STUDIES RELATING TO FECAPENTAENE-12

by

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(ABSTRACT)

The glyceryl enol ether fecapentaene-12 (FP-12) is a direct-acting mutagen that is formed by bacteria in the lower part of the gasrtrointestinal tract from a precursor of unknown structure. Two major unsolved questions concerning FP-12 are the structure of its precursor and the nature of its interaction (if any) with DNA.

The structure of the biosynthetic precursor of FP-12 is thought to be that of a plasmalogen with an intact pentaenyl ether moiety. A synthesis of the perhydro analog of the proposed precursor structure is described, and approaches to the synthesis of the precursor itself are also described. Comparison of chromatographic data for the saturated model precusor and natural precursor provided evidence for the structure of the latter.

The nature of the interactions of FP-12 with DNA was probed by model studies of the reaction of nucleoside bases with FP-12 and two proposed FP-12 metabolites. No adducts were formed between FP-12 or between the various putative polyenal metabolites and guanosine, cytosine, or thymidine. A model epoxy ether did react with a guanosine derivative, however, indicating that an epoxy ether derivative of FP-12, if formed, would be capable of reacting with DNA.

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An undertaking such as this dissertation hardly seems plausible without undying encouragement from loved ones. Therefore, the author wishes to express sincere gratitude to his brothers , , and . In addition, the author wishes to thank for being so supportive, especially when the going was rough. Their generosity is matched by few. A very special thanks goes to who not only provided the author with much needed encouragement and helpful suggestions during his graduate career but also taught the author an attitude that allowed him to become a success.

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I Introduction

1.1 Colon Cancer Incidence.

Cancer is a health disorder in which biological, physical or chemical causes are known or suspected. It is no wonder that experimental cancer research is conducted by pathologists, radiation physicists, virologists, microbiologists, toxicologists and chemists. The mortality rate due to cancer has increased from 3.3% in 1900 to 20% in 1988.[1] Epidemiologists have proposed that the higher incidence of cancer is due to the higher life expectancy; cancer is a chronic disorder, with a latency period of up to 50 years[2]. However, between 1960 and 1977 there were more deaths due to neoplasms in the United States than could be attributed to the changed age structure (Table 1).[3] Furthermore, death rate data adjusted to age structure have shown that the death rate due to cancer has increased over the last 30 years (Table 2).[4] Perhaps: "Cancer is increasing simply because those things which cause it are increasing" (K.H. Bauer).

Colon cancer is the second most common neoplasm and has the second highest mortality rate per incidence of all cancers.[5] It has been proposed that the low nerve cell density at the epithelial cells of the intestine might explain this high morbidity rate because victims are at advanced stages before significant symptoms appear. Early detection of colon cancer therefore, is essential to curtail its progression.

Cancer epidemiology provides insight into cultural and mechanistic aspects of cancer, however low levels of risk cannot be accurately determined.[6] In the case of colon cancer, international studies suggest that the incidence of colon cancer is associated with diets high in fat and low in fiber.[7] Unfortunately, the exact role that the diet plays in the etiology of colon cancer is difficult to determine. Since cancer has a long latency period, individuals could be exposed to many other hazards before the malignancy becomes detectable. Sorting out the role of each individual hazard would be almost impossible.

Table 1. Distribution of the most common causes of death in the United States from 1950 to 1977.

	Death Rate[a]			
CAUSE	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

[a]Death rates (deaths/100,000 population) are adjusted to the age structure of the population.

Table 2. 30-year trends in age-adjusted cancer death rates per 100,000 population (1953-1955 to 1983-1985).

SITES	SEX	1953-55	1983-85	PERCENT CHANGES
ALL SITES	Male Female	175.7 145	203.1 138.2	+ 16 - 5
BLADDER	Male Female	7.2 3.1	6.1 1.8	- 15 - 42
BRAIN	Male Female	3.9 2.6	4.7 3.2	+ 21 + 23
BREAST	Male Female	0.3 26.2	0.2 27.1	* + 3
COLON & RECTUM	Male Female	25.8 24.4	24.7 17.5	* - 28
ESOPHAGUS	Male Female	4.7 1.2	5.6 1.5	+ 19 *
KIDNEY	Male Female	3.6 2.2	4.9 2.3	+ 36 *
LEUKEMIA	Male Female	8.2 5.5	8.4 5.0	+ 2 - 9
LIVER	Male Female	6.2 7.1	4.9 3.3	- 21 - 54
LUNG	Male Female	28.0 5.1	73.1 25.3	+161 +396
OVARY	Female	8.6	7.8	- 9
PANCREAS	Male Female	9.1 5.7	10.2 7.2	+ 12 + 26
PROSTATE	Male	21.3	23.2	+ 9
SKIN	Male Female	3.1 1.9	4.0 1.8	+ 29 *
STOMACH	Male Female	21.3 11.2	10.2 3.5	- 52 - 69
UTERUS	Female	19.0	7.1	- 63

* Percent changes not listed because they are not meaningful.

