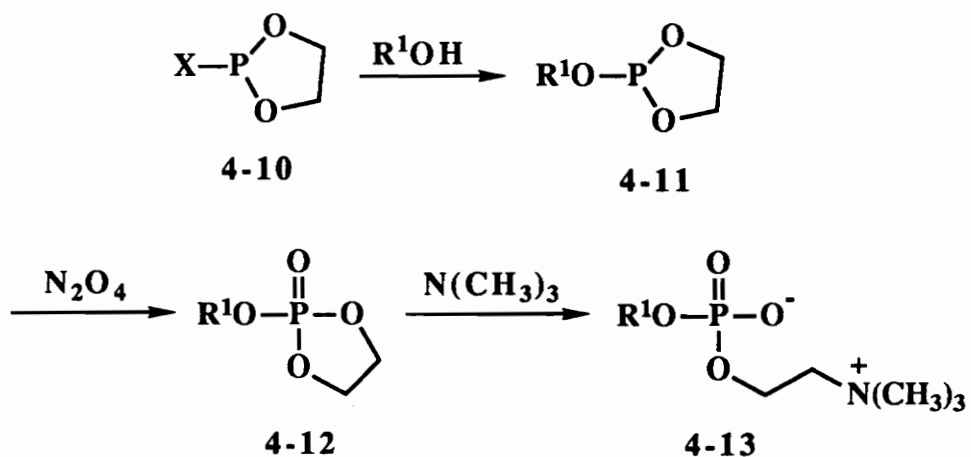
Scheme 4-3

Another useful methodology (Scheme 4-4) involves the use of two protecting groups connected as part of a ring 4-7. This phosphodiester synthon 4-8 can then be reacted with an appropriate reagent which will attack the methylene on the ring in an $\text{S}_{\text{N}}2$ fashion causing ring opening of the dioxaphospholane. This one-step reaction generates the desired alkyl groups plus the necessary acidic group 4-9.



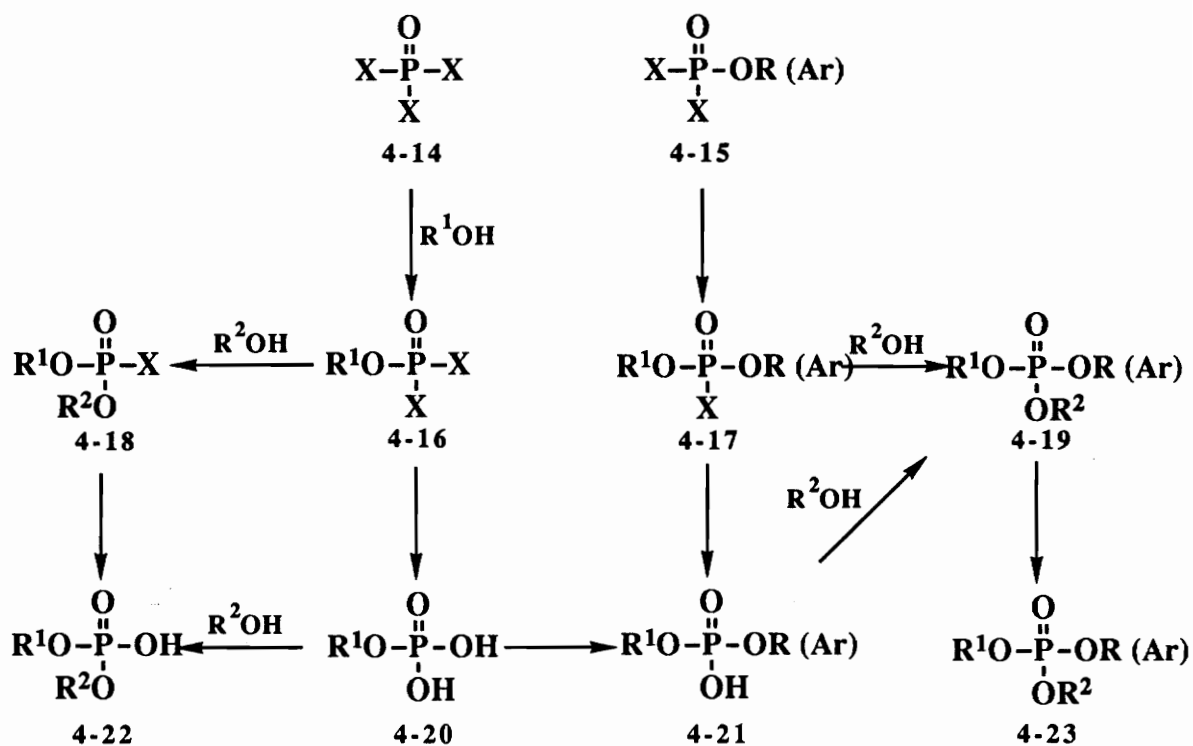
Scheme 4-4

Another strategy involves the use of tri-coordinate phosphorus (Scheme 4-5). In this approach the cyclic intermediate 4-10 is attacked by $\text{R}'\text{OH}$ at the tri-coordinate phosphorus to give 4-11. Next the use of an oxidant generates the necessary pentavalent phosphorus which can be ring opened in the same fashion as shown in Scheme 4-4 to give 4-13. This method may only be used when functional groups are present which are oxidatively stable.



Scheme 4-5

From the available strategies presented thus far, a few basic mechanistic patterns become evident which are summarized in Scheme 4-6.



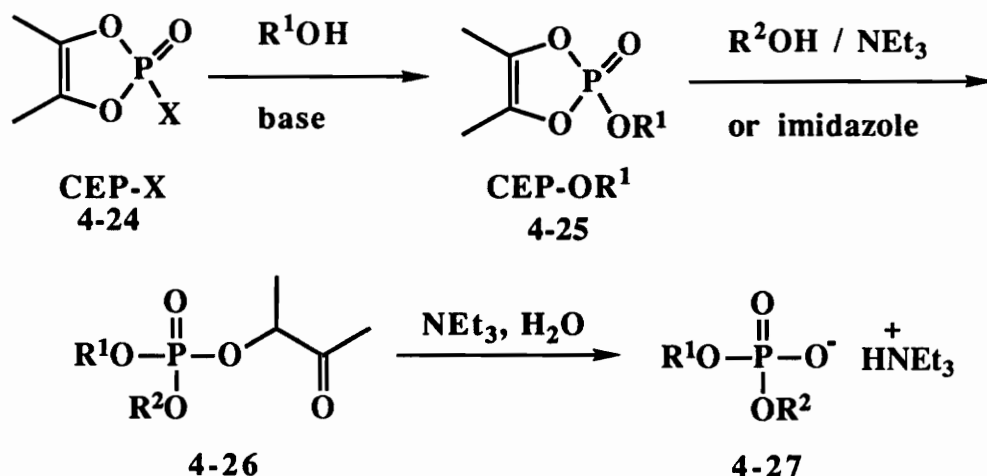
Scheme 4-6

Compounds **4-14** and **4-15** are phosphorylating reagents which lack the P-OR¹ and P-OR² bonds. X is a leaving group that is lost when the tetra-coordinate phosphorus undergoes a nucleophilic displacement. X may be the same leaving group or a different one all together. The functional groups OR or OAr are protecting groups which will eventually be converted to P(O)OH during the synthesis.

Initially R'OH is added to either phosphorylating reagent to give either **4-16** or **4-17**. These can then be hydrolyzed to give **4-20** and **4-21** then reacted with R²OH or reacted first with R²OH to give **4-18** or **4-19** then hydrolyzed to **4-22** and **4-23**. Although some syntheses do not appear to follow these mechanistic patterns and syntheses, a closer examination reveals the contrary. It was these presented strategies that provide the basic strategy for the synthesis of the protected phosphate **4-1**.

4.3 Phosphodiester Synthesis Via the Cyclic Enediol Phosphoryl Method.

Perhaps one of the most useful methodologies developed for the syntheses of phosphodiesters is via cyclic enediol phosphoryl (CEP) derivatives. Several CEP-X phosphorylating reagents where X is a good leaving group have been developed. These CEP-X reagents can then be utilized for the synthesis of unsymmetrical phosphodiesters as outlined below in Scheme **4-7**.



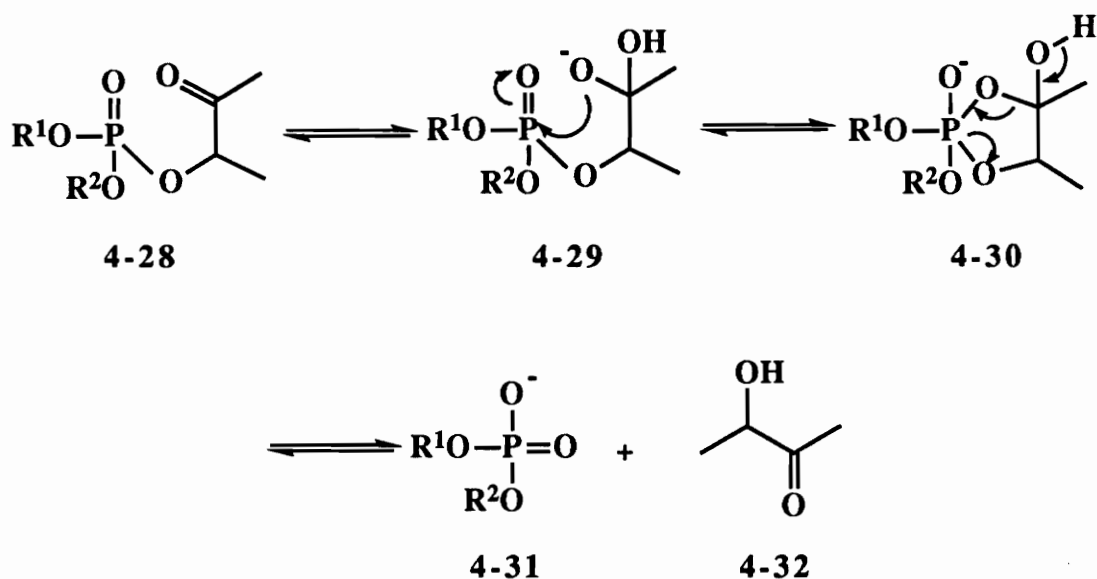
Scheme 4-7

In step 1 of Scheme 4-7, the alcohol R^1OH is phosphorylated by CEP-X 4-24 to give the cyclic triester CEP-OR¹ 4-25. This reaction proceeds with nearly complete ring retention and in quantitative yield. Initially γ -collidine was used as the base,^{115,116} but it was found that this hindered amine does not provide the nucleophilic catalysis required for optimum results in the following step, and imidazole was found to be the base of choice.

The second step, carried out in the presence of triethylamine or imidazole,^{117,118} is the phosphorylation of R^2OH by CEP-OR¹ to give the dialkyl phosphoacetoin 4-26. The amines play two important roles in this reaction. First, the rate of reaction of the alcohol, R^2OH , with the cyclic triester, CEP-OR¹, is increased. This allows a wider use of aprotic solvents since the rate of phosphorylation is solvent dependent. The use of polar-aprotic solvents causes a decrease in reaction rate. Second, the extent of ring opening in the reaction of CEP-OR¹ with alcohols is enhanced by use of these amines. This is crucial because ring retention leads to transesterification which then ultimately lends itself to symmetrical phosphodiester as shown below.



The third step of the CEP method is hydrolysis of the dialkylphosphoacetoin to the phosphodiester **4-31**. The hydrolysis is mechanistically depicted in Scheme 4-8. The hydrolysis is usually carried out in acetonitrile/water or pyridine/water solvent mixture using two equivalents of triethylamine or sodium carbonate as the base. This gives the final product as a salt of the phosphodiester **4-31**.

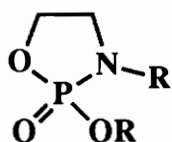


Scheme 4-8

The CEP method offers the advantage that the phosphodiester may be produced in a one-step synthesis where no intermediates are isolated, although the CEP-OR¹ intermediate or the dialkyl phosphoacetoin triester may be isolated. When utilizing the CEP method, the more hindered alcohol is used as R¹OH, and the least hindered alcohol as R²OH. This leads to a lower degree of transesterification.¹¹²

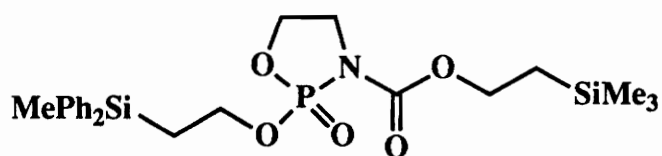
4.4 Initial Synthetic Attempt at the Protected Phosphorylethanolamine: An Electrophilic Synthon.

1,3,2-oxazaphospholidin-2-ones **4-33** have recently been employed in the synthesis of phospholipids.¹¹⁹ They have also been used to study the biological function of water soluble phosphorylethanolamine derivatives found in helminths.¹²⁰ The phosphorus atom of the oxazaphospholidinones is highly electrophilic and is readily attacked by alcohols and is easily opened up to give phosphorylethanolamines.

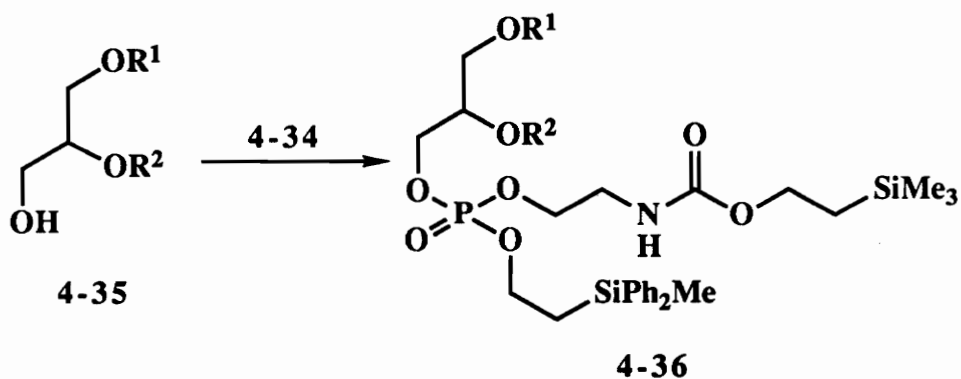


4-33

This information coupled with the fact that cyclic phosphorylating reagents are more reactive than their acyclic counterparts¹²¹ makes compounds of this type highly desirable targets as phosphorylating reagents needed for the synthesis of plasmalopentaene-12. The envisioned phosphorylating reagent **4-34** (shown below) could readily be attacked by a functionalized glycerol derivative **4-35** at the phosphorus atom (Scheme 4-9). This would then cleave the highly labile P-N bond and open the ring giving rise to the needed phosphotriester **4-36**. The protected phosphotriester can then be deprotected, as earlier described, at a later stage in the synthesis thus generating plasmalopentaene-12.



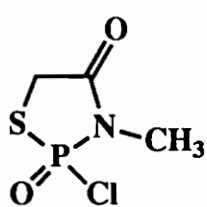
4-34



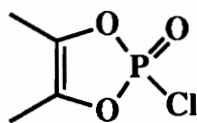
Scheme 4-9

4.4.1 Synthesis of the Protected Phosphorylethanolamine From Dichloromethoxyphosphine.

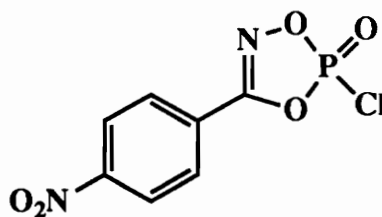
Recently, Ugi *et al.* reported a computer-aided search of potential cyclic phosphorylating reagents.¹²² Two hundred seventy-eight candidates were identified and six of these were tested as phosphorylating reagents for the synthesis of oligonucleotides and related compounds. Only one compound, 2-chloro-2,4-dioxo-3-methyl-1,3,2-thiazaphospholidine 4-37 proved effective as a phosphorylating reagent. Some others include the following 4-38 through 4-41.



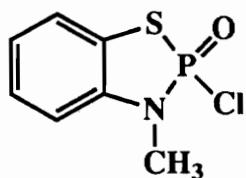
4-37



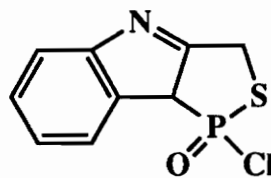
4-38



4-39

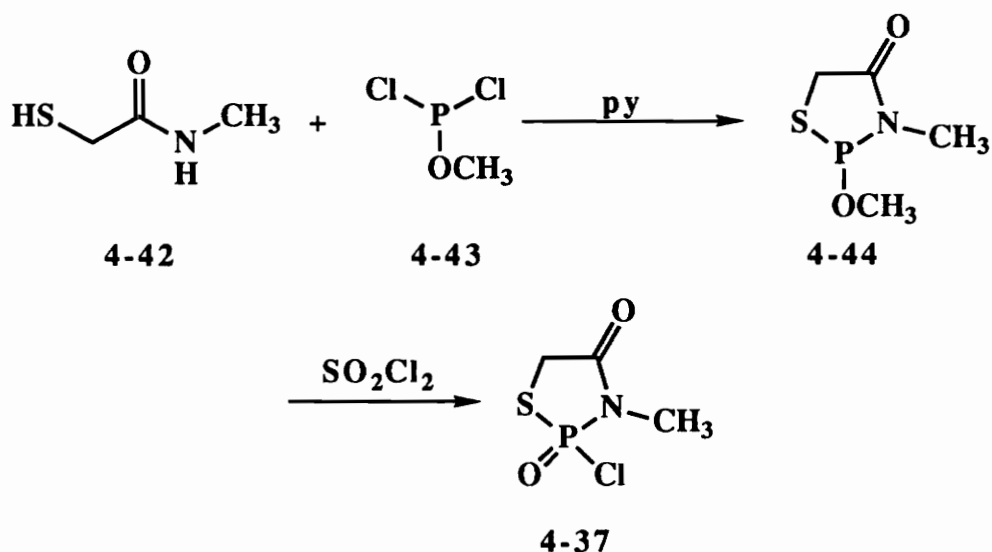


4-40



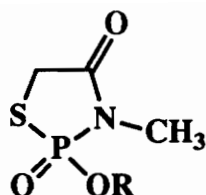
4-41

The synthesis of **4-37** by Ugi *et al.*¹²³ as shown in Scheme 4-10 could conceivably be extended to the synthesis of the desired oxazaphospholidinone **4-34**. The tri-coordinate phosphine **4-43** is cyclized to the phospholidine **4-44** using the mercaptoacetamide **4-42**. This is then reacted with sulfonyl chloride to give the target compound **4-37**.



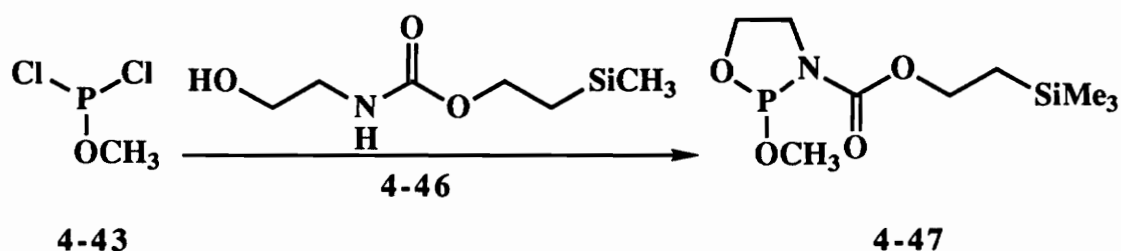
Scheme 4-10

The reaction of **4-37** with an alcohol then generates the alkoxyphospholidinone **4-45**. Careful examination of this compound reveals that it is structurally very similar to compound **4-34**. It was this observation that led to this synthetic route as the first logical entry point for the synthesis of the target oxazaphospholidinone **4-34**.

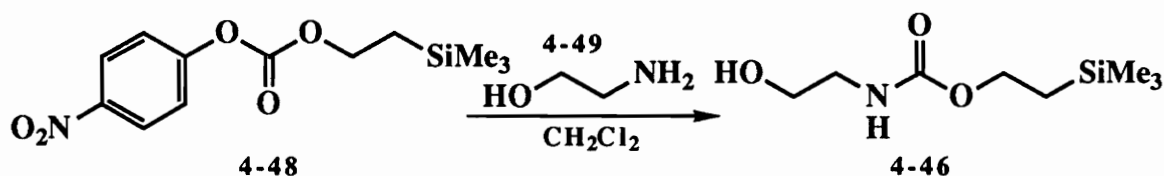


4-45

Dichloromethoxyphosphine 4-43¹²⁴ was reacted with the carbamate 4-46 (Scheme 4-11) which was prepared by reacting 2-(trimethylsilyl)ethyl-*p*-nitrophenyl carbonate 4-48 with ethanolamine 4-49 as shown below in Scheme 4-12.



Scheme 4-11

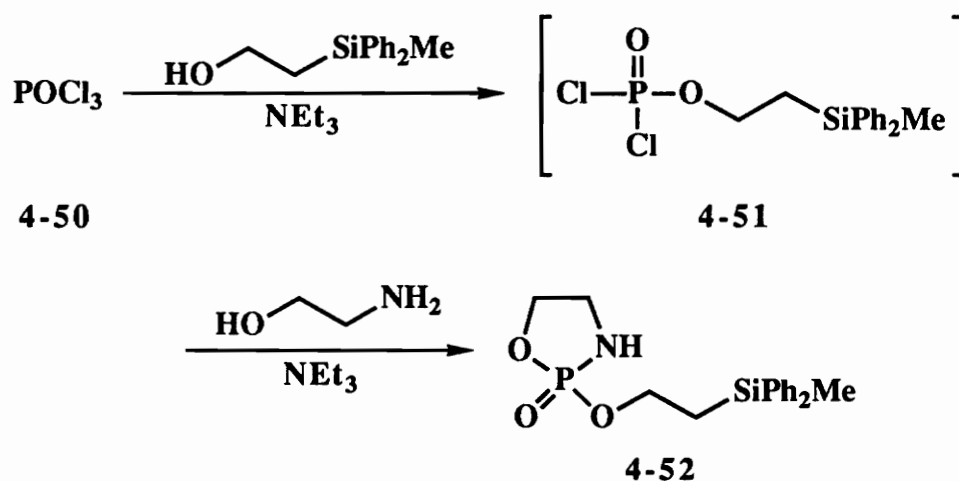


Scheme 4-12

It was not clear if the reaction of the carbamate 4-46 with the phosphine 4-43 gave the methoxyphosphine 4-47 (Scheme 4-11). It was difficult to follow the course the reaction by TLC since the product was not UV active and was not sensitive to any spray reagents used including those specific for phosphorus containing compounds. It was thus decided that this synthesis was not worthy of further pursuit. Consequently, another synthetic scheme for the synthesis of 4-34 was developed.

4.4.2 Synthesis of the Protected Phosphoryethanolamine 4-34 From Phosphorus Oxychloride.

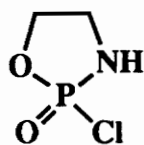
Many syntheses of phosphoesters use phosphorus oxychloride 4-50 as the phosphorylating reagent. This molecule provides a convenient starting point for the synthesis of 4-34 and was indeed used for this purpose. Following the procedure of Gibbons *et al.*, a slight excess of phosphorus oxychloride 4-50 was reacted with diphenylmethylsilyl ethanol in the presence of triethylamine under anhydrous conditions to give the phosphodichloridate 4-51, which was not isolated (Scheme 4-13).



Scheme 4-13

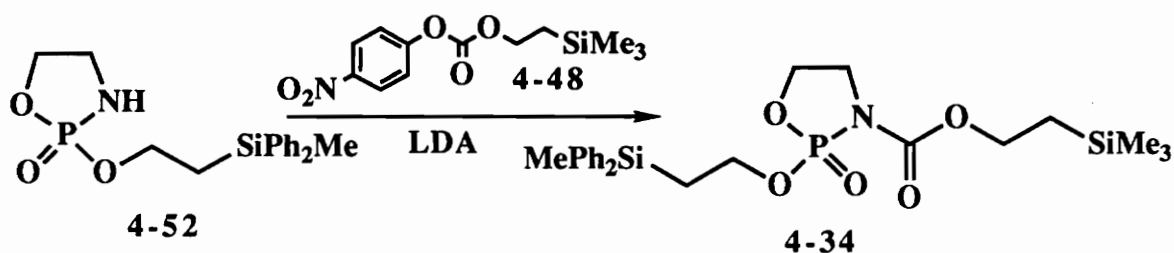
Instead the phosphodichloridate 4-51 was reacted in situ with ethanolamine in the presence of triethylamine to give the oxazaphospholidinone 4-52. The diphenylmethylsilyl ethanol was added first for two reasons. First, diphenylmethylsilyl ethanol contains a chromophore. This simplifies visual detection of reaction products on TLC. Second, by reacting a primary amino alcohol with phosphorus oxychloride as the first step would generate the cyclic product 4-53. Gibbons *et al*¹²⁵ have shown that since the

phosphoramidate proton is acidic, the presence of triethylamine causes extensive polymerization of **4-53**.



4-53

Oxazaphospholidinone **4-52** is easily purified by flash chromatography and once pure, the acidic phosphoramidate can be alkylated to complete the synthesis of **4-34**. Lithiating **4-52** with lithium diisopropyl amide and subsequent quenching of the anion with 2-(trimethylsilyl)ethyl-*p*-nitrophenyl carbonate **4-48** should then give oxazaphospholidinone **4-34** as shown in Scheme 4-15.

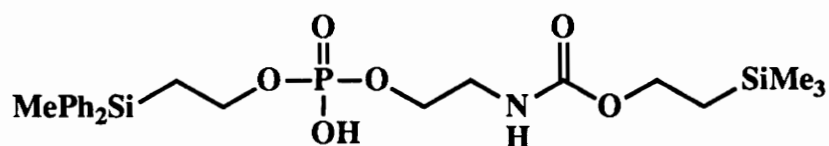


Scheme 4-14

Unfortunately the reaction did not work, possibly due to complications from the *p*-nitrophenoxide by-product. It was felt, however, that this methodology may not be the best suited for accomplishing the overall synthesis of plasmalopentaene-12, so it was therefore decided to more actively pursue the synthesis of the nucleophilic phosphate **4-1**.

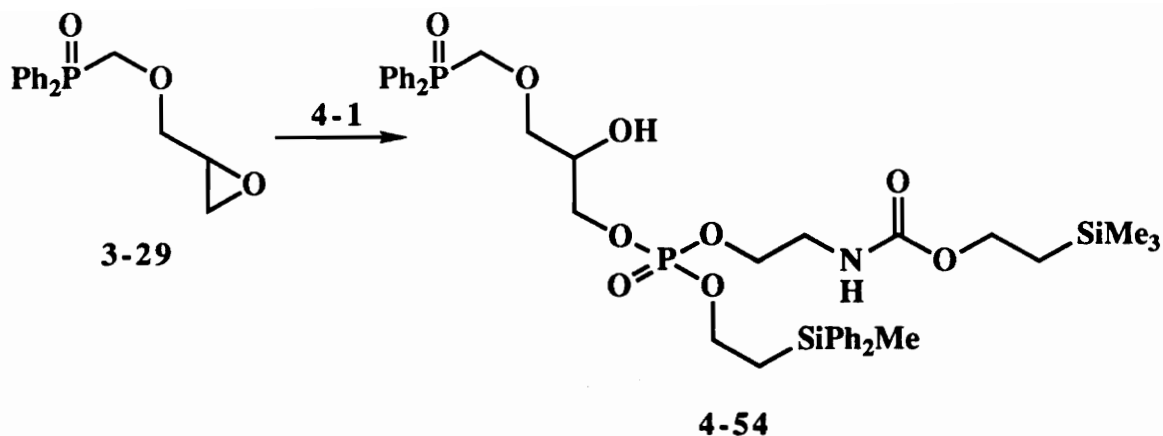
4.5 Synthesis of a Nucleophilic Protected Phosphorylethanolamine.

Previously, it was mentioned that several classes of biologically important compounds, such as nucleic acids and phospholipids, are diesters of phosphoric acid. Consequently, the conversion of two alcohols into a phosphodiester has been of great interest to the organic chemist for many years.^{126,127,128,129,130} Despite the many recent advances, there remains a need for more versatile phosphorylation procedures. The best method available to date is the method of phosphorylation via the previously discussed cyclic enediol phosphoryl (CEP) method.¹³¹ This was found to be the method of choice for the synthesis of a nucleophilic protected phosphorylethanolamine **4-1**.



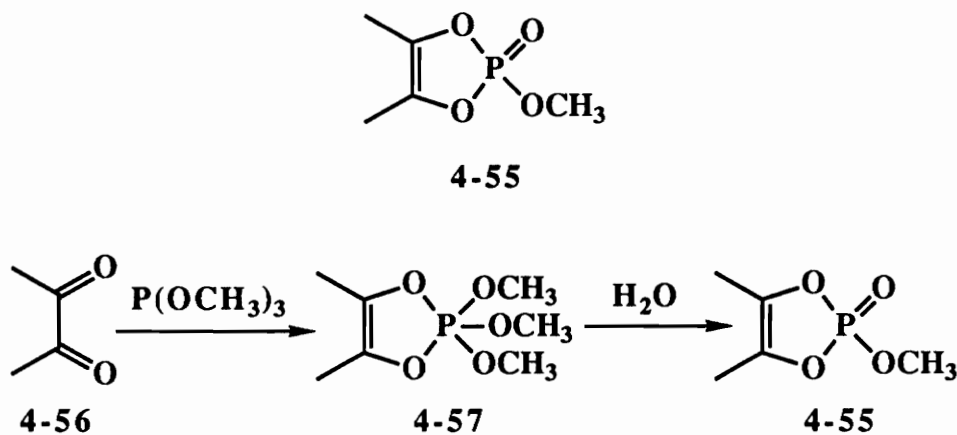
4-1

It was envisioned that phosphodiester **4-1** would ring-open epoxides in a nucleophilic fashion as previously observed in Schemes 3-15 and 3-24. This would then place the phosphorylethanolamine in the correct position needed to synthesize plasmalopentaene-12 (Scheme 4-15).



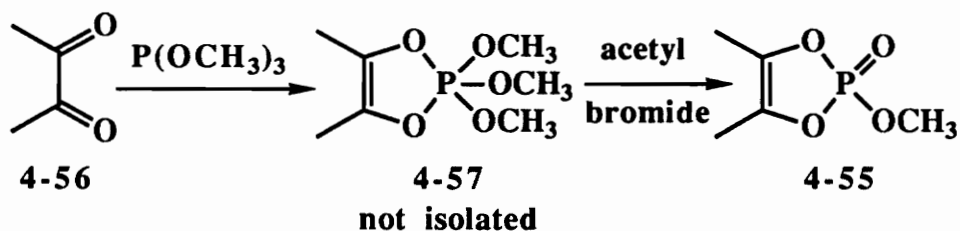
Scheme 4-15

The synthesis of 4-1 follows the standard procedure of Ramirez *et al.*¹³² CEP-OCH₃, 4-55, was first prepared by Ramirez and co-workers in low yield from the hydrolysis of oxyphosphorane 4-57¹³³ (Scheme 4-16).