The past twelve years have seen the introduction of important tests which screen for potential carcinogenic activity using mutagenacity or genotoxicity as indicators. The first such test was developed by Ames[8] in 1976 and employs special bacterial strains as sensitive indicators of DNA damage. The Ames test is an excellent general technique because mutagenicity can be quantitatively determined by counting the number of revertant colonies after incubation of the bacteria with the test compound. The importance of this work is that many known carcinogens are mutagenic by the test, thus correlating carcinogenicity with mutagenicity. It must be noted, however, that not all mutagens are carcinogens. With the advent of improved DNA assay techniques, additional data pertaining to the possible carcinogenicity of a substance can be acquired. The data is revealed by observing genotoxic effects (eg. DNA damage, chromosome aberrations) on tissues of mammalian cell cultures.

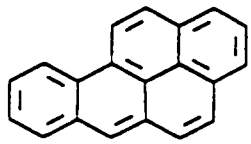
Biopsy of colonic tissue is a relatively simple procedure compared to other tissues such as the stomach, lung or liver. Therefore, it is a convenient site for performing the above mentioned assays. In addition, with the ability to perform a series of biopsies on the colon tissue, direct observation of the developing disease is possible.[9] Examination of the chemistry of the colonic lumen, microscopic observation of biopsies of colonic tissue, or the use of an endoscope are three observation techniques that also aid in acquisition of pertinent data relating to colon cancer etiology. These experimental techniques, thus, provide a rapid way of testing hypotheses for the origin of colon cancer since epidemiological studies require long periods of time before significant symptoms appear.

1.2 Possible Causes of Colon Cancer.

Many hypotheses for the origin of colon cancer are currently being studied. Three important important factors on many of the theories include calcium deficiency, high fecal pH and

fecal mutagens. It has been proposed that the presence of calcium in the colon precipitates bile acids as their calcium salts. A deficiency of calcium, thereby, leads to the increased concentration of bile acids (known tumor promoters) and fatty acids in the intestinal lumen.[10] Due to the toxicity to colonic epithelial cells by these acids, cell proliferation is increased and thus the cells are sensitized to carcinogens.[11] A high fecal pH also increases the solubility of bile acids in the colon, thereby increasing toxic effects, cell proliferation, and the potential for tumor promotion.[12]

The presence of mutagens in the body has been implicated as a possible cause of colon cancer; these mutagens originate from the diet or are naturally occurring. Proteinaceous foods that are fried contain various gentoxic compounds which include the potent carcinogens benzo(a)pyrene (**1**), 3-amino-1,4-dimethyl-5H-pyrido-(4,3-b)-indole (Trp-P-1, **2**) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ, **3**).[14]



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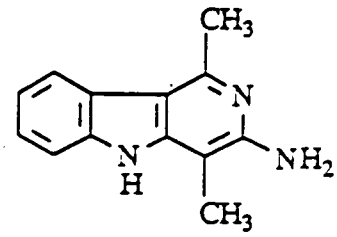

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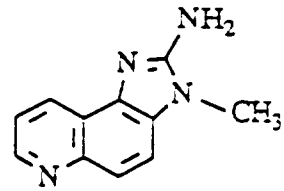

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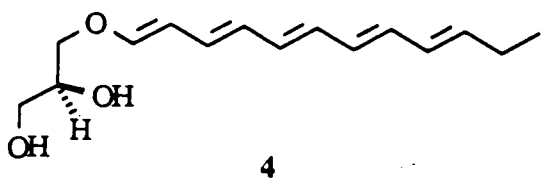
These compounds are known carcinogens[15], however their role in colon cancer remains unclear. Naturally occurring bioactive compounds found in the fecal stream include 3-ketosteroids, cytotoxic steroids, genotoxic steroids and the fecapentaenes.[16] The concentration of the bioactive

steroids in the fecal stream is varied, usually low and they presumably originate from host or colonic metabolism.[17] On the other hand, the fecapentaenes are the most prevalent and abundant mutagens in the colon and their origin is unknown.