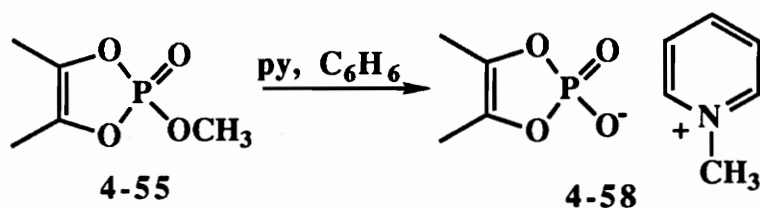
Scheme 4-16

Since then a better procedure was developed by Ramirez in which 2,3-butanedione 4-56 was reacted with trimethylphosphite to give oxyphosphorane 4-57 which is not isolated, but reacted directly in an Arbuzov reaction with acetyl bromide to give CEP-OCH₃ 4-55 in high yield (Scheme 4-17).



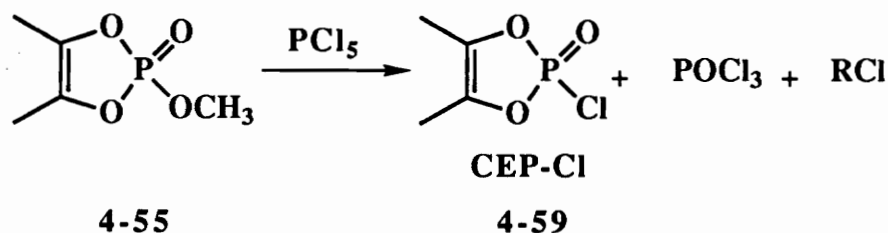
Scheme 4-17

Methyl acetoin enediol cyclophosphate CEP-OCH₃ **4-55** is then readily converted into the N-methylpyridinium salt of the CEP-O⁻ anion **4-58**^{116,117,133} (Scheme 4-18). This salt was produced in high yield and is convenient because it can be made in large quantities and when stored in dry conditions is stable for long periods of time.



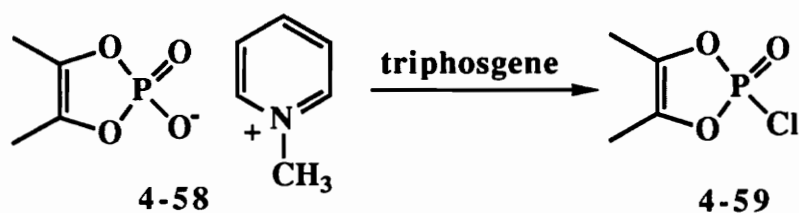
Scheme 4-18

The N-methylpyridinium salt was then converted into CEP-Cl **4-59**. Ramirez first prepared this compound by reacting **4-55** with phosphorus pentachloride¹³⁴ (Scheme 4-19). The reported yields were moderate, but the major drawback is that purification of **4-59** is difficult since the boiling points of **4-55**, **4-59** and PCl₅ are similar.



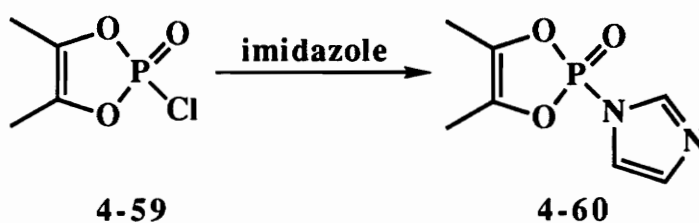
Scheme 4-19

A better method shown in Scheme 4-20 is the reaction of the N-methyl-pyridinium salt **4-58** with triphosgene to give CEP-Cl **4-59** in high yield. Initially, Ramirez called for the use of phosgene,¹³³ but due to the hazards and toxicity of phosgene, triphosgene was used in its place. This gave equal if not superior results to those reported in the literature.



Scheme 4-20

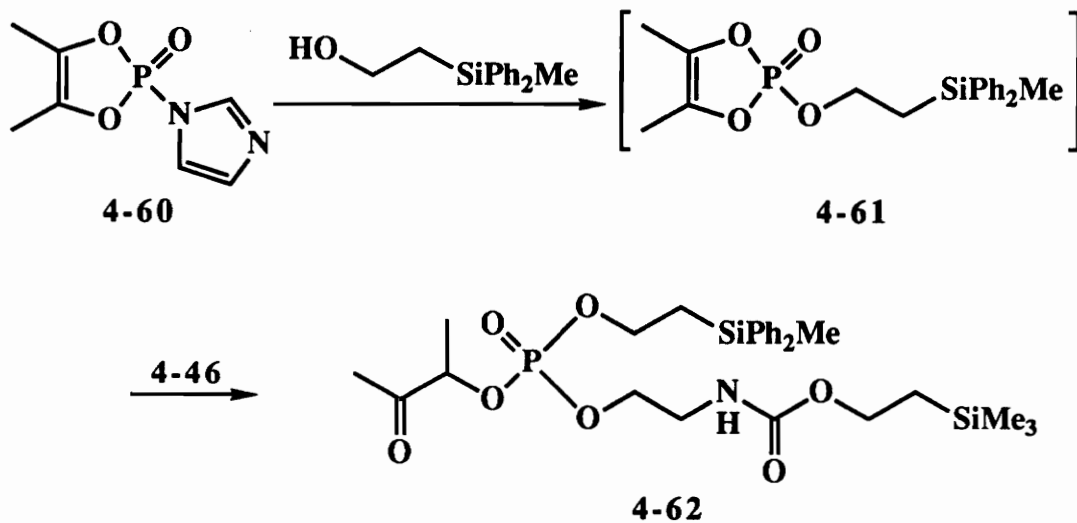
The final step in generating the necessary phosphorylating reagent by the CEP method involves reacting CEP-Cl **4-59** with imidazole to give the imidazolyl oxodioxaphosphole (CEP-Im) **4-60**^{133,135,136} (Scheme 4-21). Compound **4-60** is a convenient phosphorylating reagent in that unlike **4-59** compound **4-60** is slightly less reactive and makes the generation of unsymmetrical phosphodiester easier. It also is stable and can be stored refrigerated for long periods of time.



Scheme 4-21

Once imidazolyl oxodioxaphosphole **4-60** had been synthesized it was now possible to construct the protected phosphodiester **4-1**. The first alcohol,

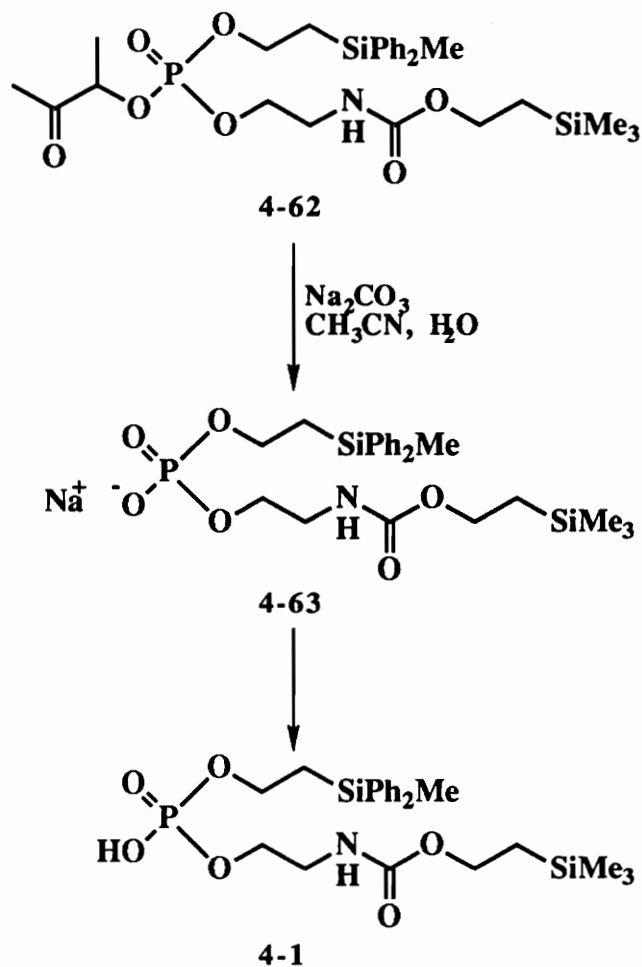
diphenylmethylsilylethanol, was reacted with **4-60** displacing imidazole to give **4-61** which was not isolated (Scheme 4-22).



Scheme 4-22

Reactions of **4-60** with alcohols are known to proceed with almost complete ring retention.¹¹² Since compound **4-61** is not isolated, it was necessary to react the diphenylmethylsilylethanol first since the chromophore of the phenyl groups provided a convenient method of monitoring the progress of the reaction by thin layer chromatography. Once this reaction was complete, the second alcohol, **4-46**, was added to give the phosphotriester **4-62**.

Finally, to generate the target compound **4-1**, it was necessary to remove the acetoin group by hydrolysis. This was accomplished by reacting **4-62** with sodium carbonate in acetonitrile/water (1:1 v/v) to give the sodium salt **4-63** which was protonated using ion exchange resin to give the protected phosphorylethanolamine target compound **4-1** (Scheme 4-23).



Scheme 4-23

4.6 Experimental.

Preparation of N-2-(trimethylsilyl)ethylcarbonyl-2-hydroxyethanolamine (4-46)

2-(Trimethylsilyl)ethyl-*p*-nitrophenyl carbonate (5.24 g, 18.50 mmol) was dissolved in 50 ml of anhydrous dichloromethane and cooled to -5°C . To this cool solution, ethanolamine (7.90 ml, 131.40 mmol) dissolved in 5.5 ml of anhydrous dichloromethane was added over 20 minutes. Once the addition was complete, the reaction was allowed to stir at room temperature for 3 hours. The reaction was diluted with 150 ml

of ethyl acetate and 100 ml of water. This mixture was washed with 2 x 100 ml of 10% HCl. The acid washings were combined and back extracted with 3 x 150 ml of ethyl acetate. The ethyl acetate fractions were combined and washed with 10% NaOH until the yellow color in the aqueous phase ceased to exist. They were then washed with 3 x 150 ml of brine, dried over Na₂SO₄, and filtered. The solvent was removed *in vacuo* to give a yellowish oil (2.97 g, 78%) which required no purification. ¹H NMR (CDCl₃) δ 5.02 (s, 1H, NH), 4.13 (t, J = 8.6 Hz, 2H, CO₂CH₂), 3.69 (t, J = 5.1 Hz, 2H, HOCH₂), 3.31 (dt, J = 5.3 Hz, 2H, NHCH₂), 2.33 (s, 1H, HO), 0.96 (t, J = 8.5 Hz, 2H, CH₂SiR₃), 0.0 (s, 9H, Si(CH₃)₃). IR (neat) 3342, 2953, 2897, 1698, 1535, 1462, 1415, 1334, 1251, 1178, 1145, 1062, 951, 860, 838, 768, 694, 664 cm⁻¹. MS (FAB) *m/z* (relative intensity) 206 (MH⁺, 50), 190 (22), 178 (100), 162 (33), 147 (16) 135 (7).

Preparation of N-2-(trimethylsilyl)ethylcarbonyl-2-methoxy-1,3,2-oxaphospholidine (4-47)

To a solution of dichloromethoxyphosphine (158 mg, 1.20 mmol) in 1.5 ml of anhydrous benzene was added anhydrous pyridine (0.233 ml, 2.90 mmol) and cooled to 0°C. Next 4-46 (250 mg, 1.20 mmol) dissolved in 0.25 ml of anhydrous benzene was added at such a rate that the temperature did not exceed 5°C. The reaction was stirred at 0°C for 15 minutes, then for 12 hours at room temperature. The precipitate was removed by filtration and the solvent was removed from the filtrate by rotary evaporation to give 288 mg (91%). ¹H NMR (CDCl₃) δ 4.17 (m, 4H, CH₂), 3.74 (m, 3H, OCH₃), 3.47 (m, 2H, NCH₂), 0.95 (t, J = 8.3 Hz, 2H, CH₂Si), 0.0 (s, 9H, Si(CH₃)₃).

Preparation of 2-[(2-diphenylmethylsilyl)ethyl]-1,3,2-oxazaphospholine-2-one (4-52)

To a solution of phosphorus oxychloride (0.204 ml, 2.20 mmol) dissolved in 5 ml of anhydrous THF cooled to 0°C was added diphenylmethylsilyl ethanol (0.470 mo, 2.10

mmol) dissolved in 5 ml of anhydrous THF containing triethylamine (0.334 ml, 2.40 mmol) over a 1.5 hour period. The reaction was slowly allowed to warm to room temperature where it was stirred for 12 hours. The reaction was cooled back to 0°C and ethanolamine (0.134 ml, 2.20 mmol) and triethylamine (0.719 ml, 5.20 mmol) dissolved together in 5 ml of anhydrous THF was added over a 1 hour period. The reaction was warmed to room temperature where it was stirred for 1 hour. The precipitate was filtered and the solvent was removed *in vacuo* from the filtrate to give a colorless oil. Purification by flash chromatography eluting with 92% CH₂Cl₂ / 8% isopropyl alcohol gave 0.132 g (17%) of **4-52**. ¹H NMR (CDCl₃) δ 7.49 (m, 4H, SiPh₂), 7.33 (m, 6H, SiPh₂), 4.13 (m, 4H, POCH₂ and SiCH₂CH₂-OP), 3.39 (m, 1H, NH), 3.20 (m, 2H, NHCH₂), 1.64 (t, J = 7.8 Hz, 2H, CH₂SiPh₂Me), 0.57 (s, 3H, SiPh₂CH₃). MS (CI) *m/z* 347 (MH⁺, 10), 320 (30), 304 (35), 270 (70), 243 (100), 197 (90), 124 (70).

Preparation of 4,5-dimethyl-2-methoxy-2-oxo-1,2,3-dioxaphosphole (4-55)

2,3-butanedione **4-56** (8.78 ml, 0.10 mol) was added dropwise over a 2 hour period to trimethylphosphite (11.79 ml, 0.10 mol) which had been cooled to 10-15°C and placed under nitrogen. Once the addition was complete, the temperature was raised to room temperature where stirring was continued for 3 hours. Next the reaction mixture was dissolved in anhydrous acetonitrile (65 ml) and acetyl bromide (2.0 ml, 0.25 mol) was added. Additional acetyl bromide (60 ml, 0.75 mol) was then added at such a rate that the temperature did not exceed 45°C. Stirring was continued for 3.5 hours after completion of the addition. The solvents were removed *in vacuo*. Purification by vacuum distillation (2 mm Hg distilling at 90-105°C) gave 11.79 g (72%) of **4-55** as a light yellow oil, (lit¹³³, 48-49°C at 0.06 mm Hg). ¹H NMR (CDCl₃) δ 3.75 (d, J = 11.0 Hz, OCH₃), 1.90 (s, 6H, CH₃). The spectral data agree with those reported in the literature.¹³³

Preparation of 1'-methylpyridinium-4,5-dimethyl-2-oxido-oxo-1,3,2-dioxaphosphole (4-58)

Dioxaphosphole 4-55 (11.79 g, 71.9 mmol) was dissolved in anhydrous benzene (35 ml). Anhydrous pyridine (18.02 ml, 222.8 mmol) was added and the mixture was heated to reflux for 7 hours. The reaction was then allowed to stir at room temperature for 12 hours. At this time the solvent was decanted from the brown precipitate and washed with 3 x 10 ml portions of anhydrous benzene. The remaining volatile materials were removed under high vacuum giving 15.85 g (90.7%) of 4-58 as a brown solid. ^1H NMR (D_2O), δ 8.80 (d, $J = 5.8$ Hz, 2H, Ar), 8.54 (t, $J = 7.8$ Hz, 1H, Ar), 8.05 (t, $J = 7.2$ Hz, 1H, Ar), 4.84 (s, 3H, Py-CH₃), 1.87 (s, 6H, CH₃). The spectral properties are identical to those previously described.¹³³

Preparation of 2-chloro-4,5-dimethyl-2-oxo-1,3,2-dioxaphosphole (4-59)

Methylpyridinium dioxaphosphole 4-58 (15.39 g, 63.3 mmol) was dissolved in anhydrous benzene (90 ml) and cooled to 0°C. To this mixture a solution of triphosgene (18.79 g, 63.3 mmol) dissolved in anhydrous benzene (24 ml) was added dropwise over a 30 minute period. Once the addition was complete, the reaction was allowed to stir at room temperature for 48 hours. At the end of this time the reaction mixture was filtered and the solvents removed *in vacuo*. Purification by vacuum distillation (2.5 mm Hg, 74-81°C) gave 6.99 g (65.7%) of 4-59 as a colorless oil, (lit¹³⁴ 48°C at 0.1 mm Hg). ^1H NMR (CDCl_3) δ 1.98 (s, 6H, CH₃). The spectral properties are identical to those previously described.¹³⁶

Preparation of 4,5-dimethyl-2-(1'-imidazolyl)-2-oxo-1,3,2-dioxaphosphole (4-60)

Chlorodioxaphosphole 4-59 (6.99 g, 41.6 mmol) dissolved in dichloromethane (15 ml) was added dropwise over a 30 minute period to a solution of imidazole (5.67 g,

83.2 mmol in dichloromethane (65 ml) at 0°C. The reaction was stirred at 0°C for 1 hour and the reaction mixture filtered. The residue was washed with dichloromethane and the solvents removed *in vacuo* to give 8.32 g (100%) of **4-60** as a thick slightly yellow oil which slowly crystallized upon refrigeration. ¹H NMR (CDCl₃, 200 MHz) δ 8.40 (s, 1H, Im), 7.22 (s, 2H, Im), 1.88 (s, 6H, CH₃). The spectral properties are identical to those previously described.¹³³

Preparation of [N-2-(trimethylsilyl)ethylcarbonylaminoethyl]-[2-(diphenylmethylsilyl)ethyl]-[2-keto-3-methylpropyl]phosphate (4-62)

Dioxaphosphole **4-60** (2.46 g, 12.3 mmol) was dissolved in 16 ml of anhydrous acetonitrile and cooled to -10°C. To this solution 2-(diphenylmethylsilyl)ethanol (2.11 ml, 9.20 mmol) dissolved in 13 ml of anhydrous acetonitrile was added dropwise with vigorous stirring over a 40 minute period. Once the addition was complete, the reaction mixture was maintained for 15 minutes at -10°C then the temperature was raised to room temperature where it was stirred for 1 hour. The reaction was cooled back to -10°C and carbamate **4-46** (1.89 g, 9.20 mmol) dissolved in 7.0 ml of anhydrous acetonitrile was added dropwise. The temperature was slowly allowed to warm to room temperature where it was held for 24 hours. The solvent was removed *in vacuo*, and the residue was dissolved in 100 ml of ether. This was washed with 3 x 100 ml of 10% HCl. The combined acid washings were back-extracted with 3 x 100 ml ether, and all the organic extracts were combined. These were washed with 2 x 100 ml H₂O followed by 1 x 100 ml brine. The extracts were dried over Na₂SO₄, filtered and the solvent removed *in vacuo* to give 5.0 g (44%) of **4-62**. ¹H NMR (CDCl₃) δ 7.48 (m, 5H, Ph), 7.35 (m, 5H, Ph), 5.17 (s, 0.5 H, diastereomer of NH), 5.06 (s, 0.5 H, diastereomer of NH), 4.70 (m, 1H, COCH(CH₃)), 4.04 (m, 6H, OCH₂CH₂SiPh₂Me, OCH₂CH₂NH, CO₂CH₂), 3.39 (m, 2H, NHCH₂), 2.15 (2 x s, 3H, diastereomers of CH₃CO), 1.65 (m, 2H, CH₂SiPh₂Me),

1.40 (2 x d, J = 7.7 Hz and J = 7.6 Hz, 3H, diastereomers of CH₃), 0.94 (t, J = 8.5, 2H, CH₂SiMe₃), 0.00 (s, 12H, SiPhCH₃ and Si(CH₃)₃). MS (CI) *m/z* 581 (M⁺, 3), 520 (10), 524 (12), 437 (30), 284 (80), 197 (100), 160 (90).

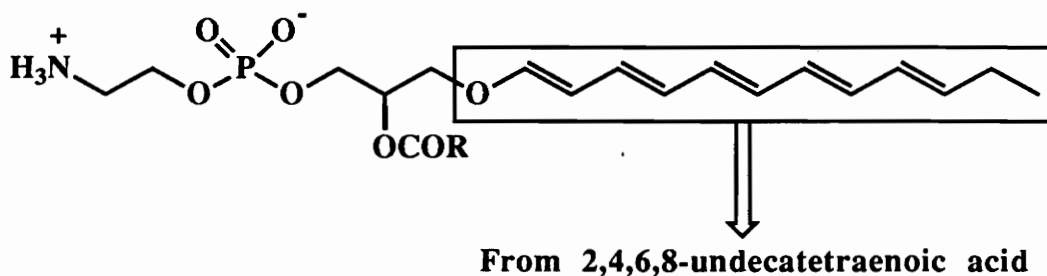
Preparation of [N-2-(trimethylsilyl)ethylcarbonylaminoethyl]-[2-(diphenylmethylsilyl)ethyl] sodium phosphate (4-63)

Phosphate 4-62 (4.97 g, 88.6 mmol) was dissolved in 90 ml of a acetonitrile / water (1:2 CH₃CN/H₂O v/v) solution. Sodium carbonate (4.19 g, 39.5 mmol) was added. The mixture was stirred at room temperature for 24 hours. The solvent was removed *in vacuo*, and the residue was taken up in ethyl acetate. The insoluble material was filtered off by vacuum filtration. The solvent was removed from the filtrate to give 4.74 g of crude 4-63. Purification by flash chromatography eluting with 30% MeOH / 80% CH₂Cl₂ gave 7.21 g (92%), mp 207.5-208°C decompose. ¹H NMR (CDCl₃; poorly resolved spectrum) δ 7.43 (m, 5H, Ph), 7.30 (m, 5H, Ph), 4.0 (m, 4H), 3.68 (m, 2H), 3.30 (m, 2H), 1.50 (m, 2H), 0.91 (s, 2H), 0.50 (m, 3H), 0.0 (s, 12H). IR (KBr) 2956, 1720, 1525, 1428, 1252, 1112, 1056, 837, 794, 732, 699 cm⁻¹.

V. Synthesis of 2,4,6,8-Undecatetraenoic Acid

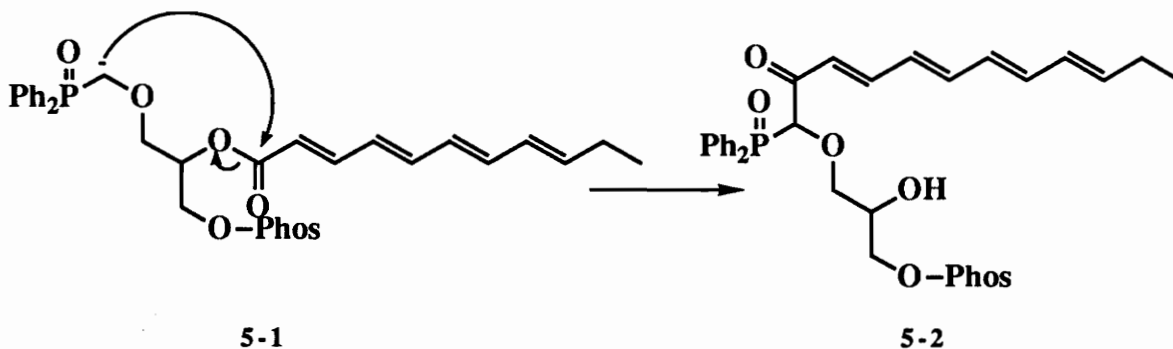
5.1 Introduction.

Previously, the synthetic methodology for the synthesis of plasmalopentaene-12 was mentioned. It was discussed how each functional group attached to the glycerol backbone was to originate, (Scheme 5-1), specifically how the pentaenyl ether was to originate.



Scheme 5-1

The synthetic methodology requires that an acyl group in the form of 2,4,6,8-tetraundecenoic acid to be migrated via generation of a lithiated species **5-1** (Scheme 5-2) which will give the ketone **5-2**.



Scheme 5-2

After several steps the ketone **5-3** is then reduced to the polyenyl alcohol **5-4** and finally eliminated in a Wittig fashion to generate the pentaenyl ether **5-5** (Figure 5-1).

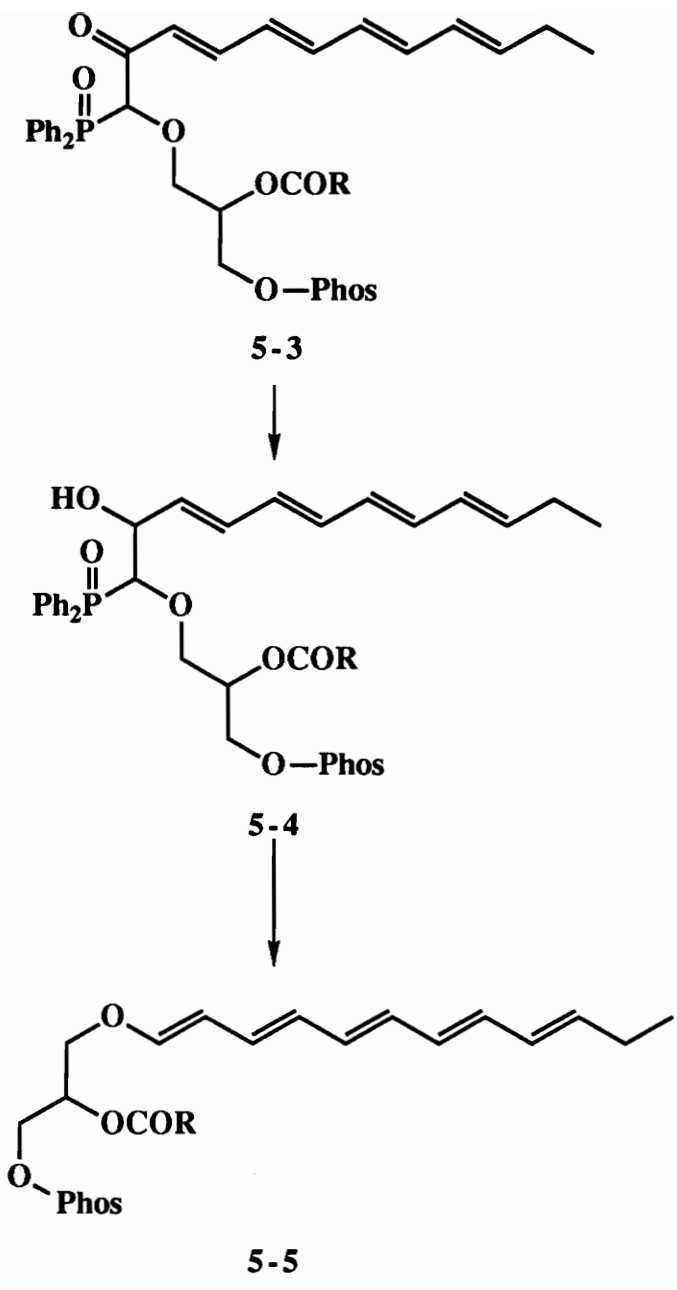
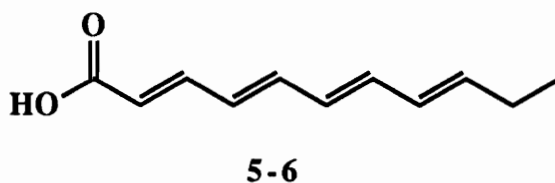


Figure 5-1. Generation of the Pentaenyl Ether of Plasmalopentaene-12.

This particular synthetic methodology enables one to produce plasmalopentaene-12 analogs with varying enol ethers i.e. dienol ethers from enoic acid, trienol ethers from dienoic acid and tetraenol ethers from trienoic acid etc. For the case of plasmalopentaene-12, the tetraenoic **5-6** acid was required for the generation of the pentaenyl ether functionality.



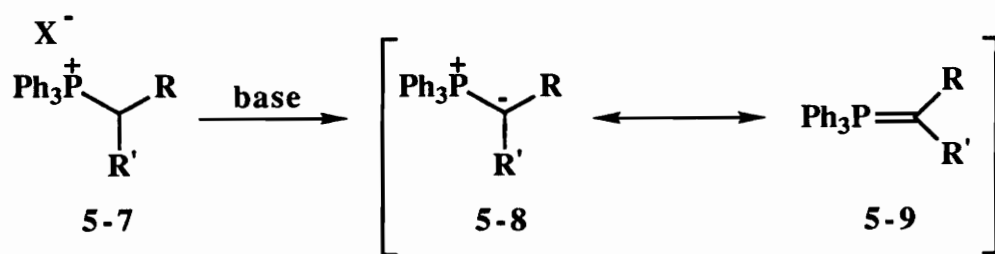
Several attempts at synthesizing the acid **5-6** utilizing some of the most common methodologies were unsuccessful. Inclusion of these failed attempts serves to provide a clearer illustration of subsequent synthetic efforts.

5.2 Synthesis of the Tetraenoic Acid Via Wittig Methodology.

Several methods exist for synthesizing olefins such as compound **5-6**. Of these methods, probably the most popular are the Peterson olefination¹³⁷ and the Wittig reaction^{138,139}. The Wittig reaction is the more versatile and popular method and is generally the method of choice to produce alkenes from carbonyl compounds.

5.2.1 Synthesis of the Tetraenoic Acid Using a Triphenylphosphine Ylide.

In the Wittig reaction an aldehyde or ketone is treated with a phosphorus ylide (also known as a phosphorane) to give an olefin. The ylides are hybrids of two canonical forms **5-8** and **5-9** which are prepared by treatment of a phosphonium salt **5-7** with base (Scheme 5-3). The mechanism of the Wittig reaction is shown in Figure 5-2.