1.3 Isolation and Structure Elucidation of Fecapentaene-12.

In 1977 it was discovered that organic extracts of feces contained mutagens that were active on tester strains TA-98 and TA-100.[18,19] Isolation of these mutagens proved difficult due to their acid, light and air lability and low concentration in feces. Incubation of feces under anaerobic conditions in the presence of bile acids increased their concentrations 20-50 fold.[20] This was a key factor in obtaining satisfactory yields for structural analysis. Stabilization of the pure compounds using mild base and the antioxidant BHT provided sufficient quantities of unoxidized mutagens for spectral analysis.[20] A mixture of closely related compounds as isomers and homologs were observed in most fecal samples as evidenced by HPLC analysis (Figure 1).[21] Isolation of the compound corresponding to peak 1 could be obtained from certain feces. Based on UV spectra ("triplet" at 320, 340 and 360 nm), mass spectra (MH^+ at *m/z* 250 mu)[22] proton NMR[23,24] and chemical degradation experiments, the mutagen was assigned the structure **4**, (S)-3-(1,3,5,7,9-dodeca-pentaenyloxy)-1,2-propanediol[25,26] hereafter to be referred to as fecapentaene-12 (FP-12). The stereochemistry of FP-12 was established by comparing the bis (+)-α-methoxy-α-(trifluoro-methyl)-α-phenylacetyl esters of hydrogenated mutagen and synthetic chiral 3-dodecyloxy-1,2-propanediol on HPLC.



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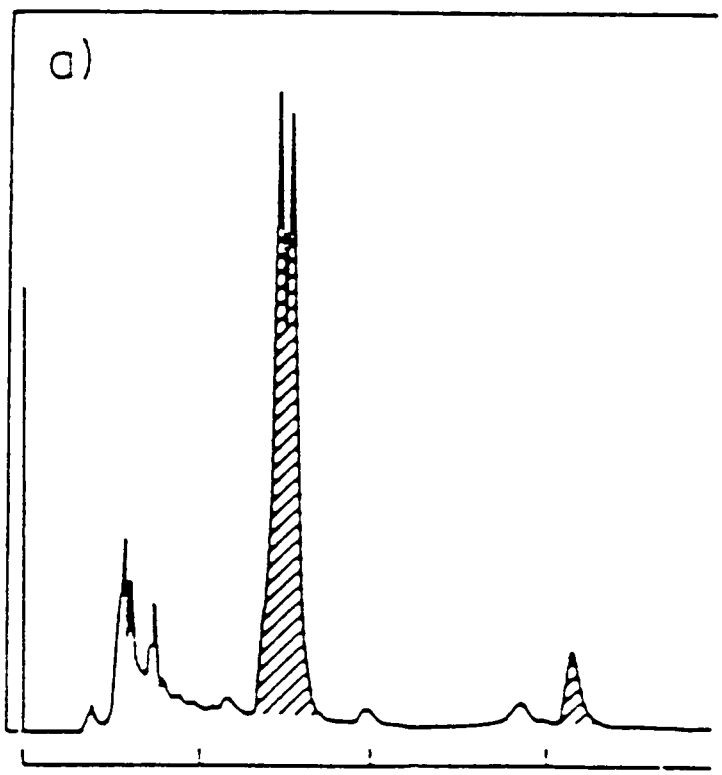
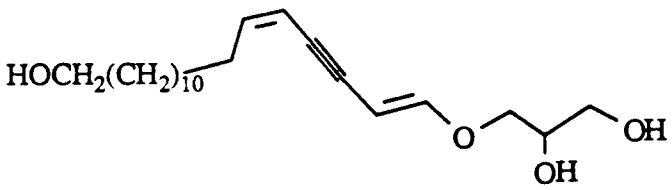


Figure 1. HPLC chromatogram of natural FP-12.

The structure of FP-12 is novel because it possesses a pentaenyl moiety on a glycerol backbone; no compound of this type has ever been characterized before. In fact, only one compound has been isolated and characterized which has any resemblance to FP-12's unusual array of functionalities. (+)-Raspailyne (**5**), was isolated from the marine sponge Rasapailia pumila.[27] This compound is similar to FP-12 in that it is an enol ether of glycerol with extended conjugation but it is not a pentaene and it is not mutagenic.



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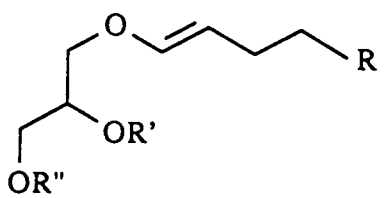
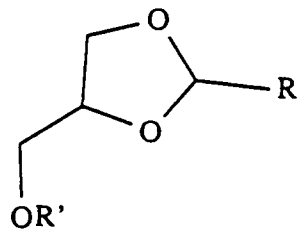
Fecapentaene-12 is a bacterial metabolite of a plasmalogen of general structure **6**. It is known that plasmalogens have been isolated from anaerobic bacteria such as Clostridium sp.[28]



6

Research has shown that plasmalogens might have a biological precursor **7** where elimination could introduce the enol ether moiety.[29] However, acetals like structure **7** are not known to exist in bacteria. Therefore, if bacteria metabolize acetals to plasmalogens they would have to do so from acetals which are available from external sources (i.e. animal or plant tissue). Fecapentaene-12 has an additional feature in that conjugation of the enol ether requires an oxidative process, a phenomenon that would be surprising in the strict anaerobic environment of the colonic microflora.