Scheme 5-3

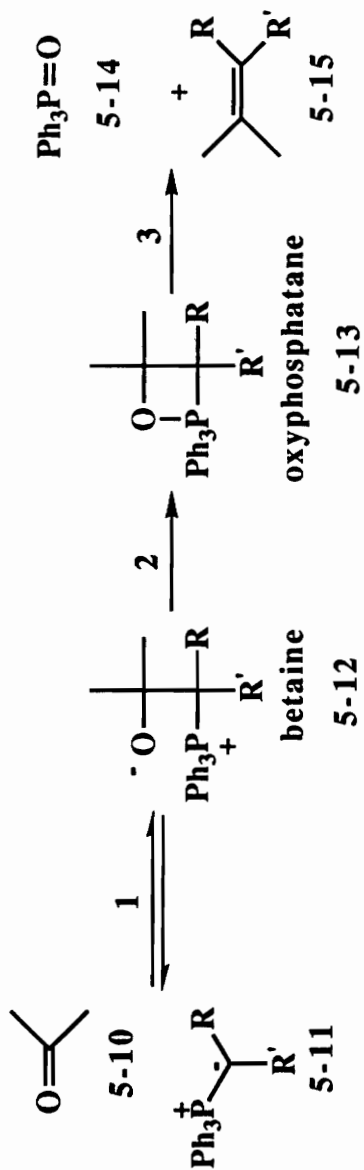
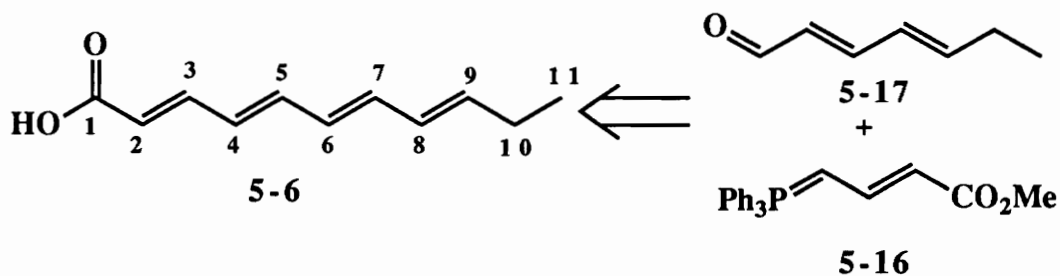


Figure 5-2. Mechanism of the Wittig Reaction

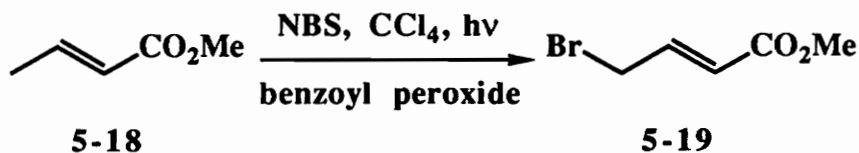
Step one, in which the aldehyde or ketone **5-10** reacts with the ylide **5-11** to give the betaine **5-12**, may or may not be an equilibrium reaction depending on the type of ylide used (i.e. the reaction is reversible for stabilized ylides--those in which R or R' is an electron withdrawing group¹⁴⁰). The second step involves formation of the oxyphosphatane **5-13** from betaine **5-12**. If the ylide used is a non-stabilized ylide, the first two steps may be simultaneous.¹⁴¹ Once the oxyphosphatane **5-13** is formed, the highly strained four-membered ring is broken to give the phosphine oxide **5-14** and the olefin **5-15**. It is the highly stable phosphorus-oxygen double bond that serves as the driving force for the formation of olefins. For this reason that the Wittig reaction has gained wide acceptance for the synthesis of alkenes from aldehydes and ketones. The utility of the Wittig reaction made it a logical starting point for the synthesis of the tetraenoic acid **5-6**.

It was envisioned that by disconnecting the bond between carbons C₄ and C₅ in **5-6** would enable a Wittig synthesis of compound **5-6** to arise from ylide **5-16** and commercially available 2,4-heptadienal **5-17** (Scheme 5-4). Since aldehyde **5-17** is readily available, the only other starting material that needs to be synthesized to attempt the synthesis is ylide **5-16**.



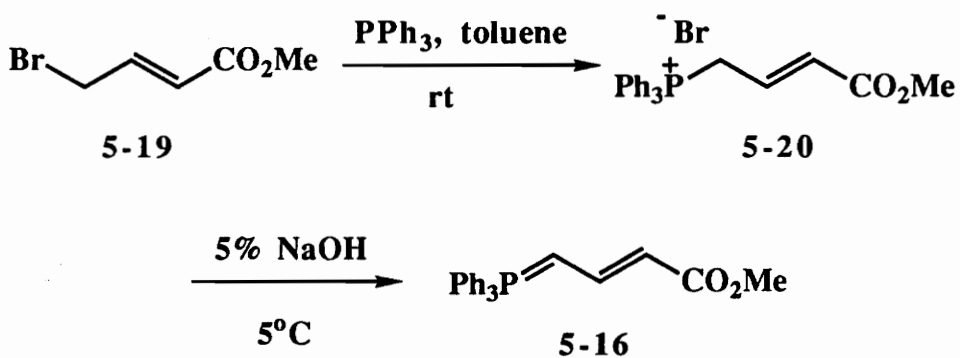
Scheme 5-4

The synthesis of compound **5-16** began by the allylic bromination of methyl crotonate **5-18** to give methyl bromocrotonate **5-19** (Scheme 5-5).

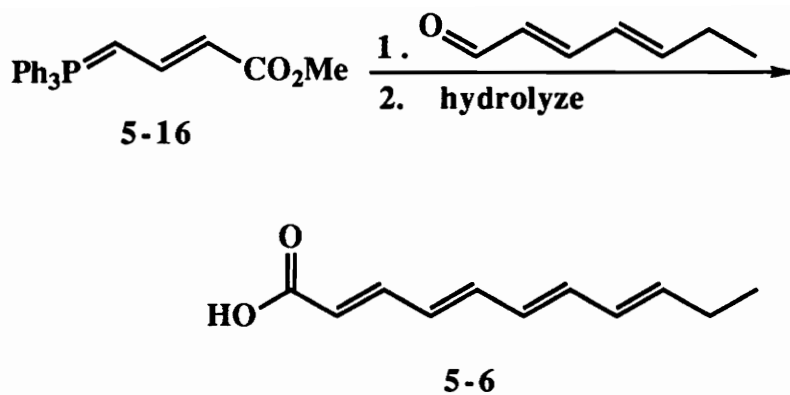


Scheme 5-5

The product was formed by standard procedures¹⁴² in which methyl crotonate **5-18** was treated with N-bromosuccinimide (NBS) in the presence of benzoyl peroxide to give methyl bromocrotonate **5-19**. Next the phosphonium salt was produced by treating bromocrotonate **5-19** with triphenyl phosphine in toluene to give methyl triphenylbromophosphonium crotonate **5-20** in a high yield. Finally **5-20** was readily converted to the ylide **5-16** by treatment with aqueous sodium hydroxide (Scheme 5-6) which was identical to an authentic sample previously prepared. With the ylide **5-16** now available, it was now possible to attempt the generation of the tetraenoic acid **5-6** (Scheme 5-7).



Scheme 5-6



Scheme 5-7

It was found that using a 1:1 stoichiometry between the ylide and aldehyde gave only starting materials. Only when increasing the amount of ylide to 2.5 equivalents did any product form. The product was formed in low yield and purification was difficult. Other conditions were sought which would avoid the high number of equivalents of the ylide. It was decided that increasing the reaction temperature may enable the olefination to occur while using a one to one stoichiometry between ylide and aldehyde. As a result the reaction (Scheme 5-7) was tried first in refluxing dichloromethane, then in refluxing toluene. In each case no product was formed. For a summary of reaction conditions tried, see Table 5-1.

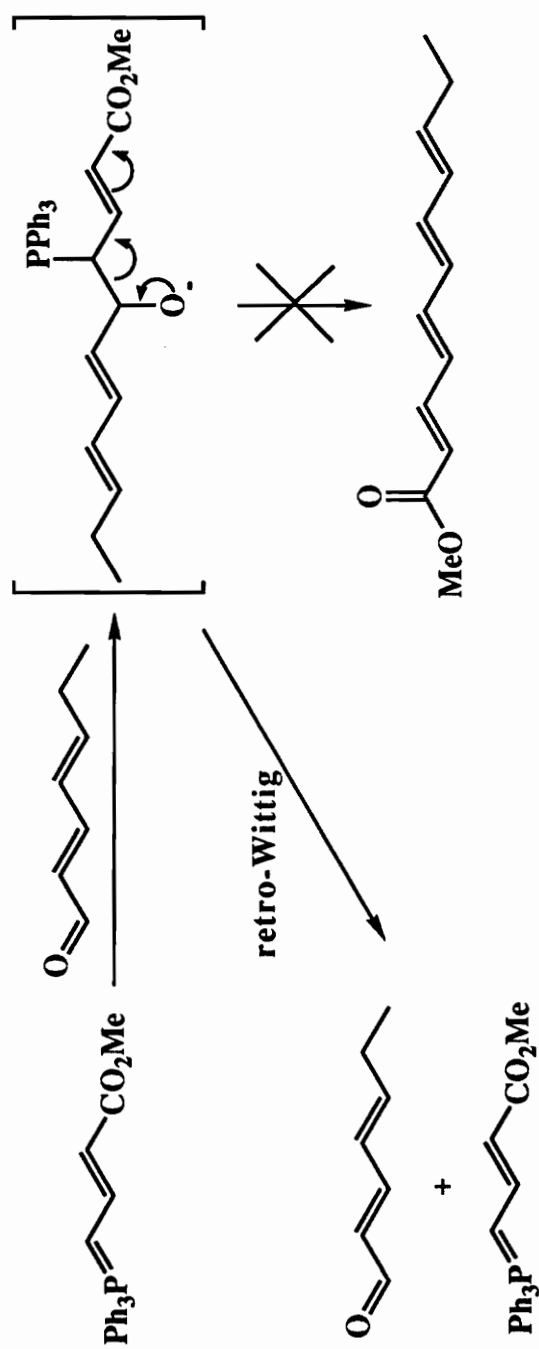
Faced with these difficulties, it became necessary to develop a synthesis that generated the product 5-6 in higher yields utilizing milder conditions.

Table 5-1. Attempted Reaction Conditions Used for the Wittig Reaction.

Solvent	Ylide Equiv.	Temperature	Time	Yield
CH ₂ Cl ₂	1.5	room	20 hour	no rxn
CH ₂ Cl ₂	2.0	room	14 hour	no rxn
CH ₂ Cl ₂	2.5	room	14 hour	low
CH ₂ Cl ₂	1.0	reflux	48 hour	no rxn
Toluene	1.0	reflux	1 week	no rxn

5.2.2 Synthesis of the Tetraenoic Acid Via the Wittig Horner-Emmons Reaction.

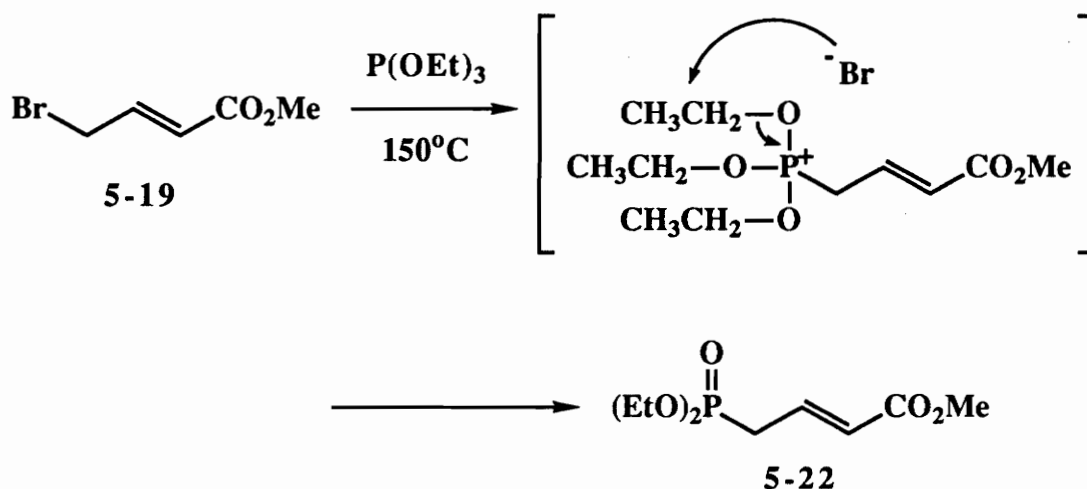
Since the ylide used for the synthesis of the acid is a stabilized ylide (i.e. conjugated through the ester carbonyl), it is less reactive than a non-stabilized ylide. With this in mind, it was felt that since the starting materials were never consumed possibly either the reaction was reaching the betaine stage and the elimination of the phosphine oxide via the oxyphosphatane was not occurring or the reaction intermediate was undergoing a retro-Wittig reaction (Scheme 5-8).



Scheme 5-8

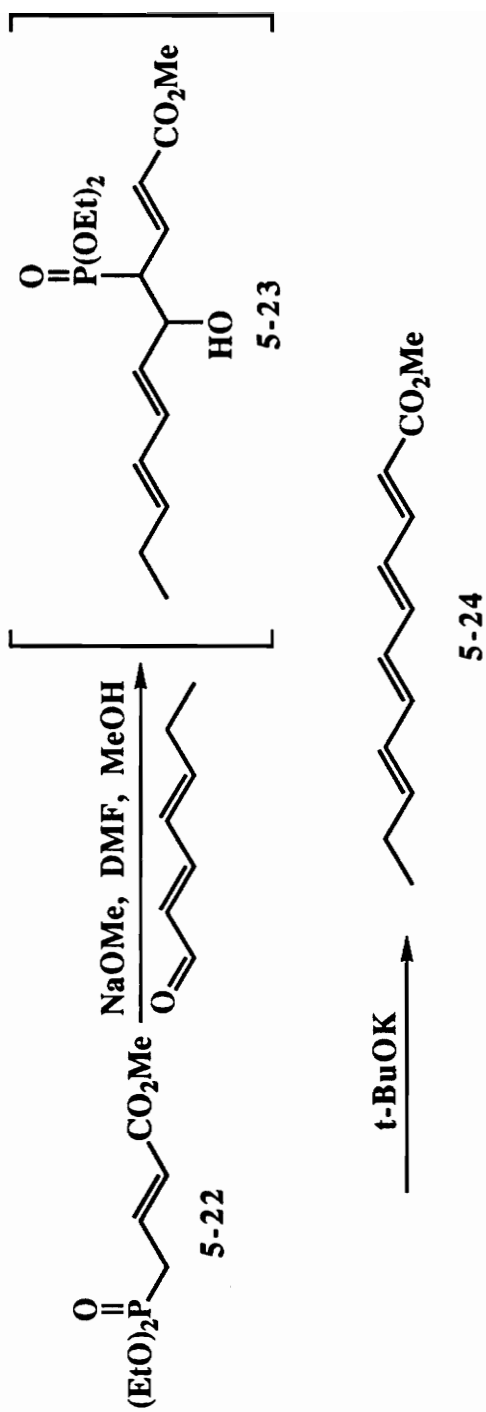
It has been shown that phosphonate ylides react with aldehydes and ketones to give olefins. This reaction, known as the Horner-Emmons or Wadsworth-Emmons reaction, offers several advantages over the use of phosphoranes in the Wittig reaction.^{143,144,145,146} First, and most importantly, the phosphonate ylides are more reactive than phosphorane ylides. Second, the phosphate ester by-product is water soluble which makes purification of the olefin less difficult. Third, phosphonates are cheaper than phosphonium salts and are easily prepared by the Arbuzov reaction.

Since the phosphonate ylides are more reactive than the triphenylphosphine ylides, it was decided to synthesize the tetraenoic acid **5-6** via the Horner-Emmons reaction. Methyl bromocrotonate **5-19** was synthesized according to Figure 5-5. The bromine of crotonate **5-19** was displaced in an S_N2 fashion using triethyl phosphite which upon heating underwent the Arbuzov reaction to give the phosphonate **5-22**¹⁴⁷ (Scheme 5-9).



Scheme 5-9

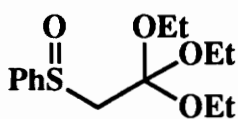
Phosphonate **5-22**, when condensed with 2,4-heptadienal **5-17** under basic conditions, should give the betaine **5-23**. It was hoped that subsequent treatment with potassium-*t*-butoxide would affect elimination of the phosphate ester and give the unsaturated ester **5-24**^{147,148,149} (Scheme 5-10).



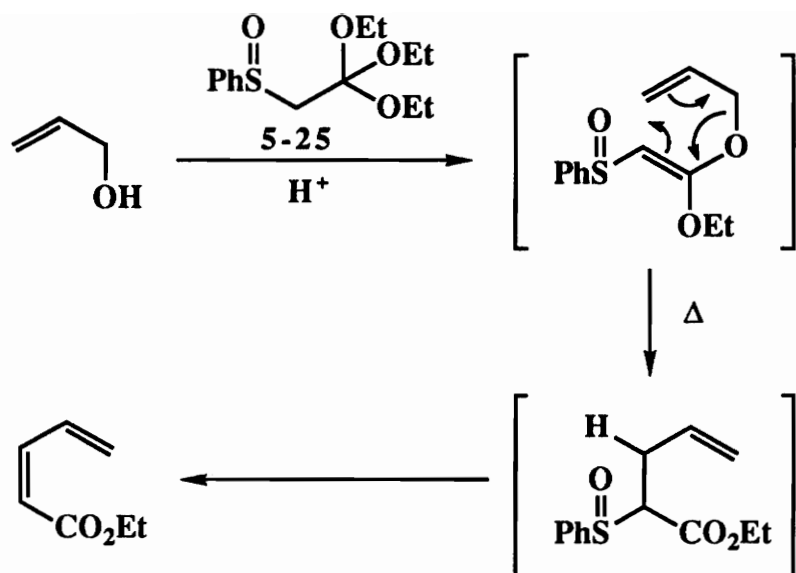
TLC examination of the intermediates indicated that no reaction occurred. It was once again felt that a retro-Wittig type of reaction was taking place, thus explaining the presence of starting materials. All other attempts to improve the reactions were met with failure and another method of producing 5-6 was sought.

5.3 Synthesis of the Tetraenoic Acid Via the Claisen Rearrangement.

In 1991, Posner *et al.* reported a conversion of allylic alcohols into conjugated dienoate esters by the use of a sulfinyl orthoester 5-25 in a Claisen rearrangement.¹⁵⁰ This method was developed as a one-flask, regiospecific, high-yielding transformation which circumvented problems associated with those of standard Claisen rearrangements^{151,152,153,154,155} such as poor yields and lack of adaptability to varying molecular architecture. These difficulties were overcome by the development of a streamlined process which utilizes the orthoester 5-25 that was designed to undergo spontaneous thermal β -elimination under the same reaction conditions used for the initial [3,3] sigmatropic rearrangement (Scheme 5-11).

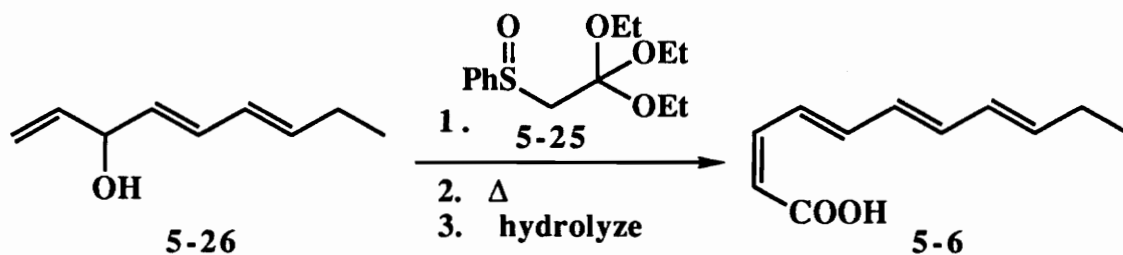


5-25

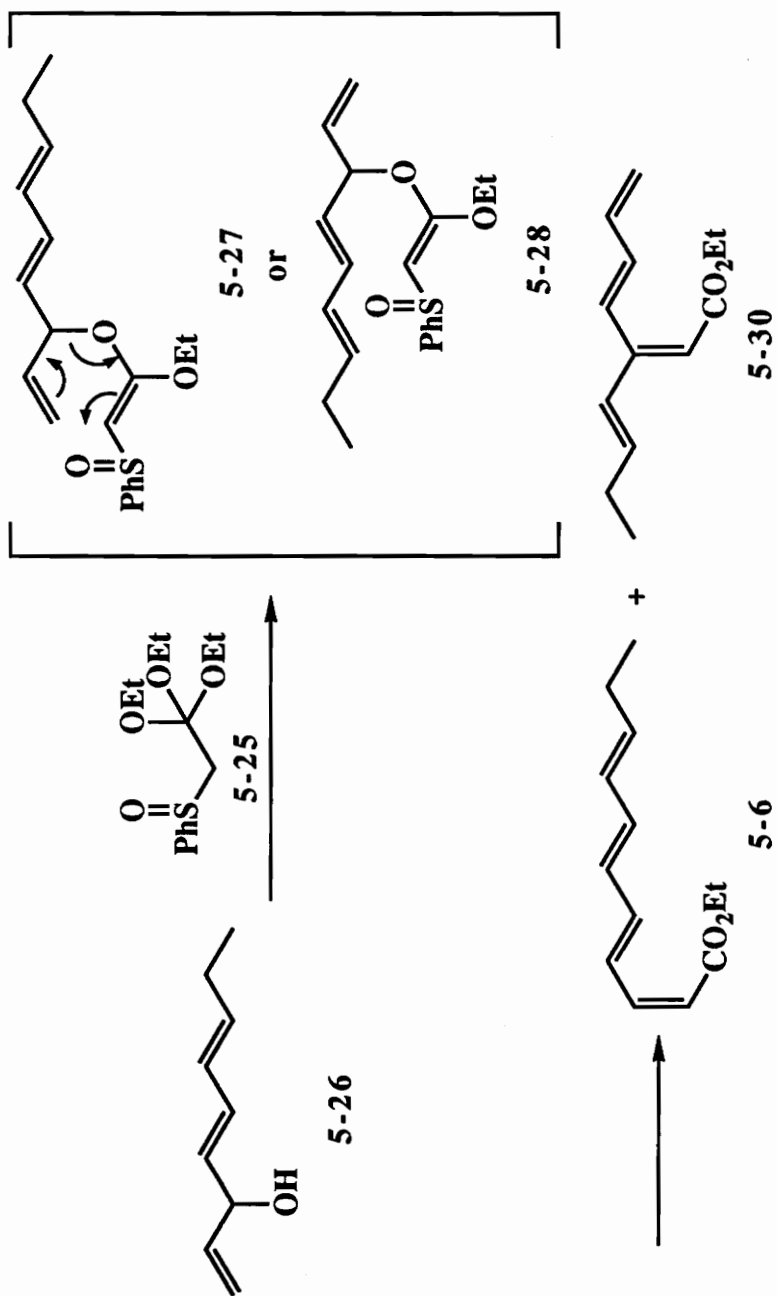


Scheme 5-11

With the knowledge of this reaction, it became desirable to investigate the use of this synthetic methodology for the synthesis of the tetraenoic acid **5-6**. It was envisioned that the above procedure (Scheme 5-11) could be modified by using allylic alcohol **5-26** to arrive at the target acid **5-6** (Scheme 5-12). This methodology may arrive at two regiochemically different products (**5-6** and **5-30**) via intermediates **5-27** and **5-28** (Scheme 5-13).

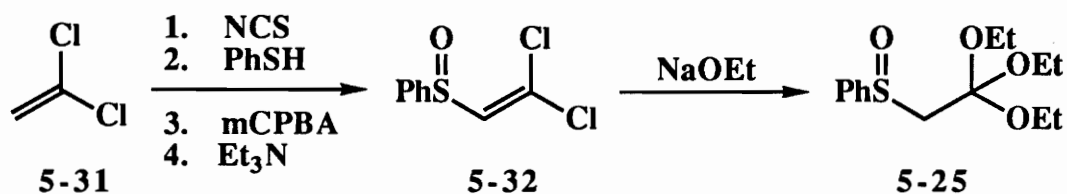


Scheme 5-12

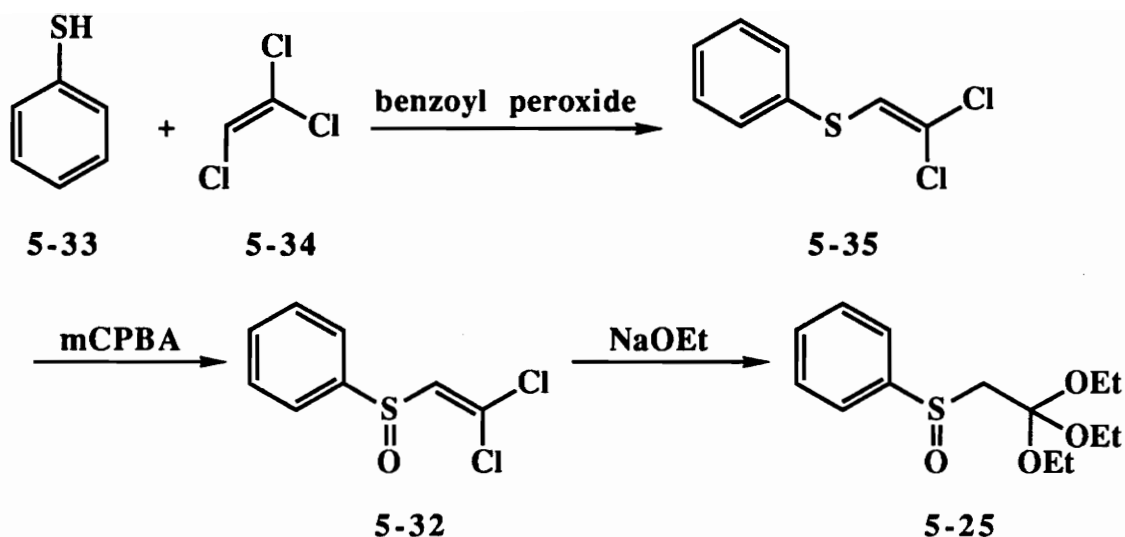


It was felt, however, based on regiochemistry that intermediate **5-27** would predominate and give the desired acid **5-6** for two reasons. First, intermediate **5-27** is less sterically crowded than **5-28** and should be the favored configuration. Secondly, when intermediate **5-28** undergoes the Claisen rearrangement some element of stabilization is lost by breaking the conjugation on the diene portion of the molecule. In view of these beliefs, it was felt that pursuit of this methodology may provide a viable route for the synthesis of tetraenoic acids.

The sulfinyl orthoester **5-25** has been prepared in two ways. First¹⁵⁶, ionic addition of benzenesulfonyl chloride to dichloroethylene **5-31** gave a trichloro thioether which when oxidized followed by dehydrochlorination gave dichlorovinyl sulfoxide **5-32**. This was then converted to **5-25** by treatment with sodium ethoxide (Scheme 5-14). The second way provides a very rapid and convenient method and thus was used to generate **5-25** (Scheme 5-15).



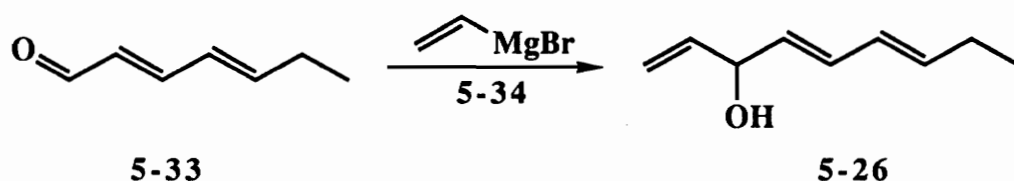
Scheme 5-14



Scheme 5-15

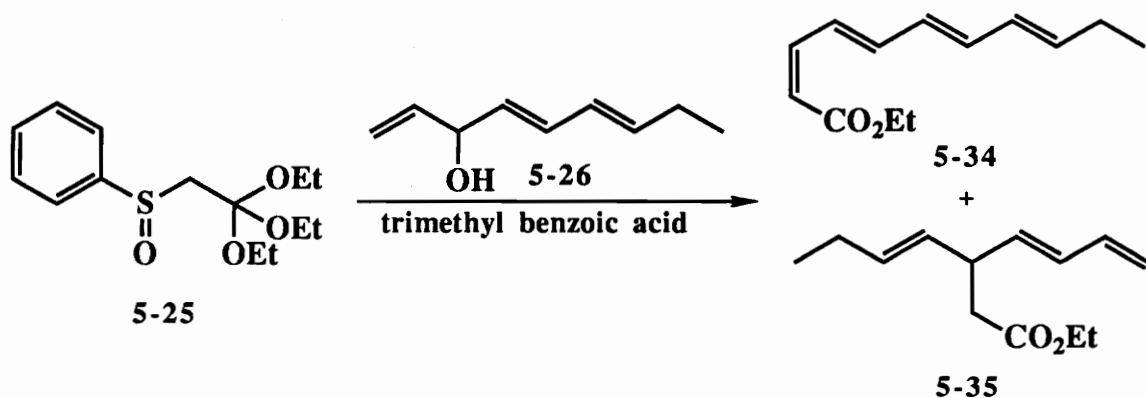
Following Posner's procedure,¹⁵⁰ radical addition of thiophenol **5-33** to trichloroethylene in the presence of benzoyl peroxide, gave the dichlorovinyl sulfide **5-35**. Oxidation of the sulfide **5-35** using MCPBA gave the dichlorovinyl sulfoxide **5-32** which, after treatment with sodium ethoxide, gave sulfanyl orthoester **5-25** as an oil in good yield. This could easily be stored at 0°C for long periods of time without any noticeable decomposition.¹⁵⁷

Having synthesized orthoester **5-25**, it was now necessary to synthesize the desired allylic alcohol **5-26** before the actual Claisen rearrangement could take place (Scheme 5-16). This was done by treating 2,4-heptadienal **5-33** with vinyl magnesium bromide **5-34** at 0°C then warming to room temperature. Quenching of the reaction with saturated ammonium chloride gave the allylic alcohol **5-26** in high yield.



Scheme 5-16

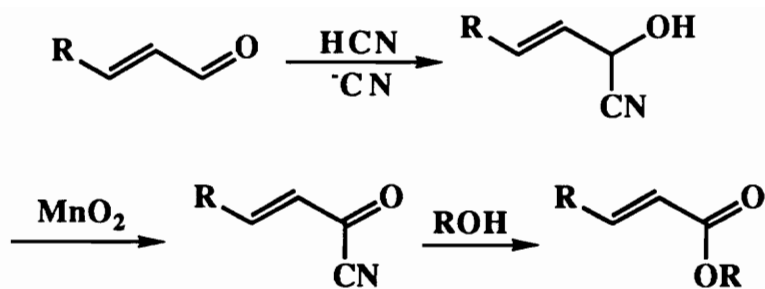
The orthoester **5-25** and allylic alcohol **5-26** could now be combined in a Claisen rearrangement to hopefully give the tetraenoic ester **5-34** without any **5-35** (Scheme 5-17). Reacting orthoester **5-25** with the allylic alcohol **5-26** in the presence of a catalytic amount of trimethylbenzoic acid in a pressure tube, unfortunately, gave a 50/50 mixture of two major compounds long with a small amount of decomposition. The identity of these products could not be determined since separation by any known chromatographic techniques was impossible.



Scheme 5-17

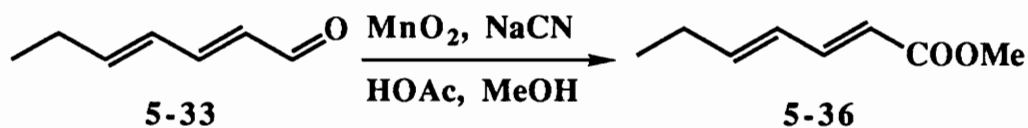
5.4 Oxidation of a Tetraenal to a Tetraenoic Acid.