Thus it is now believed that the plasmalogen precursors of the fecapentaenes originate from host metabolism.



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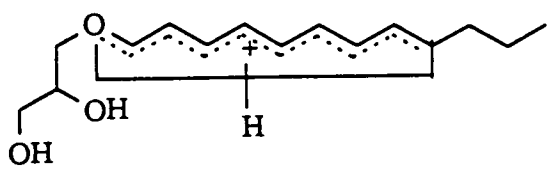
It is important to note here that the unique structure of FP-12 is responsible for its high reactivity in the presence of light, air and acid. Acid would form the unstable intermediate cation **8**. This pentaene system imparts a nucleophilic character to FP-12 which would explain its high



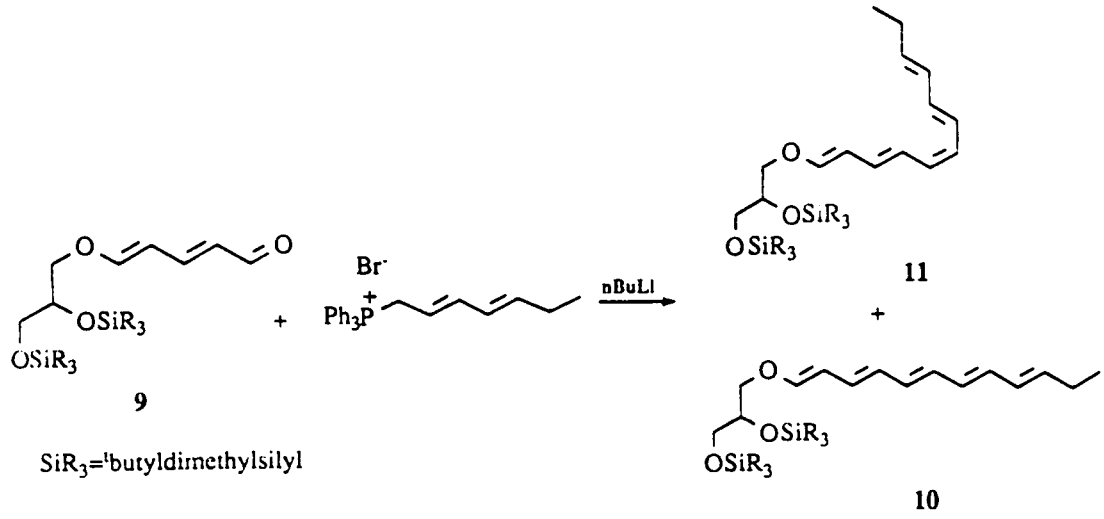
8

instability in the presence of oxygen although oxidation of FP-12 probably proceeds by a radical mechanism. A DMSO solution of FP-12 decomposed in the presence of air to the extent of about 80% after 20 minutes.[30] Light catalyzed radical formation would also form intermediate radicals stabilized through resonance. Although light induced decomposition products have not been characterized, polymerization of the pentaenyl ether moiety of FP-12 is believed to be the reaction occurring.

1.4 Fecapentaene-12 Syntheses.

Because isolation of FP-12 from natural sources provided such small quantities, carcinogenicity testing and studies of other biological activity proved impossible. For this reason gram quantities of FP-12 needed to be synthesized. The existence of the pentaenyl ether moiety made the synthesis of FP-12 a challenging project due to the aforementioned instability of this system. However, in the past 5 years, four basic approaches have provided the quantity of FP-12 needed for biological testing.

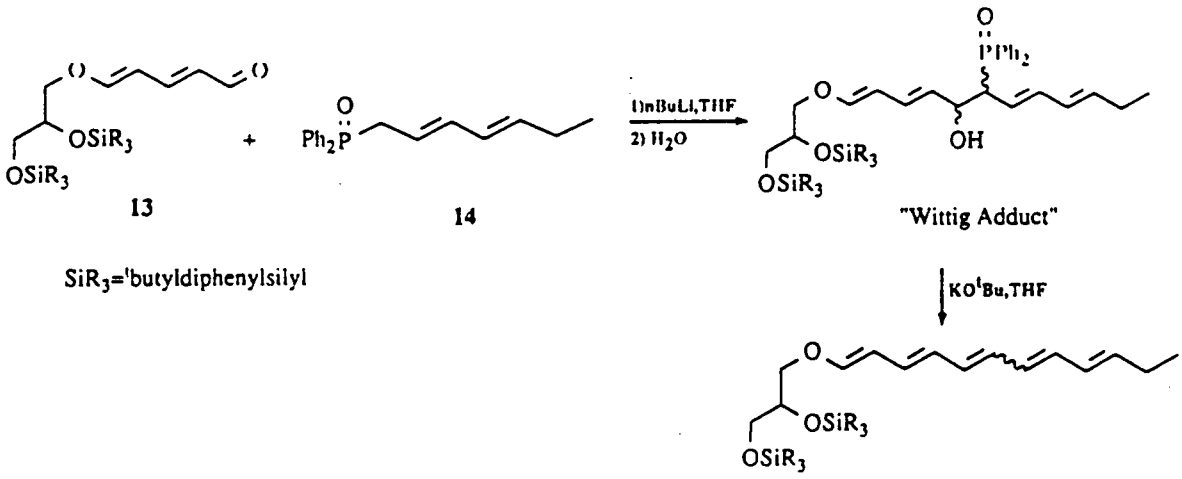
The first report of the synthesis of fecapentaene-12 was made by Kingston, et. al.[31] in 1983. The key step in the synthesis used Horner-Wittig chemistry as shown in Scheme 1. The intermediate, **9**, was made from 2,3-O-isopropylideneglycerol (solketal) in ten synthetic steps as the



Scheme 1.

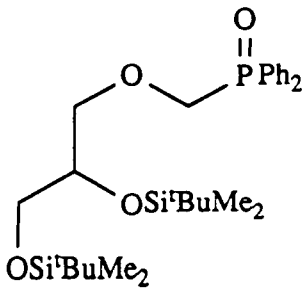
E,E isomer. Reaction of the aldehyde, **9**, with the phosphorous ylide prepared from (E,E)-2,4-heptadienyltriphenylphosphonium bromide yielded a mixture of E and Z pentaenes (**10** and **11**). This pentaene was converted to FP-12 by deprotection of the silyl ethers with fluoride ion.

The second reported synthesis of fecapentaene-12 was made by Nicolaou[32] in 1984. He applied an analogous strategy to that of Kingston. Nicolaou also applied a Horner-Wittig reaction to an intermediate (**12**) that differed from **9** only in that he used tert-butyldiphenylsilyl ethers instead of tert-butyldimethylsilyl ethers. In addition, Nicolaou used diphenylhepta-2,4-dienyl phosphine oxide (**14**) as the Wittig reagent. He isolated the "Wittig Adduct" **13** as a mixture of diastereomers. Consequently, with this methodology he was also able to get fecapentaene-12 as a mixture of 5-E and 5-Z isomers (Scheme 2).



Scheme 2.

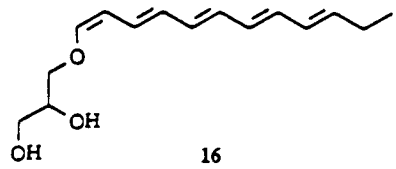
In 1984, Van der Gen and coworkers[33] developed a synthesis of FP-12 that used Horner-Wittig chemistry from a different perspective. They made the Wittig reagent, **15**, in four steps



15

starting with diphenylchlorophosphine. Subsequent reaction of the anion of **15** with undecatetra-2,4,6,8-en-1-al provided their group with fecapentaene-12 as a mixture of 1-E and 1-Z isomers.

These three syntheses of fecapentaene-12 thus provided methods of obtaining 1-cis (**16**) and 5-cis (**17**) geometric isomers in a mixture with the all trans isomer. In 1988 Pfaendler made



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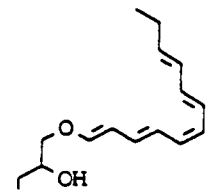

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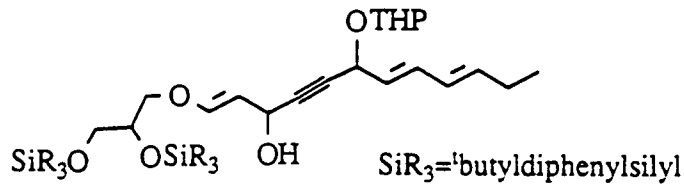
the acetylenic intermediate **18** in a total of 7 synthetic steps starting with solketal and hepta-2,4-

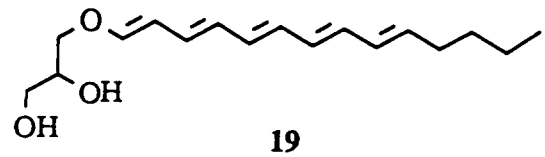


18

dien-1-al. Reduction of **18** with lithium aluminum hydride provided a method of obtaining pure, crystalline all-trans FP-12.[34] The availability of geometric isomers is important because the exact stereochemistry of natural FP-12 was not known.