Manganese dioxide (active^{158,159}) has been shown to be an effective and selective oxidizing agent for converting primary allylic alcohols to conjugated aldehydes.¹⁶⁰ Corey *et al.*¹⁶¹ in 1968 used this methodology to convert aldehydes to acids by anticipating that in the presence of HCN and -CN a conjugated aldehyde would be converted to a cyanohydrin. This would be further oxidized to the acyl cyanide by manganese dioxide and in an alcoholic medium lead to an ester (Scheme 5-18).



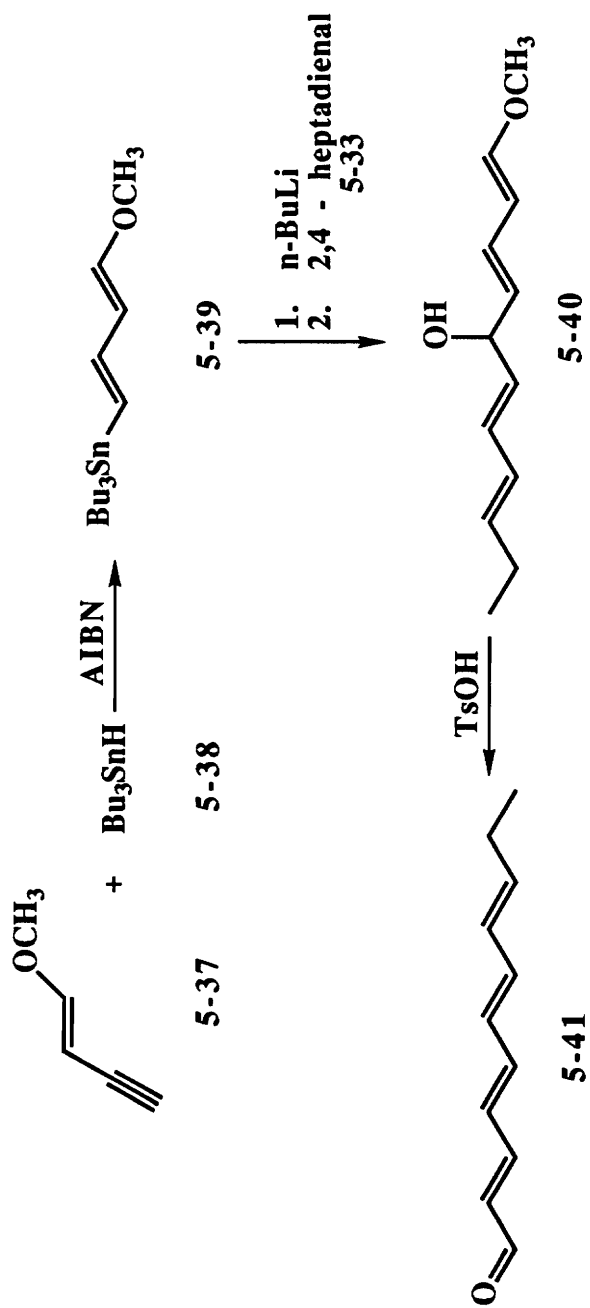
Scheme 5-18

This methodology was tested by attempting to oxidize 2,4-heptadienal to the methyl ester to see if extended conjugation would have any affect the oxidation. This reaction produced a very clean, high-yielding conversion of 2,4-heptadienal **5-33** to methyl-2,4-heptadienoate (Scheme 5-19).



Scheme 5-19

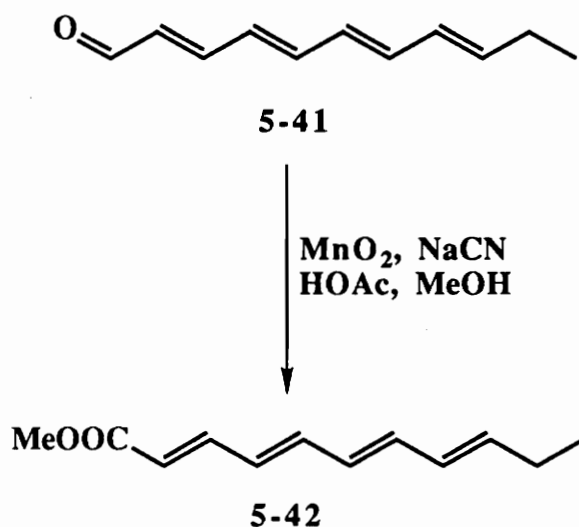
Encouraged by this result, it was decided that this may be the method needed to synthesize acid **5-6**. This could be accomplished by simply applying Corey's conditions to a tetraenal in a suitable alcoholic solvent. To attempt this synthesis, it was first necessary to synthesize 2,4,6,8-undecatetraenal **5-37**. The synthesis of this aldehyde follows a procedure reported by Wollenberg¹⁶² (Scheme 5-20).



Scheme 5-20

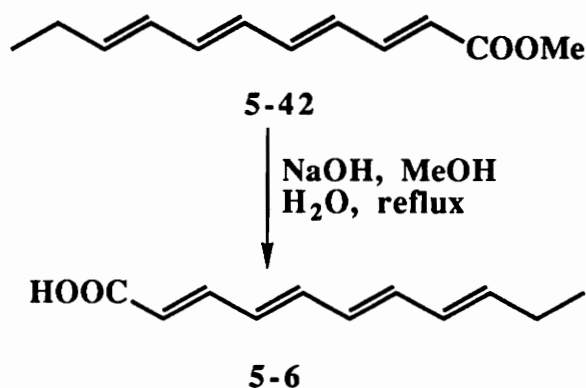
Crude 1-methoxy-2-buten-3-yne **5-37**¹⁶³ is available as a methanolic solution from Fluka. Purification by fractional vacuum distillation provided pure **5-37** needed for the synthesis. Hydrostannation of alkyne **5-37** with tri-*n*-butyltin hydride and a catalytic amount of azobisisobutyronitrile (AIBN) as a radical initiator gave the distillable vinylstannane **5-39**. Transmetalation of **5-39** with *n*-butyllithium and quenching with 2,4-heptadienal **5-33** gave the diallylic alcohol **5-40** in 83% yield. Conversion of alcohol **5-40** directly to undecatetraenal **5-41** was accomplished in a 35% yield by treatment with a catalytic amount of *p*-toluenesulfonic acid.

It was now possible to employ Corey's oxidation on the tetraenal **5-41** (Scheme 5-21). Undecatetraenal **5-41** in methyl alcohol was treated with sodium cyanide and activated manganese dioxide.^{159,160} The solution was allowed to react at room temperature for 12 hours. At the end of this time, methyl-2,4,6,8-undecatetraenoate was detected as a single spot on thin layer chromatography. This material, which eventually solidified as a brown solid, was carried on to the next step without purification.



Scheme 5-21

Hydrolysis of ester **5-42** to the acid **5-6** was carried out according to a procedure by Kucherov *et al.*¹⁶⁴ (Scheme 5-22). Ester **5-42** was treated with sodium hydroxide in a methyl alcohol-water mixture. The solution was heated to reflux during which time some insoluble beads of material formed. Acidification formed a precipitate which, after filtration, proved to be that of the desired acid, 2,4,6,8-undecatetraenoic acid **5-6**, obtained as a brown solid.



Scheme 5-22

5.5 Experimental.

Preparation of methyl-4-bromo-2-butenolate (**5-19**)

N-bromosuccimide (7.87 g , 44.2 mmol) was added to a solution of methyl crotonate (4.26 ml, 40.2 mmol) dissolved in 50 ml of anhydrous carbon tetrachloride. To this solution a catalytic amount of benzoyl peroxide was added. The reaction mixture was heated to reflux for 7 hours while being irradiated with long wave UV light (365 nm). The reaction was allowed to cool and filtered. The solvent was removed from the filtrate to give crude **5-19**. Purification by vacuum distillation (bp 63°C-72°C at 3 mm Hg) gave 4.61 g (64%) as a colorless oil. ¹H NMR (CDCl₃) δ 7.01 (m, 1H, olefinic), 6.04 (d, J = 2.4 Hz,

1H, olefinic), 4.0 (d, J = 5.4 Hz, 2H, CH_2), 3.78 (s, 3H, CH_3). ^{13}C NMR (CDCl_3) δ 141.95 (olefinic) 124.25 (olefinic), 51.83 (CH_2), 29.02 (CH_3). These spectral properties are identical to those previously described.¹⁶⁵

Preparation of [3-(methoxycarbonyl)-2-propenyl]triphenylphosphonium bromide (5-20)

Triphenylphosphine (5.75 g, 21.9 mmol) was added to a solution of methyl-4-bromocrotonate **5-19** (3.92 g, 21.9 mmol) in 50 ml of toluene. The mixture was stirred at room temperature for 60 hours. The reaction mixture was filtered by vacuum filtration and the solid washed with toluene. Drying under vacuum yielded 6.38 g (66%) of **5-20** which was used directly in the next reaction. ^1H NMR (CDCl_3) δ 7.90 (m, 15H, Ar), 6.45 (m, 2H, olefinic), 5.25 (dd, J = 7.0 and 23.0 Hz, 2H, CH_2), 3.62 (s, 3H, OCH_3). These spectral properties are identical to those previously described.¹⁶⁵

Preparation of Methyl 4-diethylphosphonate-2-butenate (5-22)

Methyl-4-bromocrotonate (4.61 g, 25.8 mmol) was added to triethylphosphite (4.42 ml, 25.8 mmol). The reaction was heated to 150°C for 3 hours. The reaction was allowed to cool to room temperature. Purification by vacuum distillation (bp 140-147°C at 2.5 mm Hg) gave 5.0 g (82%) as a colorless oil. ^1H NMR (CDCl_3) δ 6.82 (m, 1H, olefinic), 5.94 (dd, J = 5.4 and 13.5 Hz, 1H, olefinic), 4.06 (q, J = 8.1 Hz, 4H, OCH_2), 3.70 (s, 3H, OCH_3), 2.72 (dd, J = 13.5 and 23.0 Hz, 2H, PCH_2), 1.31 (t, J = 8.1 Hz, 6H, CH_2CH_3). ^{13}C NMR (CDCl_3) δ 165.97 (C=O), 137.20 (olefinic), 125.30 (olefinic), 62.14 (OCH_3), 51.34 (PCH_2), 31.95 (CH_2), 29.20 (CH_2), 16.25 (CH_3). IR (KBr) 3462, 2987, 1724, 1657, 1440, 1395, 1330, 1251, 1024, 971, 880, 836, 805, 688 cm^{-1} . These spectral properties are identical to those described in the literature.¹⁶⁶

Preparation of 2,2-dichlorovinylphenyl thioether (5-35)

Trichloroethylene (13.24 ml, 147.5 mmol) was heated to reflux and a catalytic amount of benzoylperoxide (0.033 g, 0.136 mmol) was then added. Next thiophenol (4.66 ml, 45.4 mmol) was added dropwise over a 1 hour period. Once the addition was complete, the reaction was allowed to reflux for 18 hours. The residual volatile materials were removed *in vacuo* to give an oil. Purification by vacuum distillation (bp 141-145°C at 2 mm Hg) gave 6.56 g (71%) of 5-35 as a colorless oil, (lit¹⁵⁶ 89-91°C at 0.1 mm Hg). ¹H NMR (CDCl₃) δ 7.36 (m, 5H, Ar), 6.57 (s, 1H, olefinic). The spectral data agree with that previously reported.¹⁵⁶

Preparation of 1,1-dichloro-2-(phenylsulfinyl)ethylene (5-32)

m-Chloroperbenzoic acid (9.69 g, 30.9 mmol) dissolved in 25 ml of anhydrous dichloromethane was added to a solution of 2,2-dichlorovinylphenyl thioether 5-35 (6.0 g, 29.4 mmol) dissolved in 100 ml of anhydrous dichloromethane which had been cooled to -20°C. The mixture was stirred at -15°C for 30 minutes and 50 ml of saturated sodium bicarbonate was added. The mixture was extracted with 3 x 100 ml ether and washed with 2 x 100 ml water followed by 1 x 100 ml brine. Drying over magnesium sulfate and removal of the solvent *in vacuo* gave 7.04 g of crude 5-32. Purification by flash chromatography and eluting with 40% ether / 60% hexanes gave 2.97 g of pure 5-32. ¹H NMR (CDCl₃) δ 7.70 (m, 2H, Ar), 7.57 (m, 3H, Ar), 6.67 (s, 1H, olefinic). These spectral data agree with that previously reported.¹⁵⁶

Preparation of 2-(phenylsulfinyl)-1,1,1-triethoxyethane (5-25)

21% sodium ethoxide (9.78 ml, 26.2 mmol) was added to a solution of 1,1-dichloro-2-(phenylsulfinyl) ethylene 5-32 (2.90 g, 13.1 mmol) dissolved in 15 ml of

anhydrous ethyl alcohol. The mixture was heated to reflux for 12 hours then allowed to cool to room temperature. The precipitate was separated from the supernatant by centrifugation, and the precipitate was washed with 3 x 10 ml portions of anhydrous ethyl alcohol. The supernatants were combined and the solvent removed *in vacuo* to give 4.50 g of crude (5-25). Purification by flash chromatography using 5% triethylamine / 25% ether and 70% hexanes gave 3.16 g (84%) of a brown oil. ^1H NMR (CDCl_3) δ 7.71 (m, 2H, Ar), 7.51 (m, 3H, Ar), 3.67-3.24 (m, 8H, CH_2), 1.18 (t, $J = 7.0$ Hz, 9H, CH_3). IR (KBr) 3058, 2978, 2932, 2360, 2341 1582, 1478, 1444, 1366, 1305, 1060, 753 cm^{-1} . These spectral properties are identical to those previously described.¹⁵⁰

Preparation of 1,4,6-nonatrien-3-ol (5-26)

A 2 M solution of vinyl magnesium bromide (16.32 ml, 16.3 mmol) in tetrahydrofuran was added to a solution of 2,4-heptadienal (1/70 ml, 13.6 mmol) dissolved in 25 ml of anhydrous tetrahydrofuran which had been cooled to 0°C. It was allowed to warm to room temperature over a 24 hour period. Saturated ammonium chloride (20 ml) along with 30 ml of water was added to the reaction. This was extracted with 3 x 50 ml of ether. The extracts were combined and washed with 2 x 50 ml water followed by 1 x 50 ml brine. The extracts were dried over magnesium sulfate, filtered, and the solvent removed *in vacuo* to give 1.87 (100%) of a yellow oil as a single spot on TLC. ^1H NMR (CDCl_3) δ 6.29-5.63 (m, 5H, olefinic), 5.19 (m, 2H, olefinic), 4.65 (broad s, 1H, CH), 2.11 (q, $J = 10.0$ Hz, 2H, CH_2), 1.01 (t, $J = 10.0$ Hz, 3H, CH_3). ^{13}C NMR (CDCl_3) δ 139.53 (olefinic), 137.50 (olefinic), 131.40 (olefinic), 128.43 (olefinic), 114.90 (olefinic), 73.57 (CH), 67.94 (CH), 25.60 (CH_2), 13.38 (CH_3). IR (neat); 3385, 3081, 3015, 2964, 2933, 2873, 1058, 988, 921 cm^{-1} . MS m/z (relative intensity) 138 (M^+ , 2), 123 (3), 109 (26), 95 (27), 91 (16), 81 (30), 68 (32), 55 (100).

Attempted preparation of ethyl 2,4,6,8-tetraundecenoate by the Claisen route (5-34)

Compound 5-26 (0.118 g, 8.55 mmol) along with 2-(phenylsulfinyl)-1,1,1-triethoxyethane 5-25 (0.489 g, 1.71 mmol) were placed in a hydrolysis tube. Next a catalytic amount of 2,4,6-trimethylbenzoic acid (0.015 g, 0.088 mmol) dissolved in 1.2 ml of anhydrous dichloromethane was added. The hydrolysis tube was purged with argon, sealed, and heated at 120°C for 15 hours. The reaction was allowed to cool, and the solvent was removed *in vacuo* to give 0.532 g of an inseparable mixture of products.

Preparation of methyl 2,4-heptadienoate (5-36)

Sodium cyanide (0.118 g, 2.41 mmol), activated manganese (IV) oxide (0.829 g, 9.53 mmol), and glacial acetic acid (0.042 ml, 0.726 mmol) were added to 5 ml of anhydrous methyl alcohol. To this solution 2,4-heptadienal (0.057 ml, 0.454 mmol) was added. The mixture was stirred at room temperature for 12 hours, and the contents then filtered through a pad of Celite. The solvent was removed from the filtrate *in vacuo*, and the residue taken up in 15 ml of ether. The solution was washed with 25 ml of water, and the aqueous phase was extracted with 3 x 25 ml of ether. The organic extracts were combined and washed with 2 x 25 ml water followed by 1 x 25 ml brine. The extracts were dried over magnesium sulfate, filtered, and the solvent removed *in vacuo* to give 0.064 g (100%) of 5-36 as a single spot on TLC. ¹H NMR (CDCl₃) δ 7.27 (m, 1H, olefinic), 6.17 (m, 2H, olefinic), 5.79 (d, J = 15.3 Hz, 1H, olefinic), 3.74 (s, 3H, OCH₃), 2.19 (m, 2H, CH₂), 1.05 (t, J = 7.4 Hz, 3H, CH₃). MS *m/z* (relative intensity) 140 (M⁺, 28), 125 (4), 111 (100), 109 (24), 97 (6), 81 (56), 65 (10), 53 (24). The spectral data are in agreement with previously prepared material.¹⁶⁷

Preparation of tributyl(4-methoxy-1,3-butadienyl)stannane (5-39)

Freshly distilled 1-methoxy-1-buten-3-yne (4.52 g, 55.1 mmol) and *n*-tributyltin hydride (18.51 ml, 41.3 mmol) were combined. To this solution a catalytic amount of azobisisobutyronitrile (0.068 g, 7.5 mmol) was added and the solution was heated to 90°C for 12 hours. The solution was allowed to cool, and the product was purified by vacuum distillation, (bp 120-135°C at 0.1 mm Hg) to give 17.0 g (83%) of **5-39** as a colorless oil. ¹H NMR (CDCl₃) δ 6.84 (d, J = 13.0 Hz, 1H, olefinic), 6.59 (dd, J = 9.8 Hz, 17.7 Hz, 1H olefinic), 6.09 (m, 1H, olefinic), 5.78 (dd, J = 11.3 and 21.5 Hz, 1H, olefinic), 3.80 (s, 3H, OCH₃), 1.75 (m, 6H, CH₂Sn), 1.57 (m, 6H, CH₂), 1.32 (m, 6H, CH₂), 1.13 (t, J = 6.3 Hz, 9H, CH₃). IR (neat) 2925, 2875, 1634, 1598, 1461, 1215, 1115 cm⁻¹. The spectral data are identical to previously prepared material.¹⁶²

Preparation of undeca-2,4,6,8-tetraenal (5-41)

A 2.5 M solution of *n*-butyllithium (18.21 ml, 45.5 mmol) was slowly added to a solution of tributyl (4-methoxy-1,3-butadienyl)stannane **5-39** (16.96 g, 45.5 mmol) dissolved in 60 ml of anhydrous THF which had been cooled to -78°C. The reaction was stirred at -78°C for 1 hour then 2,4-heptadienal (5.21 ml, 41.7 mmol) was added dropwise. It was allowed to stir at -78°C for 4 hours then 25 ml of saturated sodium bicarbonate was added and allowed to warm to room temperature. The reaction mixture was extracted with 3 x 150 ml of ether. The combined ether extracts were washed with 2 x 100 ml of brine and dried over magnesium sulfate. The extracts were filtered, and the solvent removed *in vacuo* to give a yellow liquid which was immediately dissolved in 40 ml of aqueous 5% tetrahydrofuran and cooled to 0°C. A catalytic amount of *p*-toluenesulfonic acid (0.317 g, 1.7 mmol) was added and stirring was continued at 0°C for 1 hour. The solvent was removed *in vacuo* to give 16.7 g of crude **5-41**. Purification by flash chromatography and eluting with 40% ether / 60% hexanes using silica gel which was pre-treated with 10%

triethyl amine in hexanes gave 2.31 g (34%) of yellow crystals. $^1\text{H NMR}$ (CDCl_3) δ 9.53 (d, $J = 7.2$ Hz, 1H, CHO), 7.11 (dd, $J = 10.7$ and 13.8 Hz, 1H, olefinic), 6.67 (dd, $J = 9.9$ and 13.6 Hz, 1H, olefinic), 6.43 (dd, $J = 9.6$ and 9.8 Hz, 1H, olefinic), 6.39 (dd, $J = 9.5$ and 10.0 Hz, 1H, olefinic), 6.22 (dd, $J = 13.4$ and 10.6 Hz, 1H, olefinic), 6.11 (m, 2H, olefinic), 5.94 (m, 1H, olefinic), 2.75 (m, 2H, CH_2), 1.02 (t, $J = 7.1$ Hz, 3H, CH_3). IR (KBr) 3024, 1693, 1609, 1131, 1011 cm^{-1} . The spectral data are identical to that previously reported.¹⁶²

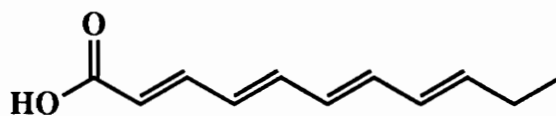
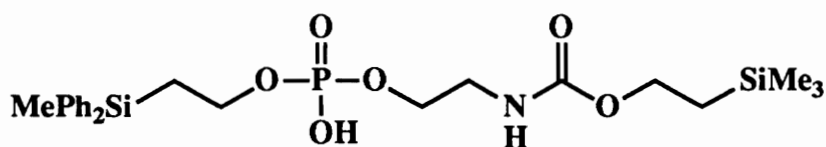
Preparation of 2,4,6,8-undecenoic acid (5-6)

Sodium cyanide (3.70 g 75.6 mmol), activated manganese (IV) oxide (26.03 g, 299.4 mmol) and glacial acetic acid (1.42 ml, 22.8 mmol) were dissolved in 120 ml anhydrous methyl alcohol. To this a solution of **5-41** (2.31 g, 14.3 mmol) dissolved in 30 ml of anhydrous methyl alcohol was added. The reaction was protected from light by wrapping in aluminum foil, and it was stirred at room temperature for 15 hours. The reaction mixture was filtered through a pad of Celite, and the solvent was removed *in vacuo* to give 2.73 g (100%) of **5-42** as a brown solid. Next sodium hydroxide (0.972 g, 24.3 mmol) dissolved in 11 ml of distilled water was added to a solution of **5-42** (2.75 g, 14.3 mmol) in 50 ml of methyl alcohol. The mixture was heated to reflux for 30 minutes. During this time some insoluble material formed. The reaction was allowed to cool to room temperature and then acidified with 10% HCl. A white precipitate formed which was filtered off and washed with water to give 1.54 g (60.4%) of a brown solid **5-22** which was sufficiently pure for the next reaction. $^1\text{H NMR}$ (CDCl_3) δ 7.32 (dd, $J = 15.0$ and 20.6 Hz, 1H, olefinic), 6.71 (dd, $J = 14.1$ and 19.9 Hz, 1H, olefinic), 6.54-6.12 (m, 4H, olefinic), 5.98-5.84 (m, 2H, olefinic), 2.14 (q, $J = 9.0$ Hz, 2H, CH_2), 1.00 (t, $J = 10.0$ Hz, 3H, CH_3). MS (EI) m/z (relative intensity) 128 (M^+ , 37), 149 (12), 133 (23), 117 (33), 105 (33), 99 (100), 77 (49), 65 (30).

VI. Attempted Synthesis of Plasmalopentaene-12

6.1 Introduction.

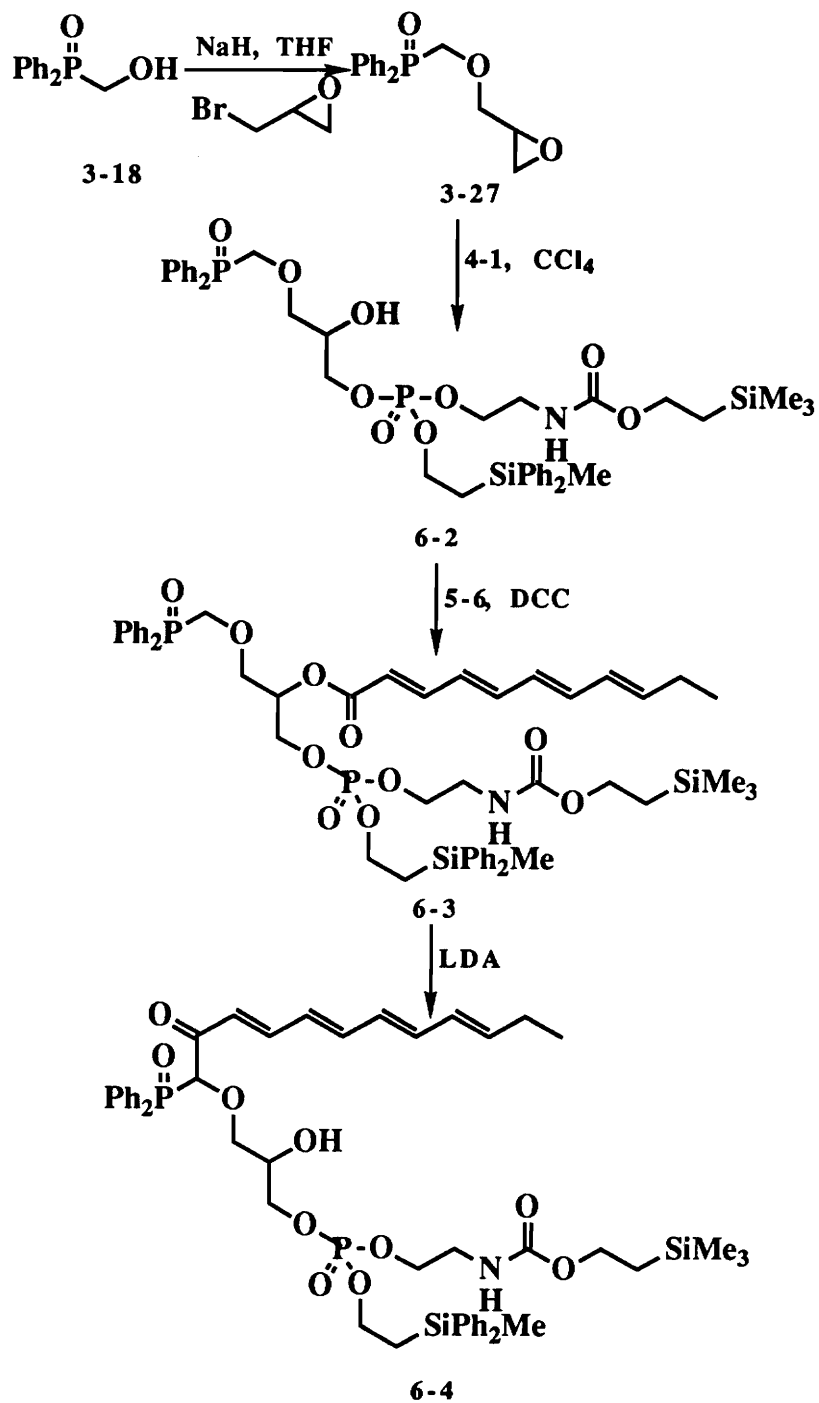
Once the protected phosphate **4-1** and the tetraenoic acid **5-6** were synthesized it was now possible to attempt the synthesis of plasmalopentaene-12 utilizing the previously discussed acyl-migration pathway. The attractiveness of the proposed synthetic scheme, demonstrated in Scheme 6-1, lies in the fact that the pentaenyl ether moiety is generated late in the synthesis. This helps alleviate any problems associated with the inherent instability of the molecule thus making the synthesis easier and less cumbersome.



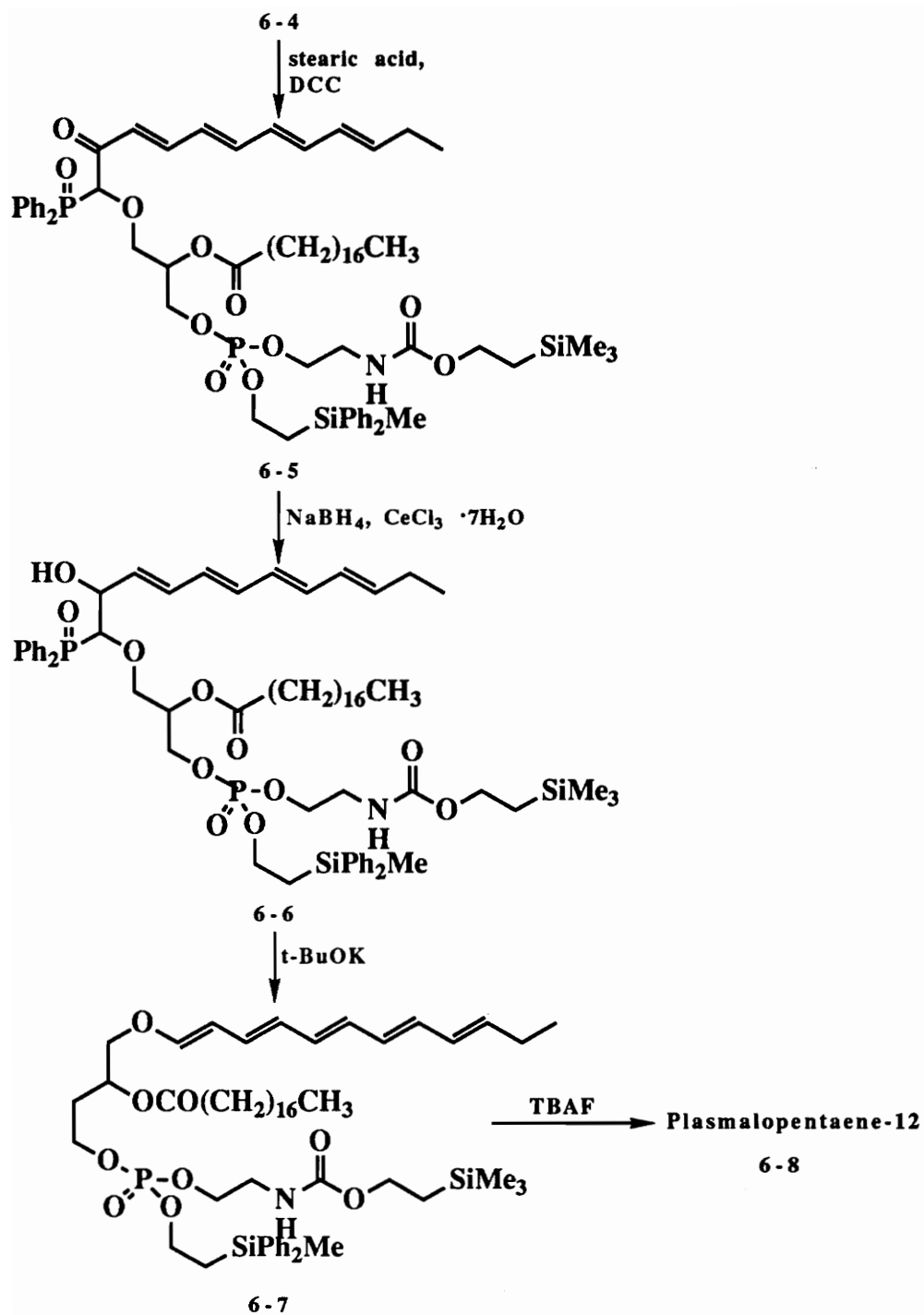
The first step of the proposed synthesis is coupling the phosphine oxide **3-18** with epibromohydrin utilizing the Williamson synthesis. This then gives the oxirane **3-27**. Next the epoxide ring is opened using the previously synthesized protected phosphate **4-1** giving the phosphotriester **6-2**. Acylation of the secondary alcohol of **6-2** with the tetraenoic acid **5-6** using dicyclohexylcarbodiimide would then afford the ester **6-3**. As the key step, abstraction of the proton α to the phosphine oxide with lithium diisopropyl amide results in intramolecular attack of the anion on the ester carbonyl. This gives the ketone **6-4** which is in reality a "masked" pentaenyl ether and not as susceptible to electrophiles and

nucleophiles. The secondary hydroxyl of **6-4** can then be reacylated with stearic acid using dicyclohexylcarbodiimide to give **6-5**. Compound **6-5** now has all the functional groups in place necessary to synthesize plasmalopentaene-12. It now becomes a matter of several synthetic manipulations to produce plasmalopentaene-12. Selective 1,2-reduction of the ketone of **6-5** using sodium borohydride and cerium chloride heptahydrate then would give the alcohol **6-6**. Treatment of the alcohol with potassium *t*-butoxide then would cause it to undergo Wittig elimination to generate the pentaenyl ether as seen in compound **6-7**. Finally, deprotecting the phosphate using fluoride ion would ultimately arrive at plasmalopentaene-12. This synthesis would not only generate the pentaenyl ether in a late step, as already mentioned, but it would offer a short and convenient synthesis to this highly challenging and unstable molecule.