1.5 Fecapentaene-12 Distribution.

Previously it was mentioned that the isolated fecal mutagen included isomers and homologs. The compound corresponding to peak 4 (Figure 1) was identified as FP-14 (**19**);[35] the other components in the chromatogram were believed to be geometric isomers of FP-12. There



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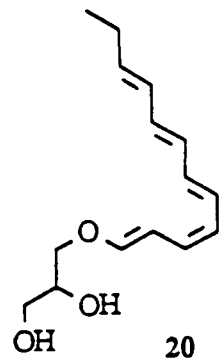
are 32 possible combinations of cis and trans geometries in the pentaene system; the all trans isomer is expected to be the most stable. Studies were carried out to determine the distribution of these isomers in naturally occurring fecal extracts. Application of the available 1-cis (**16**), 5-cis (**17**), all trans FP-12 and natural FP-12 to comparative HPLC analysis provided information on the distribution of FP-12 isomers in fecal extracts. The ratio of FP-14 to FP-12 varies with each individual fecal sample. In addition it has been determined that geometric isomers are indeed excreted. Specifically the 5-cis FP-12 (**17**) exists in certain fecal samples and in some cases exceeds the all-trans geometric isomer in concentration. In some batches it has been determined that the ratio of isomers consists of about 10% 5-cis ,(**17**), 45% 3-cis, (**20**), 35% all-trans and 10% 1-cis, (**16**).[36] Clearly these results indicate a distribution among isomers which most likely will depend on the excreter. This varied distribution might explain the difficulty in isolating natural FP-12 since Z-isomers are believed to be highly unstable.



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A more general query arises in how the distribution of fecal mutagens varies from a geographical point of view. Colon cancer displays its greatest frequency among economically

developed nations,[37] and North Americans are considered a high risk population.[38] It is interesting to note that urban South Africans show a high rate of colon cancer and a high incidence of fecal mutagenicity (19%) by the Ames test. This is in contrast to the low incidence of fecal mutagenacity found in urban blacks (2%) and in rural blacks (0%).[39] These results reemphasize the role of diet in the etiology of colon cancer. From observations such as this it is speculated that the high levels of bile acids, caused by a high fat and low fiber diet, can also facilitate generation of endogenous genotoxins.

Reddy and coworkers (1980)[40] further attempted to correlate the presence of fecal mutagens with populations at high risk for colon cancer. Three populations at varied risk were examined for dietary intake and excretion of fecal mutagens. The low risk population, which consumed a large proportion of vegetables and fruits, showed no mutagenicity on strains TA-98 or TA-100. In the high risk population, with moderate meat intake, 13% of the fecal extracts were mutagenic on tester strain TA-98. Activation by S-9 was required in all of the cases; none of the fecal extracts from this population were mutagenic on tester strain TA-100. In the high risk population with highest meat intake, 22% were active on TA-98, 11% were directly active on TA-100 and 6% were active with S-9 activation on TA-100. These results are consistent with the data reported by Ehrich, et. al., on South African populations.[39] Although these studies indicate an apparent correlation between the presence of fecal mutagens and risk for colon cancer, they also imply the existence of more than one class of mutagen due to the varied response by the tester strains.

1.6 Biological Activity of Fecapentaene-12.

Synthetic FP-12 was assayed by the Ames test using Salmonella strains TA-98 and TA-100; it proved to be highly mutagenic with over 2,000 revertant colonies per microgram[41-43]. No

metabolic activation by microsomal enzymes was required to observe the mutagenicity, indicating that FP-12 is a direct acting mutagen. In 1986, Plummer[44] cultured human fibroblasts in the presence of FP-12 and found that the genotoxicity of FP-12 was 900-fold and 300-fold more than the two direct acting mutagens N-methyl-N-nitrosourea and formaldehyde, respectively.[45] He further showed that DNA single strand breaks and sister chromatid exchanges (SCE's) occurred.[44] Later, Pfaendler observed dose dependent chromosome aberrations in the presence of FP-12, revealing for the first time that FP-12 is a direct acting mutagen in human lymphocytes.[46] In 1987 Baptista[47] prepared FP-12 suppositories and administered them to mice colon. Histological sections were examined for nuclear aberrations and they found no significant increase. Baptista explained that the triglyceride cocoa butter suppositories might have imparted a protective environment for FP-12. The above results imply the possibility of some biological or chemical activation before genotoxicity can occur.

Carcinogens in the intestine may originate from three basic sources. These sources are the diet, the host or the microflora. Although host metabolism may be the origin of the FP-12 precursor, the intestinal flora is directly responsible for FP-12 biosynthesis. Wilkins and coworkers[48] have shown that incubation of feces from an excreter increased mutagen production 10-fold, a result not observed when a similar experiment was conducted with cold or autoclaved feces or under aerobic conditions. Furthermore, upon incubating autoclaved excreter feces with fresh feces, FP-12 production was observed.

This same group developed conditions in which production of FP-12 could be enhanced. It was discovered that 5 species of the bacteria Bacteroides produced FP-12 when incubated with fecal extracts. All species are present in intestinal flora. Wilkins and coworkers also observed that addition of bile dramatically increased the production of the mutagen.[19] Although it was originally thought a precursor might exist in bile, it was felt that bile more likely solubilized the precursors

already present in feces or fecal extracts. To demonstrate the role dietary fiber has on the epidemiology of colon cancer, it was shown that addition of fermentable carbohydrates suppressed the production of fecal mutagen during incubation.[49]

In 1982 an elaborate set of experiments was conducted by Van Tassell, et. al.,[47] to determine the exact conditions required for FP-12 production. Forty species of anaerobes were screened for the ability to produce a fecal mutagen. Bacteroides thetaiotaomicron proved to be the most productive species. This species was incubated with various sources of feces, each sample independently subjected to special experimental conditions; the results are summarized in Table 3. It was shown that there was a large difference between the concentrations of fecapentaenes and their precursors in fresh feces from excreters and nonexcreters when the samples were incubated with Bacteroides spp. However, the fact that nonexcreter inoculum increased the concentration of mutagen in autoclaved excreter feces indicated the presence of the fecapentaene producing bacteria in nonexcreters. An extractable "precursor" was later found to be unique to excreters and furthermore the incubation of the precursor with appropriate bacterium in the presence of bile and fresh feces produced mutagen.

1.7 Statement of Goals.

The role of fecapentaene-12 in the etiology of colon cancer remains unclear. Research in this area is being actively pursued and could disprove or support any theories that have been proposed, or even generate new theories. Research projects that could aid in obtaining important information in this regard include DNA interactions, biogenesis studies and epidemiological studies.

Whereas epidemilogical studies have shown a correlation between diet and colon cancer and that species common to all human intestinal flora produce fecal mutagens, extensive studies have shown that diet has little to do with the distribution of microflora in the intestine. It now

Table 3. *In vitro* incubation of fresh and autoclaved feces and fecal extract broth.

Substance incubated[a]	Source	Inoculum(5%)	HPLC area[b] 0 h	72 h
Fresh feces	Excreter	None	3,200	40,000
	Nonexcreter	None	<100	<100
Autoclaved feces	Excreter	Feces(E[c])	3,300	31,000
	Nonexcreter	Feces(E)	250	400
	Excreter	Feces(N[c])	3,100	29,000
	Nonexcreter	Feces(N)	<100	<100
Fecal extract[d]	Excreter	Feces(E)	3,000	18,000
	Nonexcreter	Feces(E)	<100	<100
	Excreter	Feces(N)	2,800	19,000
	Nonexcreter	Feces(N)	<100	<100

[a]Fresh and autoclaved feces and fecal extract broths were supplemented with bile (10 mg/ml).
[b]HPLC area corresponds to the peak of FP-12 under standardized conditions.
[c]E=Excreter; N=Nonexcreter
[d]Methanolic extract.

becomes apparent that a complex scenario exists which influences the existence of mutagens (perhaps FP-12) and the biological activity of the mutagens. The presence of a biological precursor, fiber content, and bile acid concentration are all factors which seem to contribute to this scenario.

Wilkins and Van Tassell[49] have described a possible scenario of the interactions which could occur in the colon (Figure 2). They have included the liver in this scenario as a means for activation of indirect genotoxins, such as IQ and Trp-P-1. These activated genotoxins enter the colon through bile. In terms of FP-12, the plasmalogen precursor could originate from mucosal metabolism. Support of this theory is realized in recent work[50] where it was shown that germ-free pigs produced the precursors of fecapentaenes, thus the fecapentaenes are not likely of baterial origin. It should be reemphasized that the precursor may be more than one compound; this is suggested by the existence of geometric isomers of FP-12. These precursors eventually reach the colonic milieu and are then metabolized to FP-12 by colonic bacteria.

It has been shown that FP-12 is highly mutagenic and its biological precursor is converted to FP-12 with intestinal flora common to all people, excreter and nonexcreter alike. In addition, the biological precursor is only found in large amounts in individuals who excrete FP-12. What then are the characteristics of the precursor? Is the metabolic pathway from a preprecursor to plasmalogen precursor unique among excreters? Of course information on the structure of the precursor, and ultimately the precursor structure itself, must be determined before these questions can be pursued further.