All the experimental studies discussed in Chapter 3 indicate that this proposed route to plasmalopentaene-12 is a workable approach having much potential. By using this method it should be possible to synthesize the target molecule in a short efficient manner. There are, however, some potential problems which must be considered and these will be discussed at a later point in the chapter. As will be seen, this was not the most feasible methodology for the synthesis of plasmalopentaene-12. Several problems encountered will become evident which ultimately led to the failure of the synthesis. However, an alternate route for the synthesis of fecapentaene-12 and its analogs was developed as a result of this synthetic methodology.



Scheme 6-1

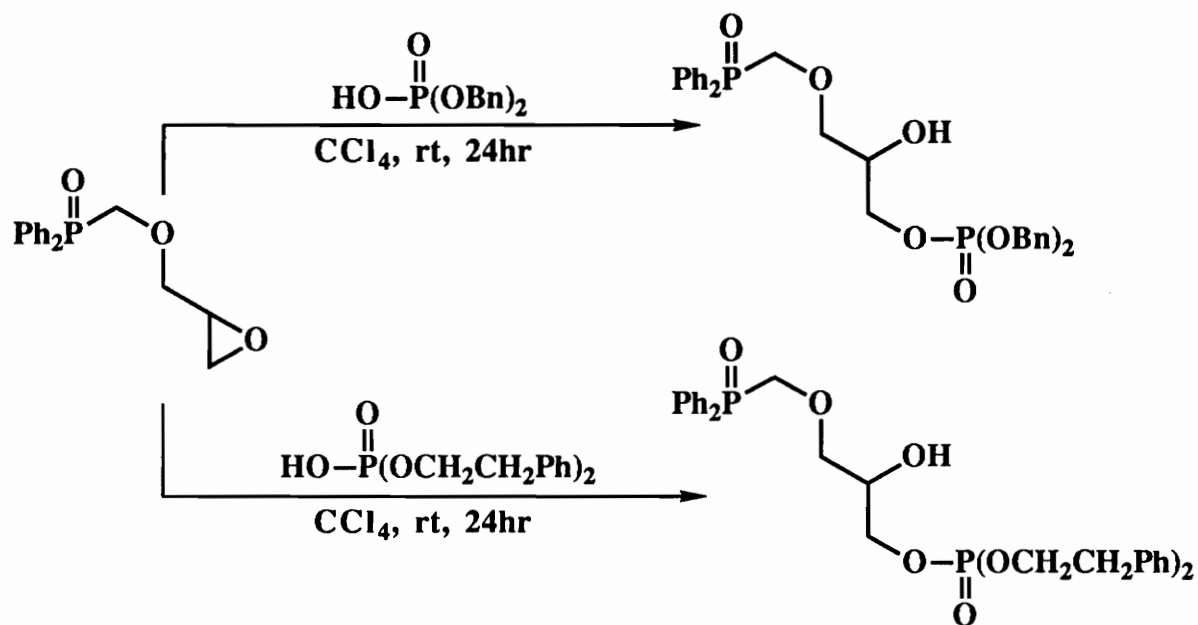


Scheme 6-1. Continued

6.2 Attachment of the Phosphate.

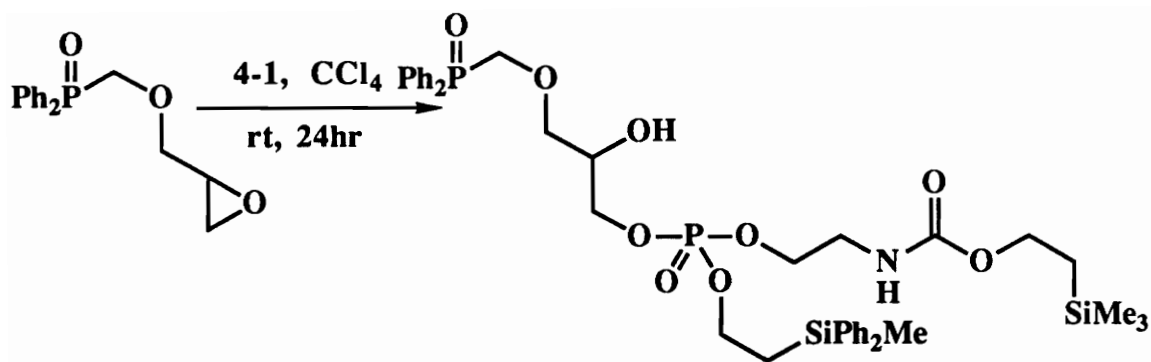
6.2.1 Ring Opening of the Oxirane With the Protected Phosphate.

The second step of the proposed synthesis as shown in Scheme 6-1 involves opening of the epoxide ring with the protected phosphate 4-1. The feasibility of this reaction was demonstrated first by opening the oxirane with dibenzylphosphate then with diphenethylphosphate (Scheme 6-2).

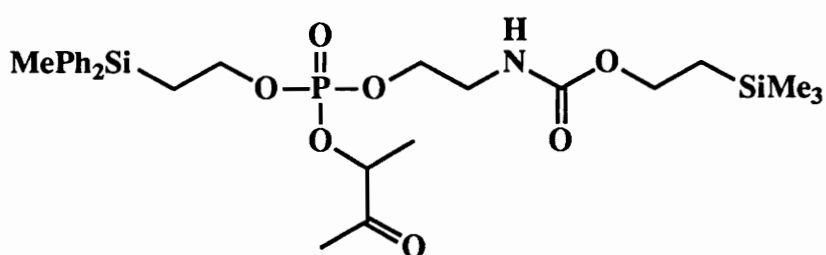


Scheme 6-2

Based on these two model studies, it should be possible to open the epoxide with the protected phosphate 4-1 using the same conditions as those previously used (Scheme 6-3). Since the synthesis of the protected phosphate 4-1 involves hydrolysis of the acetoin group of 4-64 to the sodium salt 4-65, it was necessary to first protonate the salt to the free phosphate 4-1 (Scheme 6-4).

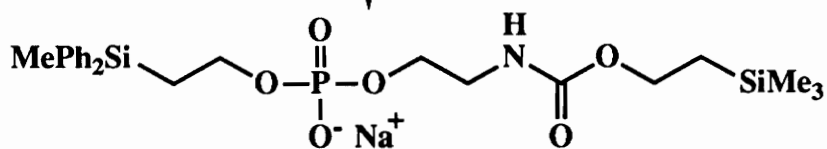


Scheme 6-3



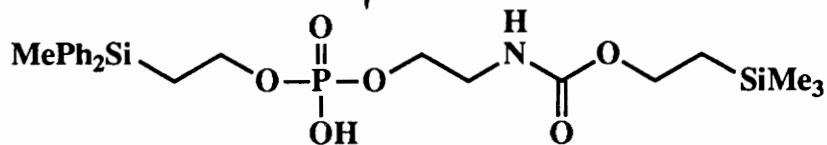
4-64

$\text{Na}_2\text{CO}_3, \text{CH}_3\text{CN} / \text{H}_2\text{O}, \text{rt}$



4-65

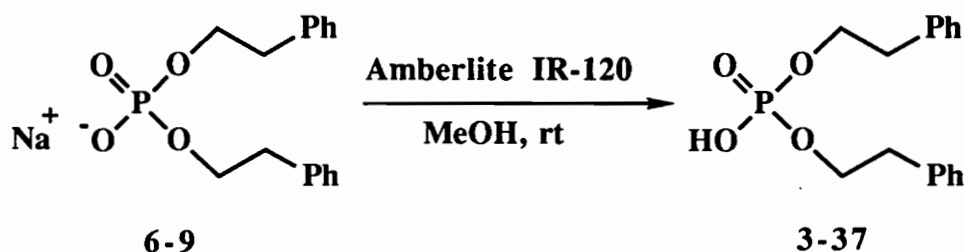
H^+



4-1

Scheme 6-4

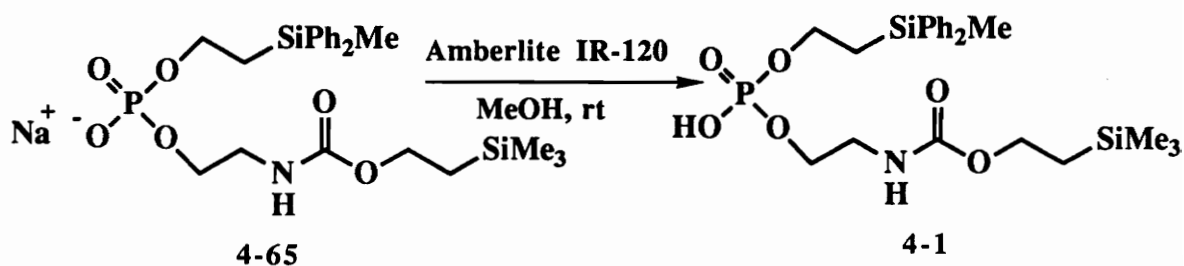
At first glance the protonation appears to be a trivial matter, but after further inspection it becomes evident that care must be exercised when the protonation is performed. The presence of the acid labile C-silyl groups makes use of very acidic conditions impractical. It was decided that the use of an acidic ion exchange resin may afford the protonation without C-silyl cleavage. The conditions for the protonation were determined using a model reaction by treating the sodium salt of diphenethylphosphate **6-9** with Amberlite IR-120 to give diphenethylphosphate **3-37** (Scheme 6-5). This was a convenient model compound since diphenethylphosphate **3-37**, synthesized by a previous method, had been fully characterized, and therefore NMR and TLC comparisons could be made with an authentic product.



Scheme 6-5

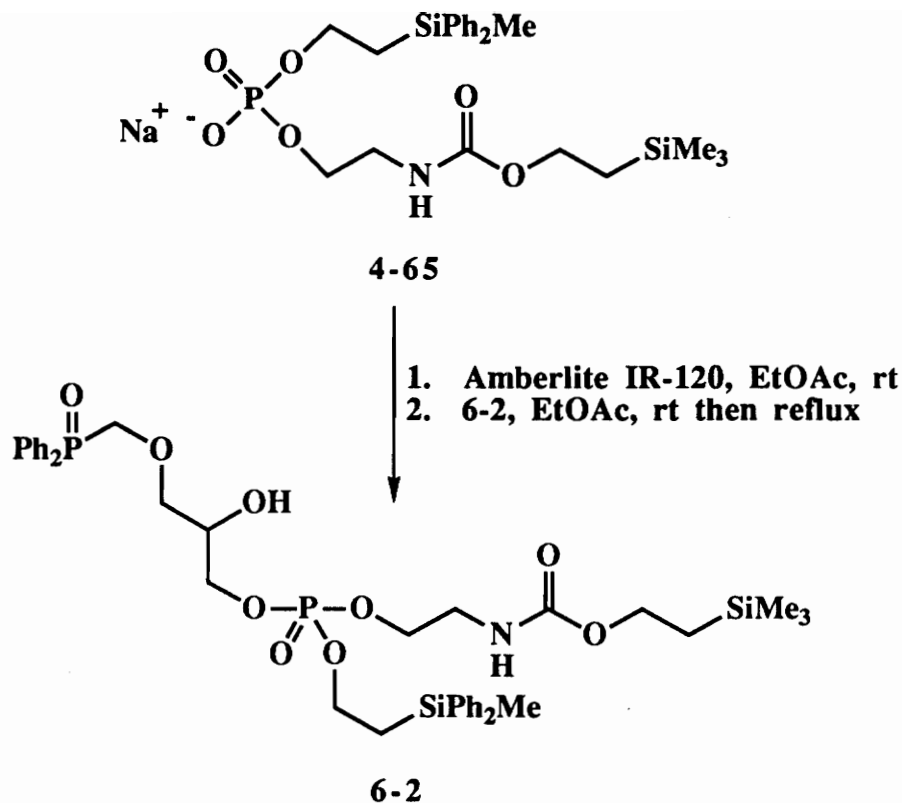
Using these conditions, it was now possible to protonate the sodium salt of the protected phosphate **4-65** and demonstrated whether or not the silyl groups will stay intact (Scheme 6-6). This reaction was hampered by the fact that the product and the starting material have the same R_f on TLC; the analytical data were inconclusive, and there was no authentic product available for comparative studies. It therefore had to be assumed that since protonation occurred under the conditions applied in Scheme 6-5 it would, by

analogy, have occurred using the same conditions as shown in Scheme 6-6. Making this assumption, it was now possible to react the epoxide with the protected phosphate as depicted in Scheme 6-3.



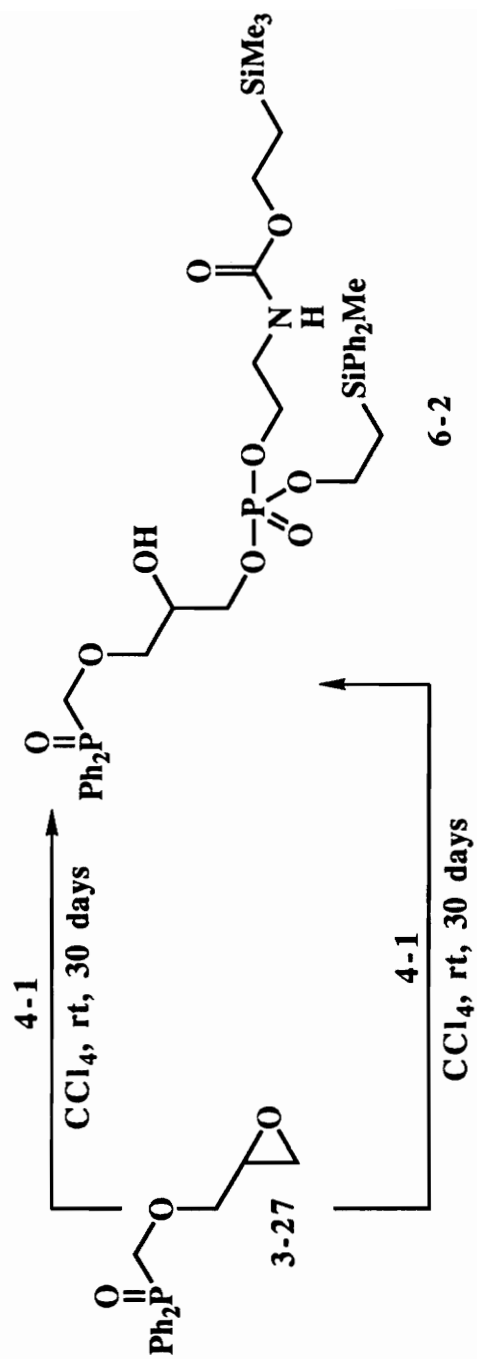
Scheme 6-6

Initial attempts focused on a one pot reaction in which the phosphate was protonated then reacted with the epoxide to give the desired product (Scheme 6-7). The sodium salt 4-65 was treated with freshly activated Amberlite IR-120 in ethyl acetate to afford the phosphate 4-1. Without isolation or purification, oxirane 3-27, in an ethyl acetate solution, was immediately added. The reaction was allowed to stir at room temperature before refluxing for several hours. Isolation of products revealed low yields of product, about 10%. The reaction was repeated several more times in attempts to improve the yields. In each case, the yields were low regardless of the reaction conditions used.



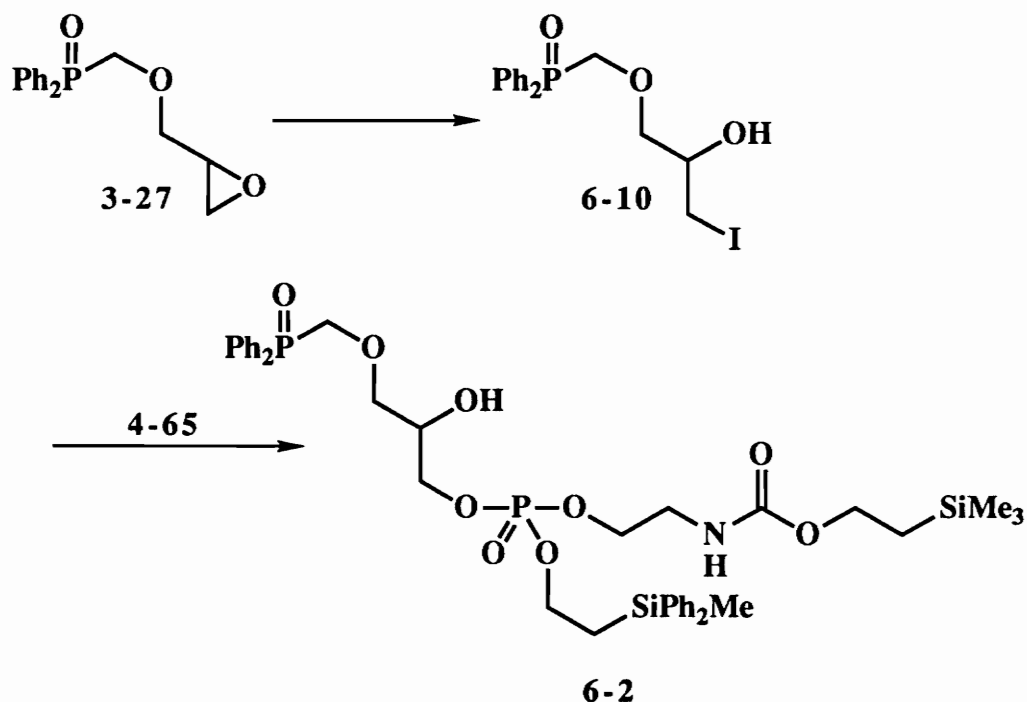
Scheme 6-7

It was decided to simplify the reaction by first protonating the sodium salt **4-65** then isolating the product. Next the protected phosphate **4-1** could be reacted with the epoxide **3-18** in a separate step. Two reactions were run. Each used the same conditions but the solvent used as the reaction medium varied. First, carbon tetrachloride was used as the solvent. This would exactly mimic the conditions used for ring opening with debenzylphosphate and diphenethylphosphate. Second, ethyl acetate was used as a solvent. From previous observations, it was noted that ethyl acetate may be a better reaction solvent (Scheme 6-8). Using ethyl acetate as a solvent produced some product, but after one month the yields were not appreciably increased. When carbon tetrachloride was used, there was no product formation.



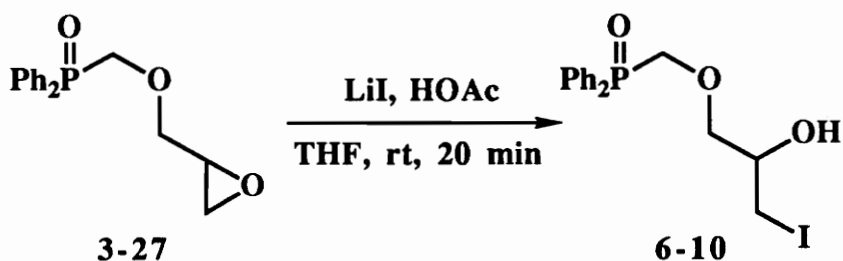
Scheme 6-8

Since the epoxide did not open with the protected phosphate **4-1**, and it was not entirely clear whether the phosphate had indeed been protonated, it was decided that possibly the salt **4-65** could be used to affect the oxirane ring opening. It was envisioned that if the iodohydrin **6-10** could be produced from the epoxide **3-27** then possibly the salt of the phosphate **4-65** would displace the iodide thus giving the desired product (Scheme 6-9).



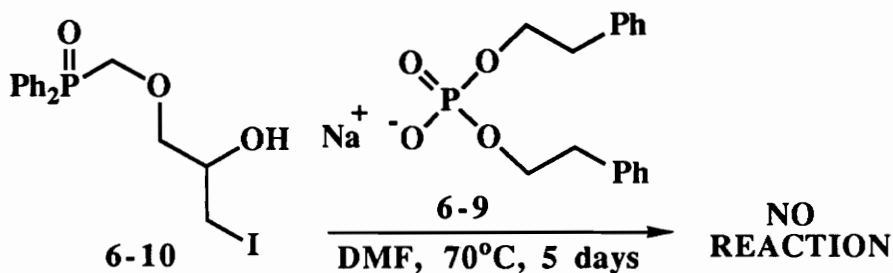
Scheme 6-9

To test the feasibility of this reaction, the readily available sodium salt of diphenylethyl phosphate **6-9** was used as a model reaction. The iodohydrin **6-10** was readily produced from epoxide **3-27** by treatment of the epoxide with lithium iodide in acetic acid using the conditions of Bajwa¹⁶⁸ (Scheme 6-10).



Scheme 6-10

When the iodohydrin **6-10** was reacted with the salt **6-9** in dimethylformamide at 70°C, no reaction occurred, even after five days (Scheme 6-11).

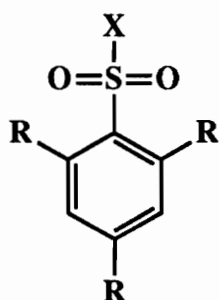


Scheme 6-11

It was felt, based on these previously discussed experiments, that possibly the large groups attached to the phosphorus on phosphate **4-1** create much more steric hindrance than realized, especially compared to that of dibenzylphosphate and diphenethylphosphate. This added bulk "hides" the nucleophilic portion of the phosphate, thus severely inhibiting its reactivity towards the epoxide. Another method of generating the phosphate **6-2** was needed.

6.2.2 Coupling the protected Phosphate with an Alcohol.

In the past decade oligodeoxyribonucleotides have played an important role in molecular biological studies. These applications as well as those found in chemistry have prompted the development of newer and more efficient synthetic methods for phosphate synthesis. Most of these synthetic methods involve the formation of a phosphotriester linkage which is subsequently converted to the desired phosphodiester bond. A key step in phosphotriester synthesis is activation of a phosphodiester by use of a coupling reagent.¹⁶⁹ The coupling reagents most commonly used are sulfonyl chlorides (6-11a)¹⁷⁰ and theirazole analogs (6-11b and 6-11c).^{171,172} These coupling reagents suffer several deficiencies which take away from their usefulness.

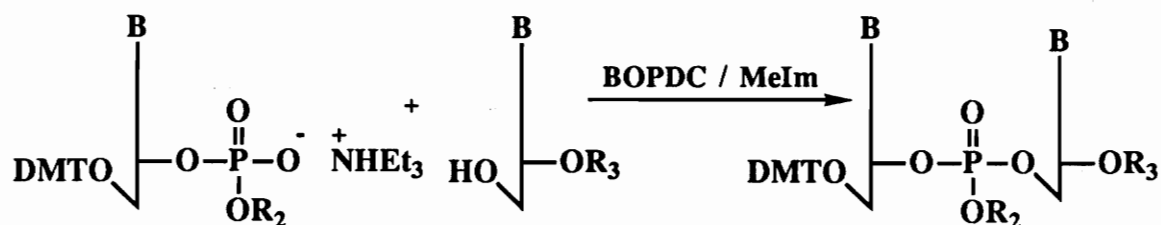


6-11a R = H, CH₃, CH(CH₃)₂, X = Cl

6-11b X = 3-nitro-1,2,4-triazole

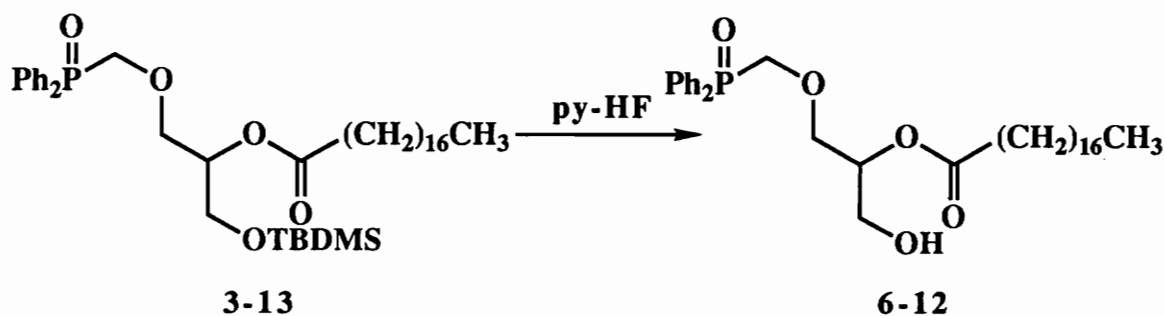
6-11c X = tetrazole

These problems were circumvented when Palomo-Coll *et al.*^{173,174} observed that carboxylic acids form symmetrical carboxylic acid anhydrides when reacted with N,N-bis[2-oxo-3-oxazolidinyl]phosphordiamidic chloride (BOPDC). Since Kan *et al.*¹⁷⁵ suggested that phosphoric anhydrides were intermediates in their synthesis of phosphotriesters Katti and co-workers¹⁷⁶ found that BOPDC was a useful coupling reagent for the synthesis of oligodeoxyribonucleotides (Scheme 6-12).



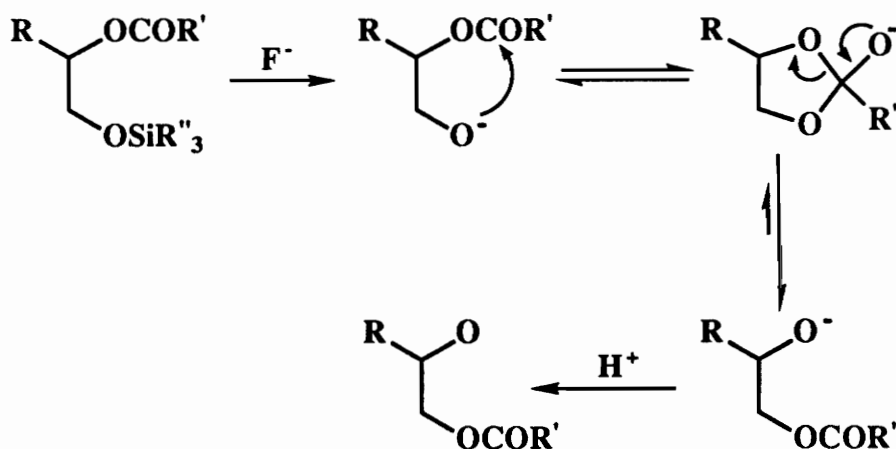
Scheme 6-12

Use of this method would require the availability of the primary alcohol **6-12** which could in principle be obtained by deprotection of the silyl ether **3-13** (Scheme 6-13).



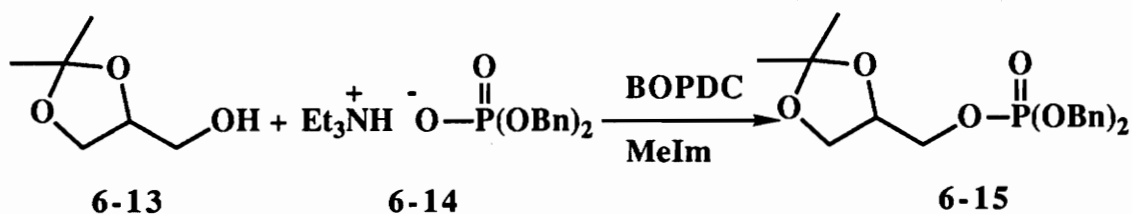
Scheme 6-13

Normally the use of conventional silyl deprotecting agents such as tetra-*n*-butyl ammonium fluoride gives the acyl-transferred product via the mechanism of Scheme 6-14, but deprotection using pyridinium hydrogen fluoride does not cause this acyl migration to occur because the alkoxide intermediate is rapidly protonated by the pyridinium salt.



Scheme 6-14

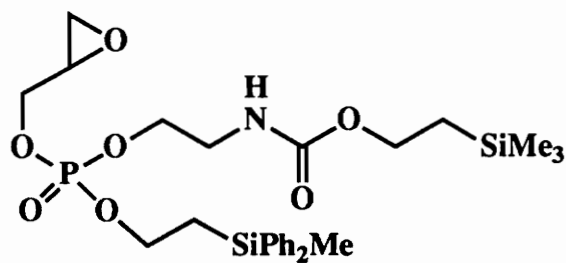
Application of this reaction to **3-13** gave the desired non-acyl transferred product **6-12** in high yield, although care must be taken to monitor the reaction, since it was found that acyl transfer did occur with lengthy reaction times. Since the primary alcohol **6-12** is readily available the BOPDC method of coupling phosphates to alcohols may prove useful. To demonstrate the utility of this reagent for our purposes a model reaction was run in which coupling between solketal **6-13** and the triethyl ammonium salt of dibenzylphosphate **6-14** to give **6-15** was attempted (Scheme 6-15). The reaction was attempted several times. Each time it failed. Since the reaction did not work on a model, no further attempts were made to generate **6-2** by this method.



Scheme 6-15

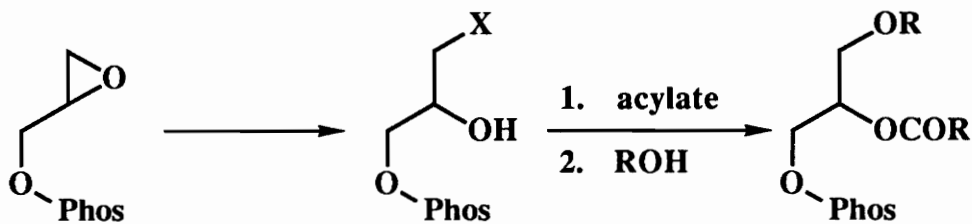
6.2.3 Synthesis of Phosphotriester 6-3 Via a Bromohydrin.

Not any of the previously described attempts to produce the phosphotriester 6-2 have worked. Since the phosphate 4-1 did not open an epoxide and since the phosphate could not be coupled to an alcohol, it was decided that possibly an alternate route to the phosphotriester 6-2 would be to choose a starting material where the protected phosphate was already in place. The envisioned candidate was the epoxide 6-16.

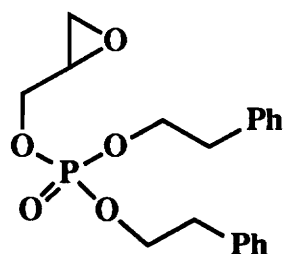


6-16

Conversion of the epoxide 6-16 to a halohydrin would then provide a method to obtain 6-2 by an indirect route (Scheme 6-16). Before the synthesis was attempted, it was decided that the phosphate model 6-17 would be used.

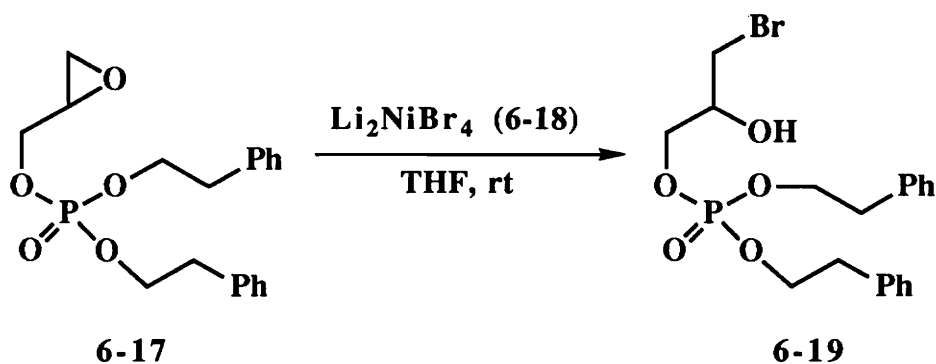


Scheme 6-16



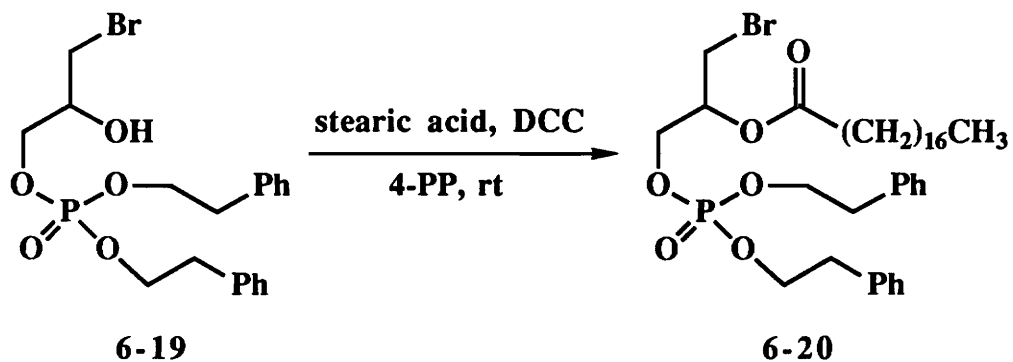
6-17

Epoxide **6-17**,¹⁷⁷ which is easily prepared from diphenethylphosphate and epibromohydrin, was reacted with dilithium tetrabromonickelate (II)¹⁷⁸ **6-18** to give in high yield bromohydrin **6-19** (Scheme 6-17).



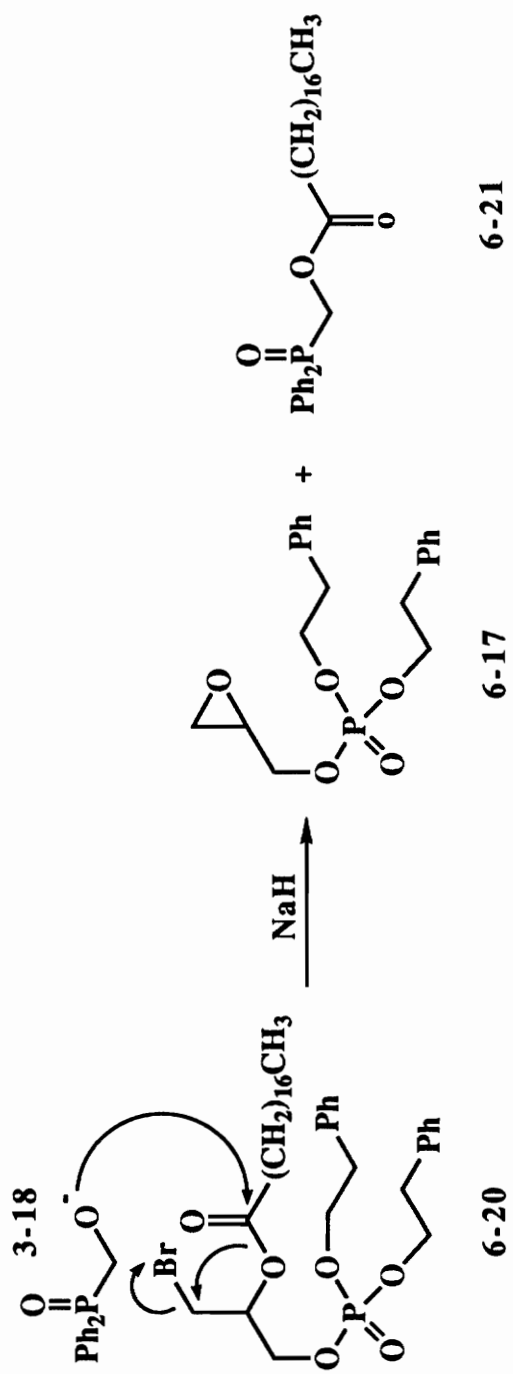
Scheme 6-17

Acylation of bromohydrin **6-19** with stearic acid using dicyclohexylcarbodiimide as a dehydration agent then gave the bromoester **6-20** which was obtained in high yield (Scheme 6-18).



Scheme 6-18

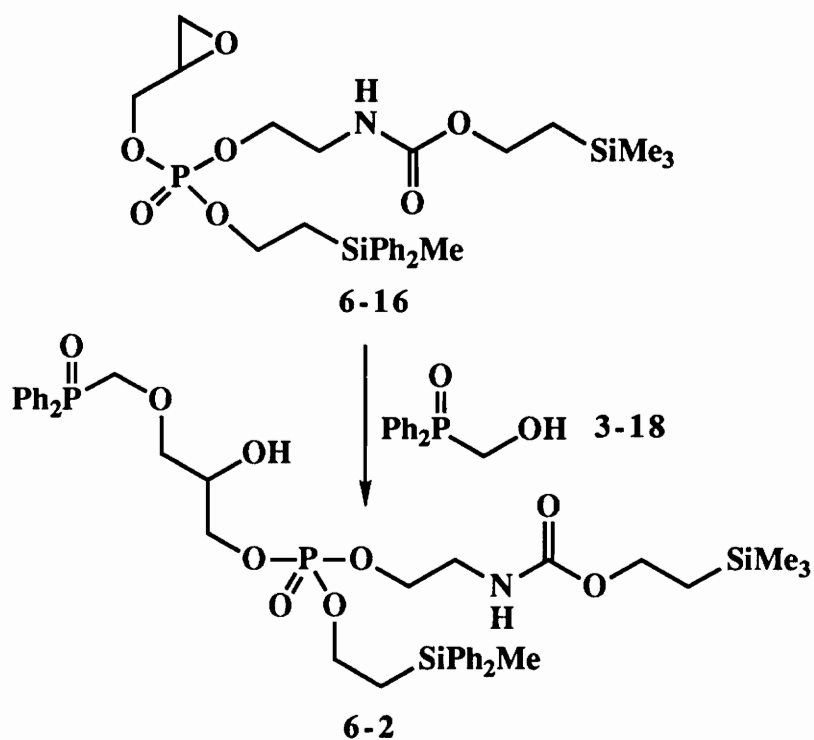
It was now hoped that the bromine of the bromoester **6-20** could be displaced in an S_N2 fashion with the alcohol **3-18**. When the bromoester **6-20** and hydroxymethyldiphenylphosphine oxide **3-18** were reacted in the presence of sodium hydride it was hoped that the displacement would predominate over elimination. To our surprise, when the reaction was run a mixture of products was obtained, in which the major products were identified as **6-21** and the epoxide **6-17** (Scheme 6-19). Apparently, the added steric bulk of the stearate ester and the diphenethylphosphate groups prevents nucleophilic displacement. Attack of the alkoxide on the more accessible ester carbonyl readily generates the epoxide **6-17** and the ester **6-21**.



6.2.4 Opening of Epoxides with Alcohols.

It has been known for a long time that epoxides can be opened by nucleophilic reagents. Most of these reactions utilize highly acidic conditions, high temperatures, and frequently extensive polymerization is encountered.^{179,180} Some of the reagents commonly used for the opening of epoxides by alcohols are Nafion-H Catalyst,¹⁸⁰ dehydrated alumina,¹⁸¹ and organotin phosphate condensates.¹⁸² Recently Iranpoor *et al.* have developed methods of opening epoxides with alcohols under neutral conditions with high regioselectivity using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ),¹⁸³ and ceric ammonium nitrate.^{184,185}

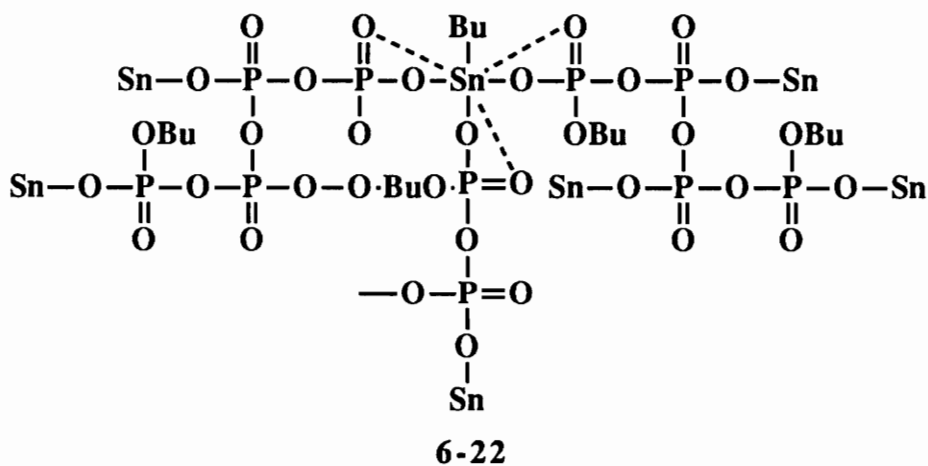
Since the bromine of **6-20** could not be displaced, then possibly the epoxide of **6-16** could be opened with the alcohol **3-18** to give **6-2** (Scheme 6-20).



Scheme 6-20

Previously, it was demonstrated in our laboratories that an epoxide could not be opened with an alcohol using DDQ without a high molar excess of the alcohol.¹⁸⁶ Ivanpoor's methodology involving the use of a large excess of alcohol was not feasible in our case so a 1:1 stoichiometric ratio of alcohol to epoxide in an inert solvent was attempted. No ring opening occurred so this methodology was discontinued.

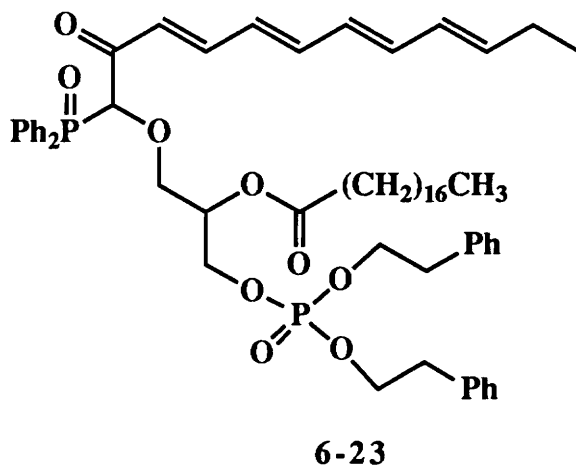
Recently, Otera *et al.*¹⁸⁷ demonstrated that epoxides can be opened with alcohols using organotin phosphate condensates **6-22**. This compound is prepared by heating dibutyltin oxide and tributyl phosphate at 250°C under reduced pressure.¹⁸⁸ It can then be used directly as a reagent for opening epoxides with alcohols. Unfortunately, all attempts at synthesizing the organotin phosphate condensate **6-22** failed, and the idea of opening the epoxide with an alcohol was discontinued. Perhaps the most promising method of generating **6-2** is by some modification of the bromide displacement reaction shown in Scheme 6-19.



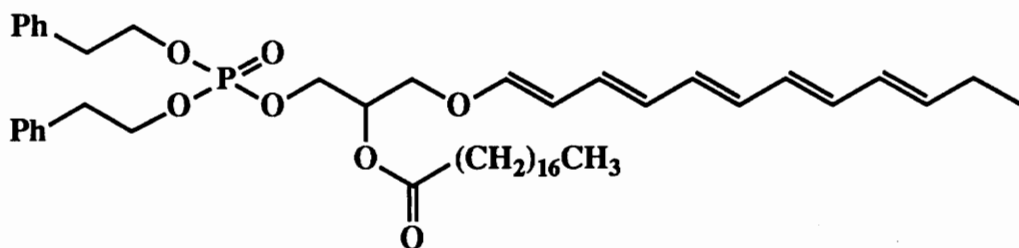
6.3 Synthesis of a Precursor Analog.

6.3.1 Synthesis of the Diphenethylphosphate Precursor Analog--Generation of the Pentaenyl Ether.

A potential problem with the proposed synthesis (Scheme 6-1) is the reduction and elimination of the phosphine oxide to generate the pentaenyl ether. It was of some concern whether or not the ketone of compound **6-5** (Scheme 6-1) could undergo a 1,2-reduction with sodium borohydride in the presence of the extended conjugation. It has been demonstrated on enones that 1,2-reduction versus 1,4-reduction predominates when sodium borohydride in the presence of cerium chloride heptahydrate is used.¹⁸⁹ To our knowledge 1,2-reduction of polyenones with this reagent has never been demonstrated. It, therefore, was deemed necessary to test this before continuing with the synthesis of plasmalopentaene-12. To determine whether or not a 1,2-reduction would occur as depicted in Scheme 6-1, a synthesis of compound **6-23** was needed.

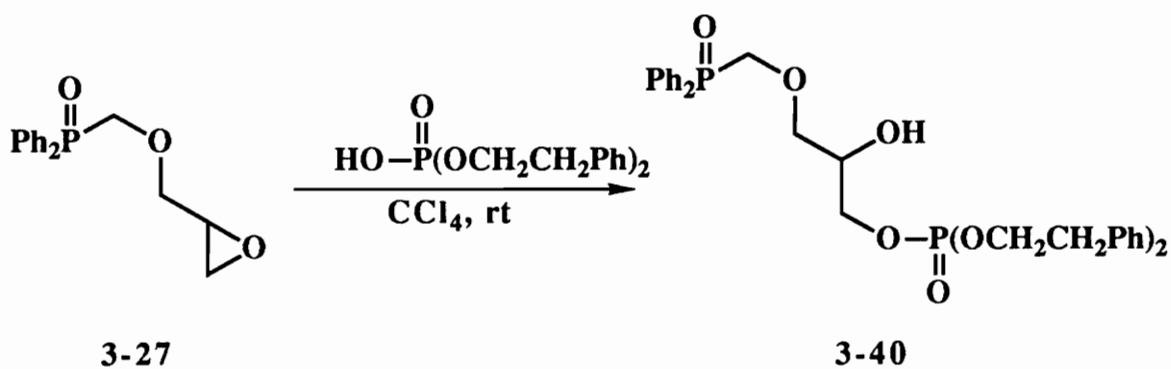


Reduction of the ketone of **6-23** followed by elimination would then give the analog **6-24**.



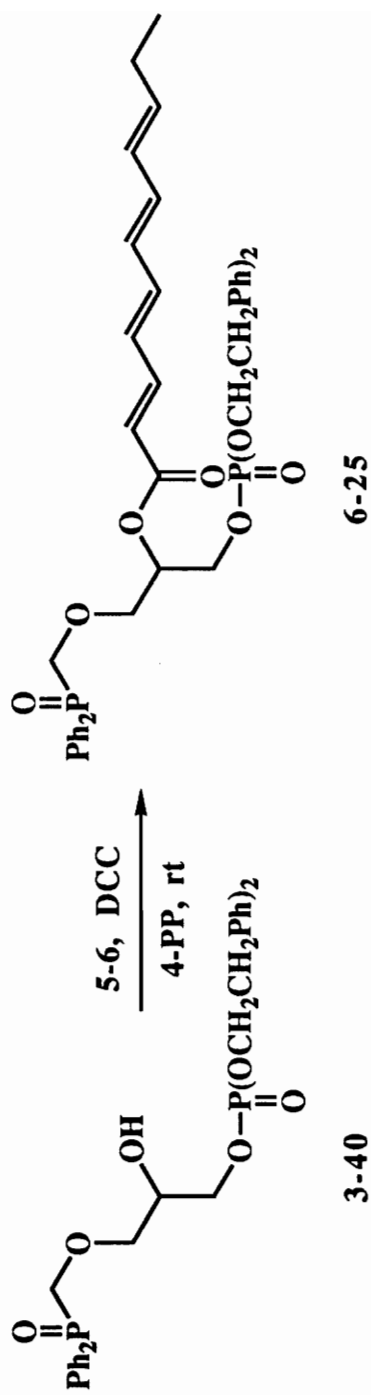
6-24

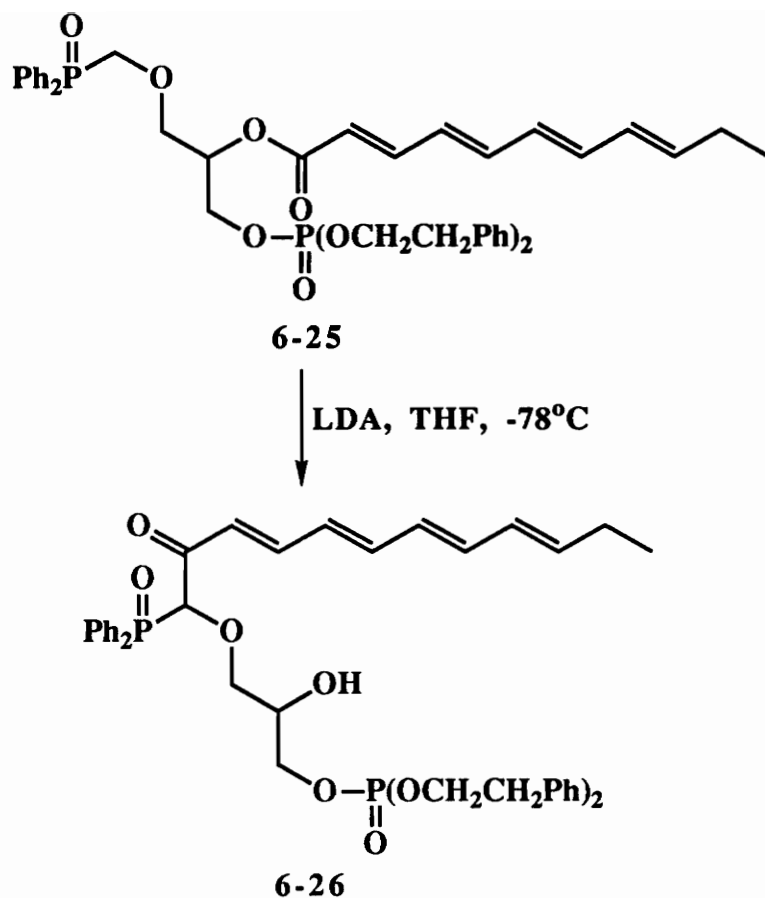
Initiation of this synthesis proceeded by reaction of diphenethylphosphate 3-37 with the oxirane 3-27 (Scheme 6-21). This reaction, which was previously discussed, involves nucleophilic attack of diphenethylphosphate at the methylene carbon of oxirane of 3-27 to open the ring giving 3-40. This reaction proceeded with extremely high regioselectivity and 3-40 was obtained good yields.



Scheme 6-21

The resulting primary alcohol was then acylated with the tetraenoic acid 5-6 using dicyclohexylcarbodiimide¹⁹⁰ to give the ester 6-25 (Scheme 6-22). When the phosphine oxide 6-25 was lithiated using lithium diisopropylamide the acyl migration took place to give the ketone 6-26 (Scheme 6-23).



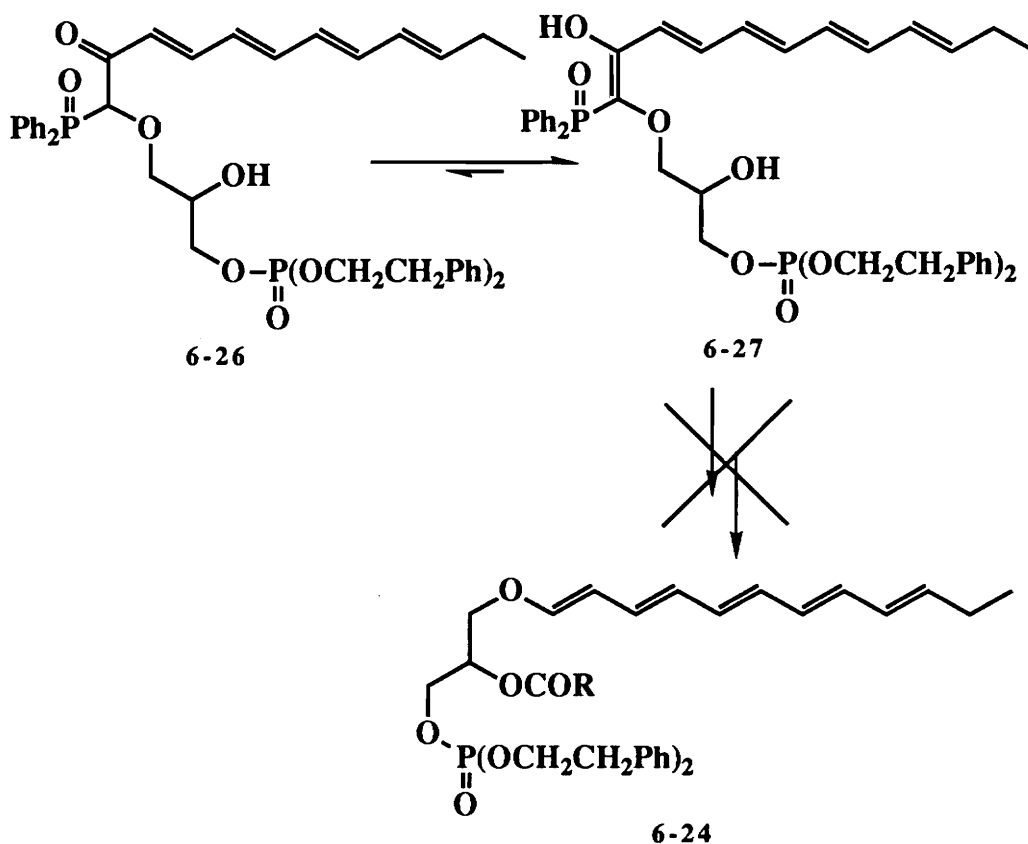


Scheme 6-23

This reaction was conducted exactly as described in Chapter 3. Unfortunately, the product **6-26** is much more unstable than any of its less conjugated analogs. The product readily enolizes to compound **6-27**. As the conjugation of the ketone increases the tendency for enolization increases to a point where the enol predominates. This becomes apparent upon close inspection of the ^1H NMR spectrum of **6-27**. This is a major obstacle since the enol cannot be eliminated to form the necessary pentaenyl ether (Scheme 6-24). The keto-enol equilibrium presumably lies further to the right since the enol form provides a more highly conjugated system by conjugating through the enol to the phosphine oxide, making it more stable than the keto form. To our discouragement this is perhaps the most difficult problem

to overcome in successfully completing the synthesis and at the time of this writing no method has been found to circumvent it.

The diagnostic signals of the migrated product were not present in the ^1H NMR spectrum, since the characteristic overlapping doublet signal normally seen at approximately 5.00 ppm was very low in intensity. Migration must have taken place however, since the methine proton on the glycerol moiety (normally found at approximately 5.25 ppm in the unmigrated material) was shifted upfield to 4.13 ppm. Since a migration took place and the overlapping doublet signal was nonexistent, it must be concluded that the product is extensively enolized and exists predominantly as **6-27** rather than **6-26**.

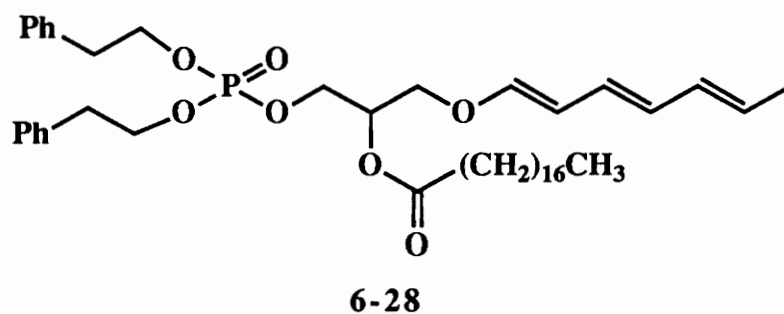


Scheme 6-24

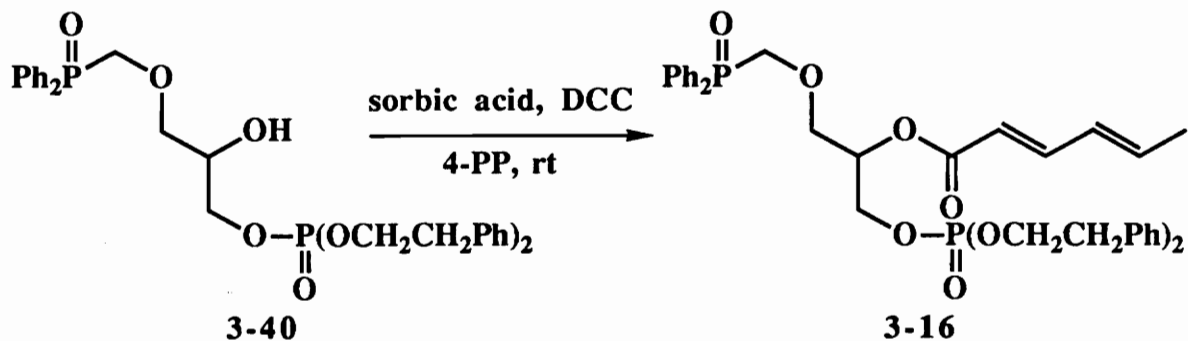
6.3.2 Synthesis of a Precursor Analog with a Trienyl Ether Moiety.

It was still of interest to explore whether the acyl migration method could be used to generate enol ethers. Perhaps a plasmalopentaene-12 analog with varying numbers of double bonds on the enol ether could be synthesized. It would still be biologically interesting to determine the effect of the extent of conjugation of the polyenyl ether that is required for plasmalopentaene-12 to exhibit its biological properties.

Since the protected phosphate **4-1** cannot be easily attached to the glycerol backbone, it was decided to pursue a model compound in which diphenethylphosphate is used as the phosphate group. A trienyl ether moiety was selected since this can originate from the readily available sorbic acid. With these considerations in mind, compound **6-28** was chosen as the target molecule.

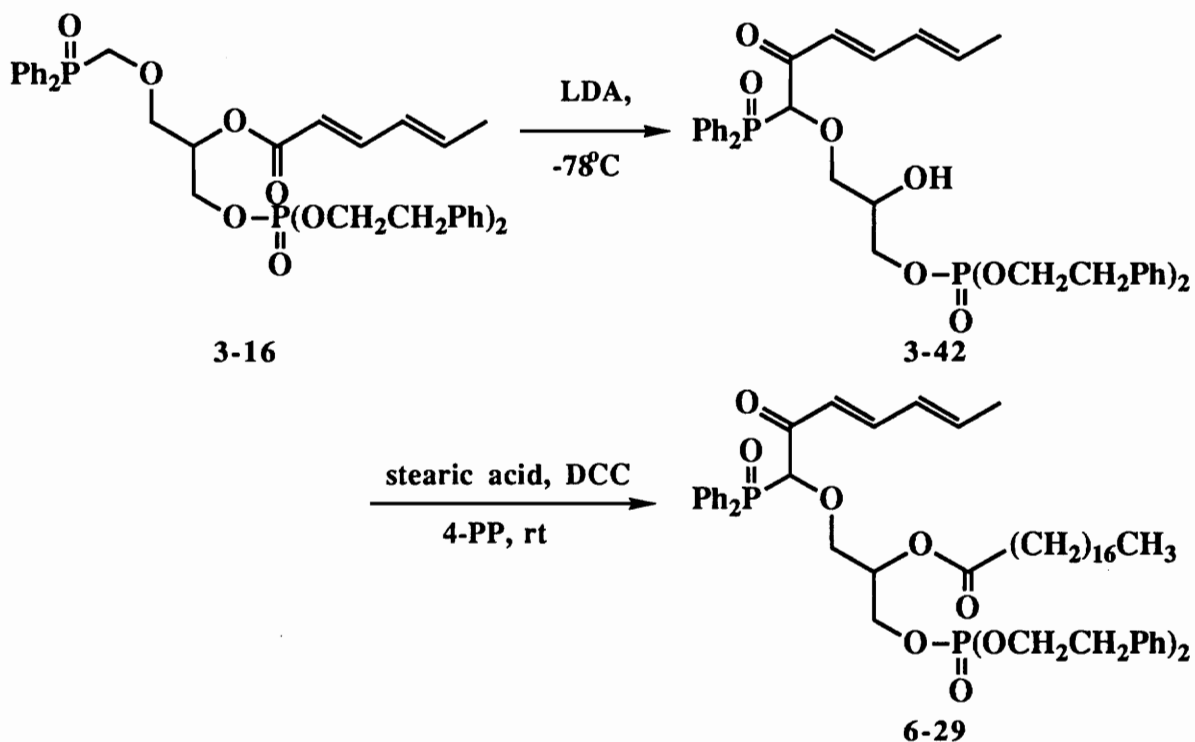


Starting with the phosphotriester **3-40**, whose synthesis was previously described (Chapter 3), acylation of the secondary alcohol with sorbic acid using dicyclohexylcarbodiimide¹⁰⁴ readily gave the dienoic ester **3-16** (Scheme 6-25).



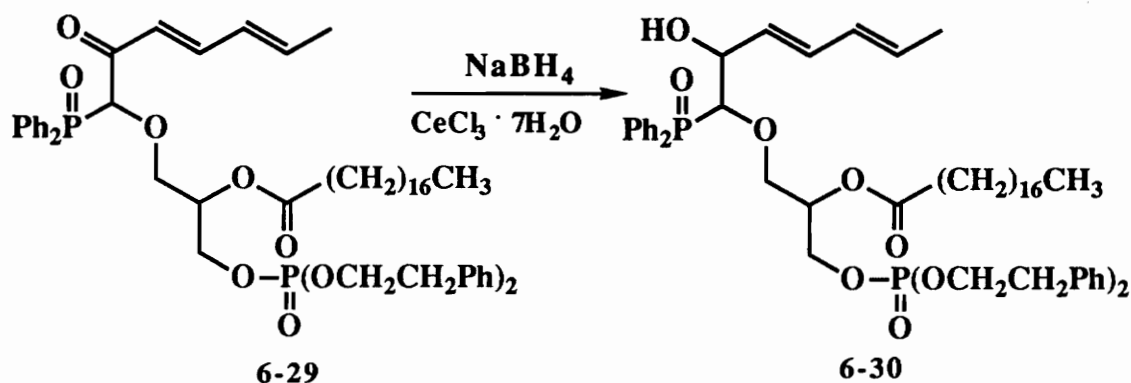
Scheme 6-25

Generation of an anion alpha to the phosphine oxide of **3-16** with lithium diisopropylamide then gave the migrated product **3-42** in very high yield, as previously described. The resulting secondary alcohol was then reacylated with stearic acid again using dicyclohexylcarbodiimide to give the ester **6-29** (Scheme 6-26).



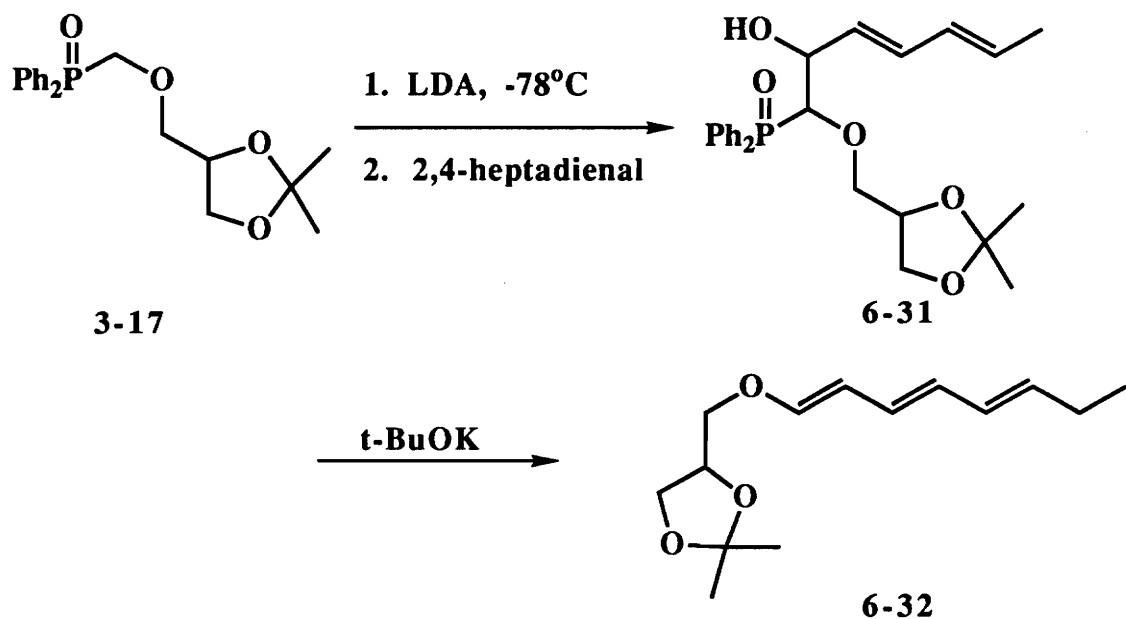
Scheme 6-26

Selective 1,2-reduction of the dienone in **6-29** with sodium borohydride and cerium chloride heptahydrate then gave the allylic alcohol **6-30** (Scheme 6-27). At this point, treatment of the allylic alcohol **6-30** with potassium *t*-butoxide should have afforded the trienol ether **6-28**, but to our surprise no reaction occurred. Other attempts yielded the same results.



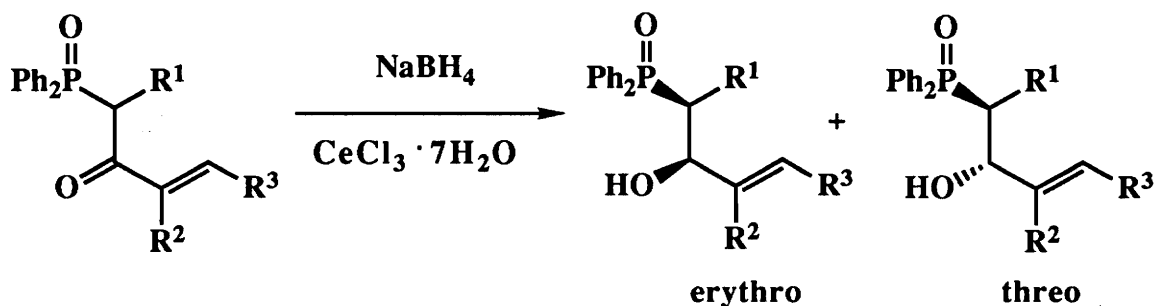
Scheme 6-27

A model compound was synthesized to test the elimination reaction. This compound, **6-32**, was produced by lithiating ketal **3-17**, which was synthesized previously (Chapter 3), with lithium diisopropylamine. Quenching of the anion with 2,4-heptadienal then gave the allylic alcohol **6-31**. Treatment with potassium *t*-butoxide then gave the enol ether **6-32** based on the thin layer chromatography evidence as a less polar spot (Scheme 6-28).



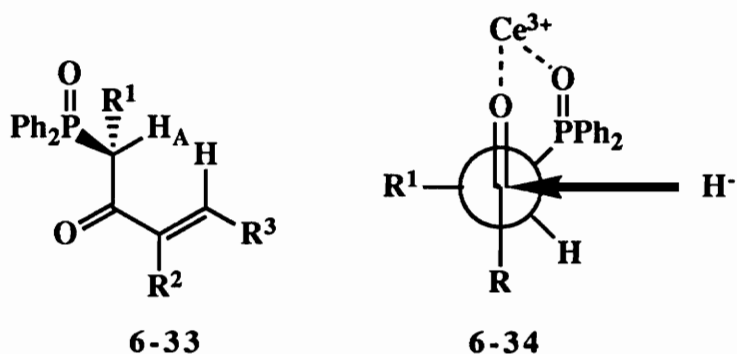
Scheme 6-28

Mechanistically the elimination should occur as demonstrated by the synthesis of 6-20, but in the case of compound 6-30 the trienol ether was not formed. This phenomenon can be explained based on the observation of Warren *et al.*¹⁹¹ In studies on the reversed stereochemical control in the regioselective reduction of hindered diphenylphosphinoyl ketones and enones Warren, observed that if R¹ is large and bulky then the reduction of enones becomes highly stereoselective (Scheme 6-29) and *erythro* alcohols are isolated in high yields.

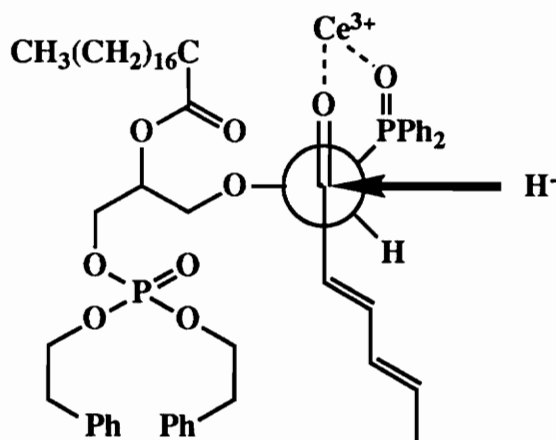


Scheme 6-29

There is NOE, IR, and X-ray evidence that enones prefer conformation **6-33** with H_A in the plane of the enone.¹⁹¹ During the reaction, cerium is chelated by $C=O$ and $P=O$ as seen in **6-34**. The *erythro* selectivity is due to attack from the hydride opposite to the large R^1 group. Elimination of the *erythro* product will then stereospecifically form E alkenes.¹⁹²



If this model is extended to compound **6-30**, then it becomes evident that due to the large substituted glycerol moiety, the 1,2-reduction would deliver a hydride to the least hindered face (i.e. opposite to the glycerol group) and give exclusively the *erythro* product (Scheme 6-30).



Scheme 6-30

If the *erythro* product is formed in very high stereochemical excess then when the allylic alcohol is treated with base the formation of the oxyphosphatane intermediate will not occur. This is because the necessary rotation of the C₁-C₂ bond needed to obtain the correct geometry to form the oxyphosphatane would be prevented by steric hindrance since the double bonds of the allylic alcohol would be forced to rotate into the region of the bulky glycerol moiety (Figure 6-1).

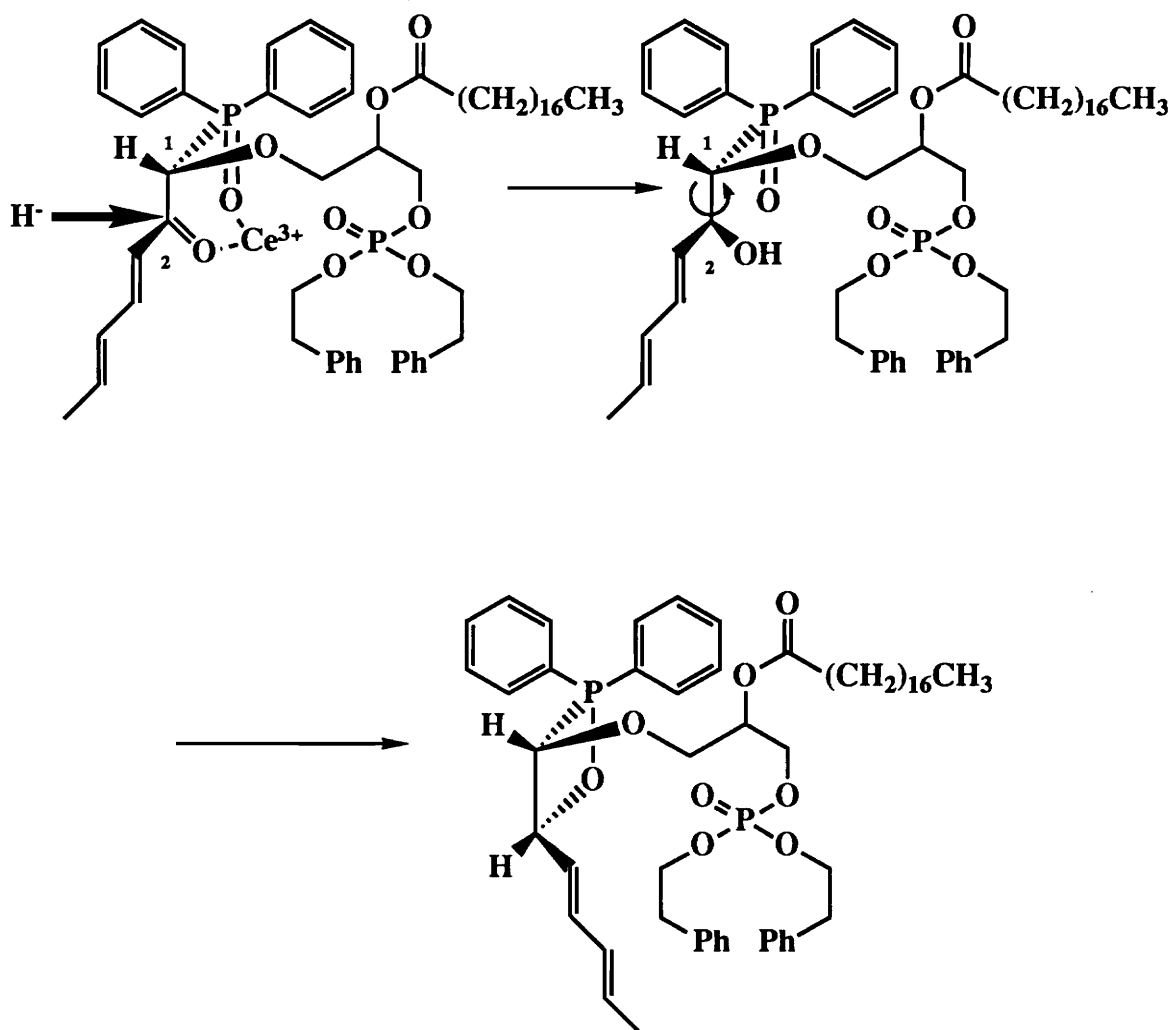
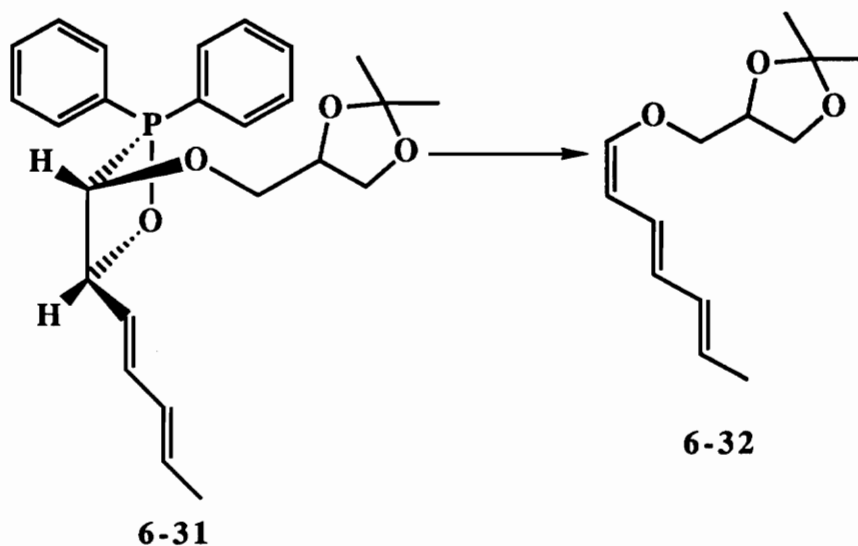


Figure 6-1. 1,2-Reduction of the Precursor Analog.

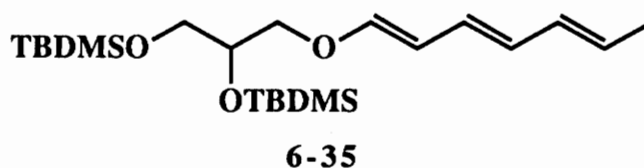
For this reason the elimination did not occur with compound **6-30**. It did, however, occur with compound **6-31** to give **6-32** since the ketal is much less sterically demanding (Scheme 6-31).



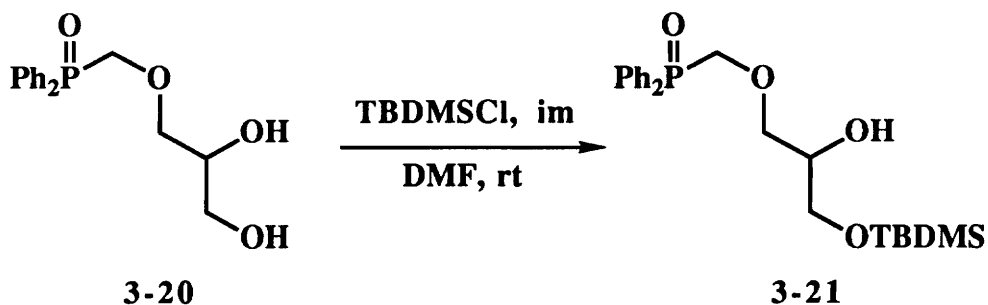
Scheme 6-31

6.4 Synthesis of a Fecapentaene-12 Analog Via the Acyl Migration Strategy.

Without many modifications, the acyl migration did not seem to be a suitable method for the synthesis of plasmalopentaene-12. It may, however, prove to be a useful and alternate method for the synthesis of the fecal mutagen, fecapentaene-12. To test this utility it was decided to synthesize the protected analog of fecapentaene-12, **6-35**.

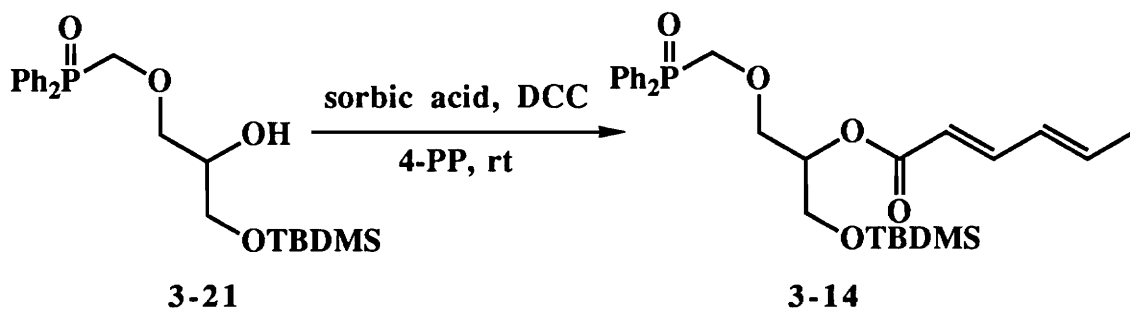


The synthesis commenced by silylating the diol **3-20**, then reacting with *t*-butyldimethylchlorosilane in the presence of imidazole to give the monosilylphosphine oxide **3-21** (Scheme 6-32).



Scheme 6-32

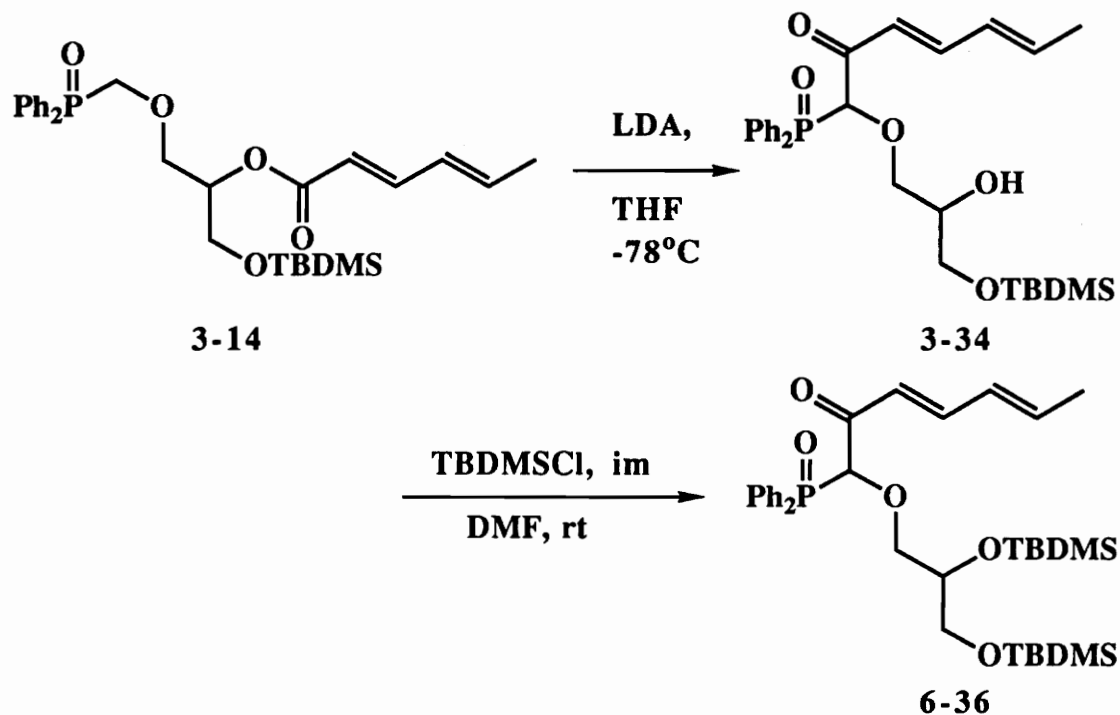
In the same fashion as used previously, the secondary alcohol of **3-21** is coupled with sorbic acid using dicyclohexylcarbodiimide with 4-pyrrolidinopyridine as a catalyst to give the dienoate ester **3-14** (Scheme 6-33).



Scheme 6-33

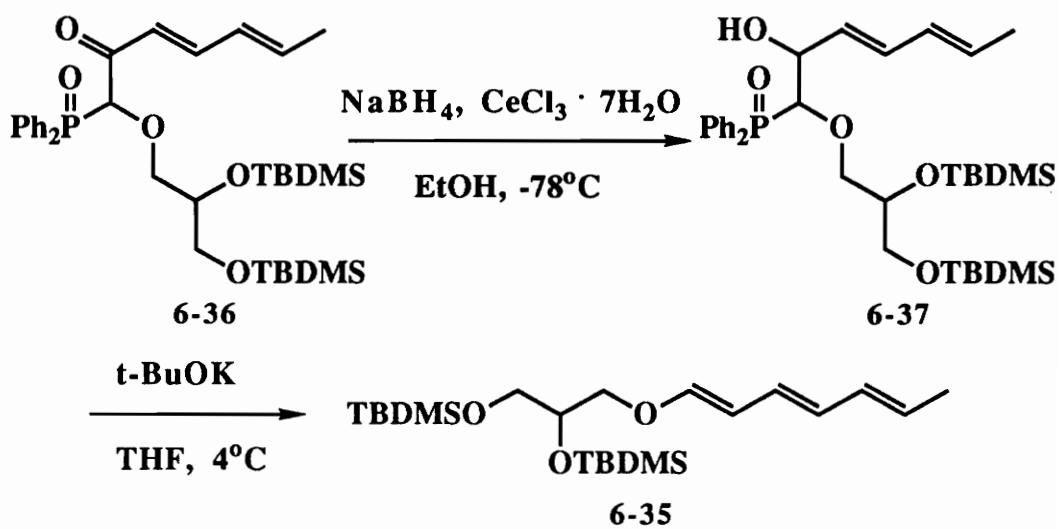
As the key step of the synthesis the proton alpha to the phosphine oxide of **3-14** was abstracted using lithium diisopropylamide. The resulting anion intramolecularly attacks the ester carbonyl giving the desired dienone **3-34**. the resulting secondary alcohol is then

protected with *t*-butyldimethylchlorosilane in the presence of imidazole to give the disilyl derivative **6-36** (Scheme 6-34).



Scheme 6-34

Stereospecific 1,2-reduction of dienone **6-36** using the conditions of Locke¹⁸⁹ i.e. sodium borohydride and cerium chloride heptahydrate gave in high yield the allylic alcohol **6-37**. Treatment of the alcohol **6-37** with potassium *t*-butoxide then affords by way of Wittig elimination, the feca-pentaene-12 analog **6-35** (Scheme 6-35). This compound exhibited a virtually identical ultraviolet spectrum to that of fecatriene-6¹⁹³ (Figure 6-2). The UV spectrum gave one adsorption at 273 nm in methyl alcohol.



Scheme 6-35

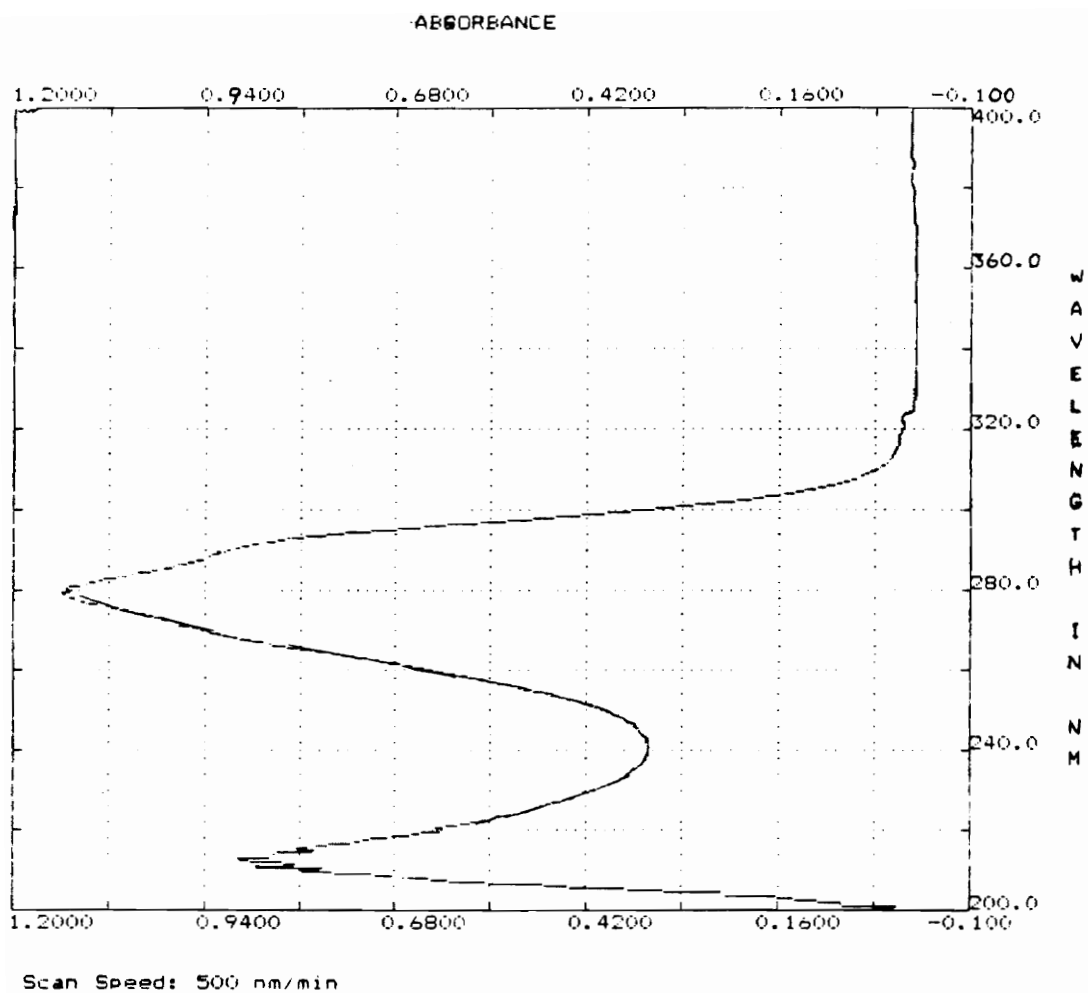
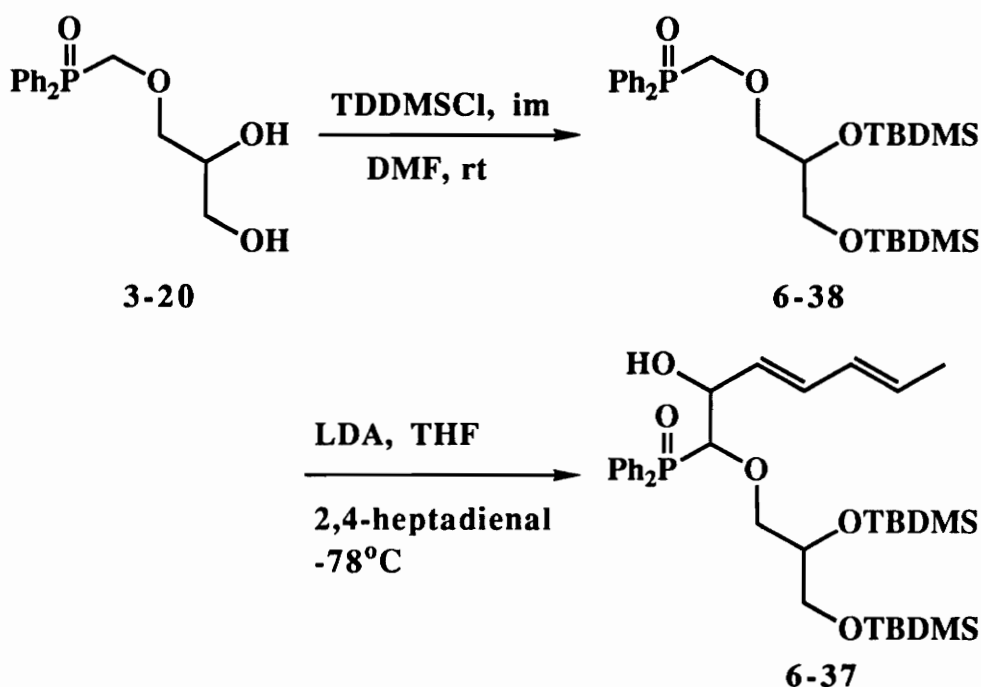


Figure 6-2. UV Spectrum of the Fecapentaene-12 Analog.

The structure of **6-35** was independently confirmed by the synthesis of **6-37** by an alternate route. Silylation of the diol **3-20** with *t*-butyldimethylchlorosilane gave the disilylphosphine oxide **6-38**. Lithiation of **6-38** with lithium diisopropylamide followed by quenching with 2,4-hexadienal then gave **6-37** in high yield (Scheme 6-36). The allylic alcohol was identical in every respect to the one produced by the acyl migration methodology. When treated with potassium *t*-butoxide the fecapentaene-12 analog **6-35** was obtained.

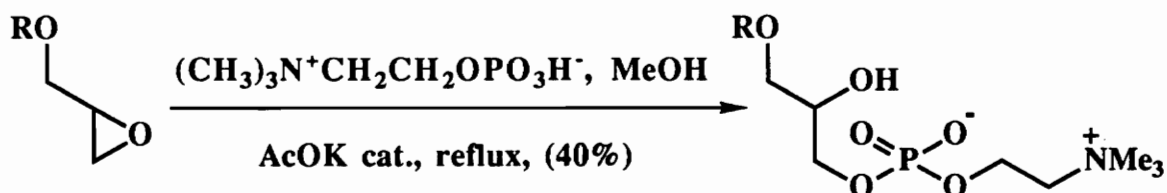


Scheme 6-36

6.5 Conclusions.

At this point in time it does not seem that the acyl migration pathway is the best method to synthesize plasmalopentaene-12. Although preliminary studies on model compounds indicated that this was a viable method, several unforeseen obstacles were

encountered when attempts were made to construct the molecule. First, no suitable method was found to attach the protected phosphate. Epoxide ring opening reactions did not work as they did with model phosphates. Neither did coupling reactions nor did displacement reactions. It is felt that this is perhaps the least difficult obstacle to overcome. With the proper choice of reaction conditions the phosphate should be able to be attached. Recently Julia *et al.*¹¹¹ have opened up an epoxide with a phosphate to form a phosphotriester (Scheme 6-37). Perhaps the target compound will be realized by some modification of this procedure. The second obstacle encountered was the problem of enolization. This would be much more difficult to overcome, although perhaps not impossible.



Scheme 6-37

The last obstacle encountered was that of the elimination reaction to form the polyenyl ether. This is a serious problem that is inherent with the synthesis. In order for the elimination to occur the bulk of the glycerol moiety must be reduced. If this is reduced, say to a protected glycerol derivative, then in order to form the pentaenyl ether the protected glycerol must be deprotected and functionalized in the presence of the pentaenyl ether. This would not be an easy task in light of the instability associated with the pentaenyl ether. Furthermore, it would take away from one of the main advantages of this synthetic design, the construction of the pentaenyl ether in one of the last steps of the synthesis.

These obstacles coupled with the constraints that we felt were necessary (i.e. avoidance of basic conditions or acidic conditions) limited the methodologies available for

the work. There is, however, a promising synthetic methodology currently being developed in our laboratories which may prove to be the method of choice for the synthesis of plasmalopentaene-12.

Even though this methodology developed to synthesize plasmalopentaene-12 ultimately failed, the work did yield useful results. First, a new synthesis of tetraundeconic acid was developed. Second, a new protected phosphorylethanolamine was developed which should prove generally useful in the synthesis of other phosphorylethanolamine derivatives. Finally, it demonstrates the limitations of the acyl migration approach in the synthesis of complex enol ethers.

6.6 Experimental.

Preparation of diphenethylphosphate (3-37)

Amberlite IR-120 (0.052 g, 0.288 mmol) was added to a solution of sodium diphenethylphosphate (0.10 g, 0.262 mmol) dissolved in 2 ml of methyl alcohol. The reaction was stirred at room temperature for 1.5 hours. The Amberlite IR-120 was removed by filtration and then washed with methyl alcohol. Removal of the solvent *in vacuo* gave 80 mg (100%) of diphenethylphosphate. $^1\text{H NMR}$ (CDCl_3) δ 11.25 (s, 1H, POH), 7.29-7.11 (m, 10H, Ph), 4.04 (q, 4H, $J = 5.4$ Hz, POCH₂), 2.90 (t, 4H, $J = 5.4$ Hz, CH₂Ph). The spectral data are in agreement with those previously described.¹¹³

Preparation of 1-[[[2-hydroxy-3-[[N-2-(trimethylsilyl)ethylcarbonyl-ethyloxy][2-(diphenylmethylsilyl)ethyloxy]]phosphinoyloxy]propoxy]-methyl]diphenylphosphine oxide (6-2)

Compound 4-65 (0.030 g, 0.057 mmol) was dissolved in 0.5 ml of anhydrous ethyl acetate. To this solution Amberlite IR-120 (0.011 g, 0.057 mmol) was added and the mixture was stirred at room temperature for 2 hours. At this time compound 3-27 (0.014 g, 0.047 mmol) which was dissolved in 0.1 ml of anhydrous ethyl acetate was added.

The reaction was allowed to stir at room temperature for 48 hours then stirring was continued at reflux for 24 hours. The reaction mixture was filtered and the solvents removed *in vacuo*. Purification by prep TLC eluting with 97% hexanes / 3% methanol gave 4.7 mg. (10.7%) of **6-3**. ^1H NMR (CDCl_3) δ 7.74 (m, 5H, Ar), 7.48 (m, 10H, Ar), 7.35 (m, 5H, Ar), 5.4 (br s, 1H, CHOH), 4.33 (m, 2H, Ph_2POCH_2), 4.12 (m, 6H, $\text{OCH}_2\text{CH}_2\text{NH}$, $\text{OCH}_2\text{CH}_2\text{SiPh}_2\text{Me}$, CO_2CH_2), 3.99 (m, 1H, CHOH), 3.93 (m, 2H, CH_2), 3.63 (m, 2H, CH_2), 3.34 (m, 2H, $\text{OCH}_2\text{CH}_2\text{NH}$), 1.66 (m, 2H, $\text{CH}_2\text{SiPh}_2\text{Me}$), 0.57 (s, 3H, SiPh_2CH_3), 0.0 (s, 9H, $\text{Si}(\text{CH}_3)_3$).

Preparation of 1-[[[2-octadecyloxycarbonyl-3-hydroxy]propoxy]methyl]-diphenylphosphine oxide (6-12)

Hydrogen fluoride-pyridine (173 μl) was added dropwise to a solution of **3-13** (0.083 g, 0.121 mmol) dissolved in 1.75 ml of anhydrous tetrahydrofuran which had been cooled to 0°C . Once the addition was complete the reaction was allowed to stir at room temperature for 1 hour. A 10% pyridine / water (v/v) solution (4 ml) was added. The resulting solution was extracted with 5 x 20 ml of ethyl acetate. The combined extracts were washed with 5 x 20 ml of water followed by 1 x 20 ml brine. The extracts were dried over sodium sulfate, filtered, and the solvent removed *in vacuo* to give 37.4 mg (54%) as a single spot on TLC (94% dichloromethane / 6% methanol). ^1H NMR (CDCl_3) δ 7.71 (m, 4H, Ph), 7.44 (m, 6H, Ph), 4.93 (m, 1H, CH), 4.23 (m, 2H, Ph_2POCH_2), 3.74 (d, $J = 5.4$ Hz, 2H, CH_2), 3.53 (d, $J = 5.4$ Hz, 2H, CH_2), 2.18 (t, $J = 8.1$ Hz, 2H, COCH_2), 1.50 (m, 2H, COCH_2CH_2), 1.17 (s, 28H, CH_2), 0.80 (t, $J = 8.1$ Hz, 3H, CH_3). IR (neat) 3338, 2926, 2854, 1736, 1466, 1439, 1177, 1123, 1100, 910, 734, 695 cm^{-1} .

Preparation of dilithium tetrabromonickelate (II) (6-18)

Anhydrous nickel bromide (0.872 g, 4.0 mmol) was added to a solution of anhydrous lithium bromide (0.696 g, 8.0 mmol) dissolved in 9.5 mol of anhydrous tetrahydrofuran¹⁷⁸. The mixture was stirred at room temperature for 60 hours. At this time the undissolved material was allowed to settle. The dark blue-green liquid is approximately a 0.4 M solution of 6-18.

Preparation of 1-bromo-2-hydroxy-3-[di(phenethyloxy)phosphinoyloxy]propane (6-19)

A 0.4 M solution of dilithium tetrabromonickelate (II) 6-18 in tetrahydrofuran (10.18 ml, 4.1 mmol) was added to a solution of diphenethylphosphoryl glycidol 6-17 (0.764 g, 2.1 mmol) dissolved in 6 ml of anhydrous tetrahydrofuran. The reaction was stirred at room temperature for 4 hours then poured into 50 ml of a 10% solution of pH 7 buffer. This solution was extracted with 3 x 50 ml of dichloromethane. The combined extracts were washed with 3 x 50 ml of brine, dried over sodium sulfate, and filtered. The solvent was removed *in vacuo* to give 0.759 g (82%) of 6-19, which needed no further purification. ¹H NMR (CDCl₃) δ 7.26 (m, 10H, Ph), 4.19 (overlapping t, J = 9.4 Hz, 4H, POCH₂CH₂Ph), 3.96 (m, 3H, CH and CHCH₂OP), 3.36 (d, J = 7.5 Hz, 2H, CH₂Br), 2.95 (t, J = 9.2 Hz, 4H, POCH₂CH₂Ph).

Preparation of 1-bromo-2-octadecyloxycarbonyl-3-[di(phenethyloxy)phosphinoyloxy]propane (6-20)

Stearic acid (0.852 g, 3.4 mmol), dicyclohexylcarbodiimide (0.707 g, 3.4 mmol), and a catalytic amount of 4-pyrrolidinopyridine (0.025 g, 0.17 mmol) were added to a solution of 6-19 (0.759 g, 1.7 mmol) dissolved in 11.0 ml of anhydrous tetrahydrofuran. The reaction was allowed to stir at room temperature for 15 hours. At this time the solvent was removed *in vacuo*. The residue was taken up in ethyl acetate and filtered. The solvent

was removed from the filtrate and purified by flash chromatography and eluting with 95% dichloromethane / 5% ethyl acetate to give 0.886 g (74%). $^1\text{H NMR}$ (CDCl_3) δ 7.27 (m, 10H, Ph), 5.06 (quintet, $J = 5.3$ Hz, 1H, CH), 4.17 (overlapping t, $J = 2.2$ and 7.2 Hz, 4H, $\text{POCH}_2\text{CH}_2\text{Ph}$), 4.01 (m, 2H, CH_2OP), 3.42 (m, $J = 5.6$ Hz, 2H, CH_2Br), 2.95 (t, $J = 6.9$ Hz, 4H, POCH_2CH_2), 2.31 (t, $J = 7.6$ Hz, 2H, COCH_2), 1.25 (s, 30H, CH_2), 0.88 (t, $J = 6.4$ Hz, 3H, CH_3).

Preparation of 1-[[2-(2,4,6,8-undecatetraenyl)oxycarbonyl]-3-[di-(phenethyloxy)phosphinoyloxy]propoxy]methyl]diphenylphosphine oxide (6-25)

Compound 5-6 (0.053 g, 0.299 mmol) was added to a solution of 3-40 (0.118 g, 0.199 mmol) dissolved in 1.3 ml of anhydrous tetrahydrofuran. To this solution dicyclohexylcarbodiimide (0.062 g, 0.299 mmol) was added followed by a catalytic amount of 4-pyrrolidinopyridine (0.003 g, 0.299 mmol). The reaction was stirred at room temperature for 10 hours and an additional 0.5 eq of 2,4,6,8-tetraenoic acid 5-6 (0.018 g, 0.099 mmol) and dicyclohexylcarbodiimide (0.021 g, 0.099 mmol) was added. Stirring was continued for an additional 10 hours at room temperature. The solvent was removed *in vacuo* and the residue was suspended in ethyl acetate and filtered. The solvent was again removed *in vacuo*. Purification by flash chromatography and eluting with 94% dichloromethane / 6% isopropyl alcohol gave 0.095 g (63%) of 6-25 as an orange oil. $^1\text{H NMR}$ (CDCl_3) δ 7.77 (m, 4H, Ph), 7.45 (m, 6H, Ph), 7.19 (m, 10H, Ph), 6.56 (dd, $J = 10.8$ and 14.7 Hz, 1H, olefinic), 6.40 (dd, $J = 10.5$ and 14.6 Hz, 1H, olefinic), 6.19 (m, 2H, olefinic), 5.92 (m, 1H, olefinic), 5.74 (d, $J = 15.2$ Hz, 1H, olefinic), 5.13 (quintet, $J = 3.0$ Hz, 1H, CHOCOR), 4.25 (m, 2H, Ph_2POCH_2), 4.11 (m, 4H, $\text{OCH}_2\text{CH}_2\text{Ph}$), 3.91 (overlapping t, $J = 6.7$ Hz, 4H, CH_2), 3.66 (d, $J = 5.6$ Hz, CH_2), 2.88 (overlapping t, $J = 7.0$ Hz, 4H, $\text{OCH}_2\text{CH}_2\text{Ph}$), 2.16 (dq, $J = 7.2$ and 7.2 Hz, 2H, $\text{CH}=\text{CHCH}_2$), 1.03 (t, $J = 7.4$ Hz, 3H, CH_3).

Preparation of 1-[2-hydroxy-3-[di(phenethyloxy)phosphinoyloxy]propoxy]-1-(diphenylphosphinyl)-1,3,5,7,9-dodecapentaen-2-ol (6-27)

1.5 M lithium diisopropylamide (monotetrahydrofuran) (0.10 ml, 0.150 mmol) was added dropwise to a solution of **6-25** (0.086 g, 0.114 mmol) dissolved in 1.25 ml of anhydrous tetrahydrofuran which had been cooled to -78°C . Stirring was maintained at -78°C for 45 minutes then 3 ml saturated ammonium chloride was added and the temperature was allowed to warm to room temperature. 10 ml of distilled water was added and the solution was extracted with 3 x 25 ml of diethyl ether. The combined extracts were washed with 2 x 25 ml of water followed by 1 x 25 ml of brine. The extracts were dried over magnesium sulfate, filtered and the solvent removed *in vacuo*. Purification by flash chromatography and eluting with 95% dichloromethane / 5% isopropyl alcohol gave 29 mg (34%) of **6-27** as an oil. $^1\text{H NMR}$ (CDCl_3) δ 7.80 (m, 4H, Ph), 7.50 (m, 6H, Ph), 7.20 (m, 10H, Ph), 6.64, (dd, $J = 8.7$ and 15.1 Hz, 1H, olefinic), 6.26 (m, 6H, olefinic), 5.90 (m, 1H, olefinic), 4.94 (d, $J = 15.8$ Hz, 0.09H, diastereomer of Ph_2POCH), 4.93 (d, $J = 15.8$ Hz, 0.09H, diastereomer of Ph_2POCH), 4.13 (m, $J = 7.1$ Hz, 1H, CHOCOR), 3.86 (m, 4H, $\text{OCH}_2\text{CH}_2\text{Ph}$), 3.62 (m, 4H, CH_2), 2.91 (t, $J = 7.1$ Hz, 4H, CH_2Ph), 2.17 (dq, $J = 7.4$ and $J = 7.4$ Hz, $\text{CH}=\text{CHCH}_2$), 1.03 (t, $J = 7.5$ Hz, 3H, CH_3).

Preparation of 1-[[2-octadecyloxyacetyl-3-[di(phenethyloxy)phosphinoyloxy]propoxy]-1-(diphenylphosphinyl)-3,5-heptadien-2-one (6-29)

Stearic acid (0.255 g, 1.027 mmol) was added to a solution of **3-42** (0.471 g, 0.684 mmol) dissolved in 4.5 ml of anhydrous tetrahydrofuran. To this solution dicyclohexylcarbodiimide (0.212 g, 1.027 mmol) and a catalytic amount of 4-pyrrolidinopyridine (0.01 g, 0.068 mmol) were added. The reaction was stirred at room temperature for 24 hours. An additional 0.5 eq of stearic acid (0.085 g, 0.034 mmol) and dicyclohexylcarbodiimide (0.071 g, 0.034 mmol) was added. Stirring was continued at room temperature for 6 hours. The solvent was removed *in vacuo* to leave a white residue.

The residue was taken up in ethyl acetate and filtered. The solvent was removed *in vacuo* to leave an oil which was purified by flash chromatography. Eluting with 100% ethyl acetate gave 0.439 g (67%) of **6-29**. $^1\text{H NMR}$ (CDCl_3) δ 7.49 (m, 4H, Ph), 7.41 (m, 6H, Ph), 7.19 (m, 10H, Ph), 6.85 (m, 1H, olefinic), 6.47 (d, $J = 2.7$ Hz, 1H, olefinic), 6.20 (m, 1H, olefinic), 4.95 (m, 1H, olefinic), 4.88 (d, 1H, $J = 3.0$ Hz, 0.5 H, diastereomer of Ph_2POCH), 4.86 (d, $J = 3.0$ Hz, 0.5 H, diastereomer of Ph_2POCH), 4.45 (m, 1H, CHOCOR), 4.10 (m, 4H, $\text{OCH}_2\text{CH}_2\text{Ph}$), 3.94-3.61 (m, 4H, CH_2), 2.88 (t, $J = 7.0$, 4H, $\text{OCH}_2\text{CH}_2\text{Ph}$), 2.10 (m, 2H, CO_2CH_2), 1.46 (m, 2H, $\text{CO}_2\text{CH}_2\text{CH}_2$), 1.22 (s, 28H, CH_2), 0.90 (t, $J = 8.5$ Hz, 3H, CH_3). IR (neat) 3344, 3061, 3028, 2923, 2852, 1738, 1693, 1632, 1592, 1174, 1119, 1012, 784, 722 cm^{-1} .

Preparation of 1-[[2-octadecyloxycarbonyl-3-[di(phenethyloxy)-phosphinoyloxy]propoxy]-1-diphenylphosphinyl-3,5-heptadien-2-ol (6-30)

Cerium chloride heptahydrate (0.143 g, 0.383 mmol) dissolved in 0.1 ml of anhydrous ethanol was added to a solution of **6-29** (0.365 g, 0.383 mmol) dissolved in 0.1 ml of anhydrous ethanol which had been cooled to -78°C . Next sodium borohydride (0.044 g, 1.149 mmol) was added portionwise. The reaction was stirred at -78°C for 30 minutes. Water (2 ml) was added and the reaction was allowed to warm to room temperature. An additional 15 ml of distilled water was added to the solution, then it was extracted with 3 x 25 ml of dichloromethane. 1N HCl (10 ml) was added and combined extracts were dried over sodium sulfate, filtered, and the solvent removed *in vacuo*. Purification by flash chromatography and eluting with 100% ethyl acetate gave 0.085 g (23%) of **6-30**. $^1\text{H NMR}$ (CDCl_3) δ 7.84 (m, 4H, Ph), 7.49 (m, 6H, Ph), 7.24 (m, 10H, Ph), 6.19 (m, 1H, olefinic), 5.64 (m, 2H, olefinic), 5.45 (m, 1H, olefinic), 4.97 (t, $J = 5.4$ Hz, 0.5H, diastereomer of Ph_2POCH), 4.86 (t, $J = 5.4$ Hz, 0.5 H, diastereomer of Ph_2POCH), 4.62 (m, 1H, CHOCOR), 4.12 (m, 4H, $\text{OCH}_2\text{CH}_2\text{Ph}$), 3.88-3.28 (m, 5H, CH_2 , CH_2 , CHOH), 2.91 (overlapping t, $J = 7.0$ Hz, 4H, $\text{OCH}_2\text{CH}_2\text{Ph}$), 2.18 (t, $J = 7.5$

Hz, COCH₂), 1.68 (d, J = 6.1 Hz, 3H, CH=CH₃CH₃), 1.59 (m, 2H, COCH₂CH₂), 1.25 (s, 28 H, CH₂), 0.88 (t, J = 6.5 Hz, 3H, CH₃). ¹³C NMR (CDCl₃) δ 175.00 (C=O), 136.84 (Ar), 132.21 (Ar), 128.67 (Ar), 126.76 (olefinic), 106.0 (olefinic), 68.0 (OPOCH₂), 49.0 (CH₂Ph), 24.0 (CH=CHCH₃), 30.0 (CH₂), 14.0 (CH₃). IR (neat) 3374, 2923, 2852, 1739, 1437, 1269, 1170, 1117, 1015, 698. MS *m/z* 957 (MH⁺, 100), 940 (90), 921 (30), 891 (20), 860 (50), 838 (20) cm⁻¹.

Preparation of [2,2-dimethyl-1,3-dioxolane-4-yl-methoxy],2,4,6-octadecatriene (6-32)

1.5 M lithium diisopropylamine (tetrahydrofuran) (2.59 ml, 3.90 mmol) was added dropwise to a solution of **3-17** (1.12 g, 3.2 mmol) dissolved in 9.0 ml of anhydrous tetrahydrofuran which had been cooled to -50°C. It was reacted at this temperature for 15 minutes. The temperature was lowered to -78°C and 2,4-heptadienal (0.45 ml, 3.6 mmol) dissolved in 5.4 ml of anhydrous tetrahydrofuran was added dropwise. Stirring was continued at -78°C for 1 hour then 10 ml of saturated ammonium chloride was added and the temperature allowed to warm to room temperature. The mixture was extracted with 3 x 50 ml of diethyl ether. The combined extracts were washed with 2 x 100 ml of water and 1 x 100 of brine. The extracts were dried over magnesium sulfate, filtered, and the solvent removed *in vacuo*. Purification by flash chromatography eluting with 90% ethyl acetate / 10% dichloromethane gave 1.27 (87%) of **6-31**. This material was used directly in the next reaction. Next potassium *t*-butoxide (0.031 g, 0.273 mmol) was added portionwise to a solution of **6-31** (0.113 g, 0.248 mmol) dissolved in 2 ml of anhydrous tetrahydrofuran which had been cooled to -50°C. The reaction was stirred at 4°C for 12 hours. Next 2 ml of brine was added and this solution was extracted with 3 x 25 ml of diethyl ether. The combined extracts were washed with 2 x 25 ml of water followed by 1 x 25 ml brine. The extracts were dried over sodium sulfate, filtered, and the solvents

removed *in vacuo*. The TLC of the reaction was similar to that of an authentic compound reported in the literature.¹⁹³

Preparation of 1-[[2,3-di[(1,1-dimethylethyl)dimethylsilyl]oxy]propoxy]-1-(diphenylphosphinyl)-3,5-heptadien-2-one (6-36)

t-Butyldimethylsilylchloride (0.284 g, 1.9 mmol) and imidazole (0.244 g, 3.6 mmol) were added to a solution of **3-34** (0.990 g, 1.7 mmol) dissolved in 5.0 ml of dimethylformamide. The reaction was stirred at room temperature for 10 hours. Additional *t*-butyldimethylsilylchloride (0.284 g, 1.9 mmol) and imidazole (0.244 g, 3.6 mmol) were added and stirring was continued at room temperature for 10 hours. Water (5 ml) was added and this was extracted with 3 x 25 ml of diethyl ether. The combined extracts were washed with 5 x 25 ml of water followed by 1 x 25 ml of brine. The extracts were dried over sodium sulfate, filtered, and the solvent removed *in vacuo*. Purification by flash chromatography eluting with 96% dichloromethane / 4% methyl alcohol gave 0.113 g (10.5%). ¹H NMR (CDCl₃) δ 7.81 (m, 4H, Ph), 7.44 (m, 6H, Ph), 7.20 (m, 1H, olefinic), 6.49 (d, J = 15.1 Hz, 0.5H, diastereomer of COCH=C), 6.47 (d, J = 15.1 Hz, 0.5H, diastereomer of COCH=C), 6.14 (m, 2H, olefinic), 5.03 (d, J = 14.9 Hz, 0.5 H, diastereomer of Ph₂POCH), 4.84 (d, J = 14.6 Hz, 0.5H, diastereomer of Ph₂POCH), 3.68-3.18 (m, 5H, CH₂CHCH₂), 1.81 (d, J = 4.9 Hz, 3H, CH=CHCH₃), 0.80 (s, 18H, 2 x SiC(CH₃)₃), 0.0 (s, 12H, 2 x SiC(CH₃)₃). ¹³C NMR (CDCl₃) δ 193.96 (C=O), 114.33 (olefinic), 142.30 (olefinic), 138.32 (olefinic), 131.76 (Ph), 130.69 (olefinic), 128.21 (Ph), 86.83 (Ph₂POCH), 74.41 (CH₂CHCH₂), 72.98 (CH₂CHCH₂), 64.49 (CH₂CHCH₂), 31.04 (CH=CH-CH₃), 18.41 (Si(H)C(CH₃)₃), -4.66 (SiCH₃). IR (neat) 2954, 2929, 2890, 2884, 1699, 1696, 1690, 1685, 1472, 1438, 1254, 1192, 1119, 1005, 929 cm⁻¹. MS (DI) *m/z* 630 (4), 629 (8), 613 (2), 571 (4), 311 (25), 203 (100), 187 (10), 109 (20).

Preparation of 1-[[2,3-di[(1,1-dimethylethyl)dimethylsilyl]oxy]propoxy]-1-(diphenylphosphinyl)-3,5-heptadien-2-ol (6-37)

Cerium chloride heptahydrate (0.019 g, 0.0521 mmol) dissolved in 0.5 ml of anhydrous ethyl alcohol was added to a solution of **6-36** (0.033 g, 0.0521 mmol) dissolved in 0.5 ml of anhydrous ethyl alcohol. The mixture was cooled to -78°C and sodium borohydride (0.0059 g, 0.156 mmol) was added. The reaction was stirred at -78°C for 30 minutes and 2 ml of brine was added. The reaction was allowed to warm to room temperature and extracted with 3 x 10 ml of diethyl ether. The combined extracts were washed with 2 x 10 ml of water followed by 1 x 10 ml of brine. The extracts were dried over sodium sulfate, filtered, and the solvent removed *in vacuo*. Purification by prep TLC eluting with 50% diethyl ether / 50% hexanes gave 0.029 g (88%) of **6-37**. ^1H NMR (CDCl_3) δ 7.92 (m, 2H, Ph), 7.78 (m, 2H, Ph), 7.46 (m, 6H, Ph), 6.15 (m, 1H, olefinic), 5.71-5.43 (m, 3H, olefinic), 4.58 (m, 1H, Ph_2POCH), 4.11 (overlapping dd, $J = 5.4$ and 20.1 Hz, 0.5 H, HOCH), 3.63-3.31 (m, 5H, CH_2CHCH_2), 1.65 (d, $J = 6.7$ Hz, 3H, $\text{CH}=\text{CHCH}_3$), 0.82 (s, 18H, $\text{SiC}(\text{CH}_3)_3$), 0.0 (s, 12H, $\text{SiC}(\text{CH}_3)_2$). ^{13}C NMR (CDCl_3) δ 132.0 (Ph), 128 (Ph), 83.71 (olefinic), 82.86 (olefinic), 81.97 (olefinic), 81.10 (olefinic), 74.53 (HOCH), 71.23 (CH_2CHCH_2), 71.68 (CH_2CHCH_2), 71.10 (CH_2CHCH_2), 64.20 (PhPOCH), 30.92 (CH_3), 29.27 ($\text{SiC}(\text{CH}_3)_3$), 18.12 ($\text{SiC}(\text{CH}_3)_3$), -5.34 ($\text{Si}(\text{CH}_3)_2$). IR (neat) 3325, 3056, 2955, 2928, 2884, 2856, 2359, 1592, 1463, 1438, 1252, 1144, 1117, 1096, 991, 836, 812, 778, 747 cm^{-1} . MS (CI) m/z 631 (MH^+ , 6), 613 (4), 563 (2), 535 (4), 321 (9), 245 (10), 203 (100).

Preparation of 3-(2,4,6-heptatrienyloxy)-1,2-bis[*t*-butyldimethylsiloxy]-propane (6-35)

1 M Potassium *t*-butoxide (0.160 ml, 0.160 mmol) was added dropwise to a solution of **6-37** (0.0092 g, 0.146 mmol) dissolved in 1.2 ml of anhydrous tetrahydrofuran which had been cooled to -50°C . The reaction was stirred at -50°C for 10

minutes then the temperature was raised to 4°C where it was stirred for 12 hours. The solvent was removed *in vacuo* and was purified by prep TLC which was pre-treated with 5% triethylamine / 95% hexanes. Eluting with 95% hexanes / 5% ethyl acetate gave 0.049 g (82%) of **6-35**. ¹H NMR (CDCl₃) δ 6.42 (m, 1H, olefinic), 6.05 (m, 3H, olefinic), 5.94 (d, J = 8.0 Hz, 1H, olefinic), 5.50 (dd, J = 8.0 and 14.0 Hz, 1H, olefinic), 3.86 (m, 2H, CH₂CHCH₂O), 3.72 (m, 1H, CH₂CHCH₂), 3.53 (m, 2H, CH₂CHCH₂OR), 1.68 (d, J = 5.0 Hz, 3H, CH=CHCH₃), 0.87 (s, 18H, SiC(CH₃)₃), 0.04 (s, 12H, Si(CH₃)₂). ¹³C NMR (CDCl₃) δ 146.93 (olefinic), 132.46 (olefinic), 129.23 (olefinic), 127.89 (olefinic), 127.83 (olefinic), 123.81 (olefinic), 75.15 (CH₂CHCH₂), 75.53 (CH₂CHCH₂), 64.54 (CH₂CHCH₂), 26.02 (SiC(CH₃)₃), 18.48 (CH=CHCH₃), 13.52 (SiC(CH₃)₃), -4.62 (Si(CH₃)₂). IR (neat) 2956, 2929, 2885, 2857, 2360, 2342, 1646, 1591, 1472, 1463, 1256, 1215, 1103, 987, 837, 777 cm⁻¹. MS (CI) 413 (MHH, 35), 349 (15), 321 (55), 303 (100), 281 (35), 189 (20), 171 (30), 149 (35), 133 (40), 109 (55). UV (MeOH) 280 nm.

Alternate preparation of 1-[[2,3-di[(1,1-dimethylethyl)dimethylsilyl]-oxy]propoxy]-1-(diphenylphosphinyl)-3,5-heptadien-2-ol (6-37)

0.5 M lithium diisopropylamide (1.45 ml, 0.721 mmol) was added dropwise to a solution of **6-38** (0.323 g, 0.604 mmol) in anhydrous tetrahydrofuran which had been cooled to -50°C. The reaction was stirred at -50°C for 15 minutes then cooled to -78°C. Next 2,4-heptadienal (0.073 ml, 0.665 mmol) dissolved in 1 ml of anhydrous tetrahydrofuran was added dropwise. Stirring was continued at -78°C for 1 hour. Saturated ammonium chloride (10 ml) was added and the reaction was allowed to warm to room temperature. The layers were separated and the aqueous layer extracted with 3 x 25 ml of diethyl ether. The combined organic extracts were washed with 2 x 25 ml of water followed by 1 x 25 ml of brine. The extracts were dried over sodium sulfate, filtered, and the solvent removed *in vacuo*. Purification by prep TLC eluting with 50% ethyl acetate / 50%

hexanes gave 0.341 g (90%) of **6-37**. ^1H NMR (CDCl_3) δ 7.92 (m, 2H, Ph), 7.77 (m, 2H, Ph), 7.46 (m, 6H, Ph), 6.15 (m, 1H, olefinic), 5.69-5.42 (m, 3H, olefinic), 4.58 (m, 1H, Ph_2POCH), 4.11 (overlapping dd, $J = 5.4$ and 20.0 Hz, 1H, HOCH), 3.63-3.31 (m, 5H, CH_3CHCH_2), 1.63 (d, $J = 6.7$ Hz, 3H, $\text{CH}=\text{CHCH}_3$), 0.84 (s, 18H, $\text{SiC}(\text{CH}_3)_3$), 0.0 (s, 12H, $\text{Si}(\text{CH}_3)_2$). ^{13}C NMR (CDCl_3) δ 133.0 (Ph), 127 (Ph), 83.7 (olefinic), 81.3 (olefinic), 81.8 (olefinic), 81.1 (olefinic), 74.51 (HOCH), 71.25 (CH_3CHCH_2), 71.65 (CH_2CHCH_2), 71.12 (CH_2CHCH_2), 64.18 (Ph_2POCH), 31.0 (CH_3), 29.3 ($\text{SiC}(\text{CH}_3)_3$), 18.95 ($\text{SiC}(\text{CH}_3)_3$), -5.4 ($\text{Si}(\text{CH}_3)_2$). IR (neat) 3323, 3053, 2953, 2930, 2882, 2360, 1438, 1252, 1144, 1096, 991, 840, 812, 747 cm^{-1} . MS (CI) m/z 631 (MH^+ , 8), 613 (5), 563 (3), 535 (4), 321 (10), 245 (10), 203 (100). This compound and the compound prepared by the migration route were found to be identical in all respects.

VII. Literature Cited

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