The etiology of a disease can be defined as the origin and cause of the disease. Thus, determining structures of precursors to certain biologically active compounds aids in the discovery of the origin and causes of the disease associated with these compounds. Studies relating to a substance's interaction with the cell nucleus provide information about the potential of the substance

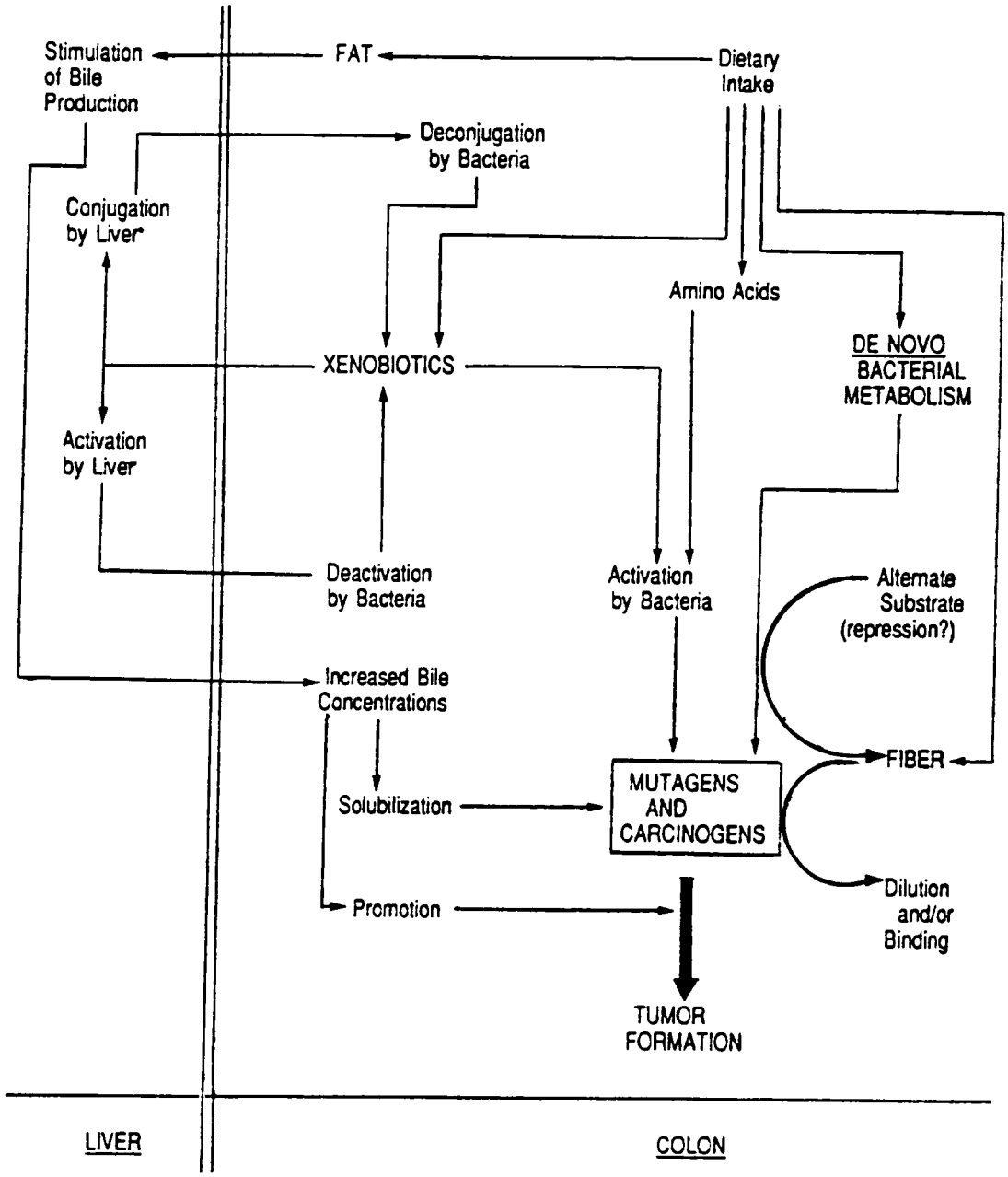


Figure 2. Possible biochemical scenario of FP-12 and its etiology in colon cancer.

to cause cancer. It is known that regulation of sequences of DNA determines a cell's phenotype and malignant cells contain abnormal phenotypes and therefore their DNA has been altered in some way.[51] Thus, it is reasonable to consider DNA as the most critical target for carcinogens. Ames' correlation between mutagenicity and carcinogenicity supports this idea. Carcinogens can effect DNA by a mode of action resulting from covalent binding, intercalation, chromosomal protein binding or altering the DNA precursor pool. Fecapentaene-12 is mutagenic, damages DNA and causes chromosome aberrations; the mechanism of interaction of FP-12 with DNA is not known. This information is needed to further uncover the role FP-12 has in colon cancer.

The evidence thus far suggests that fecapentaene-12 may have a role in the etiology of colon cancer. We elected to pursue two major aspects of this role. Our first study was directed towards the structure elucidation and synthesis of the biological precursor of FP-12, and our second study was designed to test one possible way in which FP-12 could bind to DNA.

II Fecapentaene-12 Precursor Structure-Initial Studies.

2.1 Precursor Isolation

Wilkins and coworkers[52] separated freeze-dried feces extracts into several aliquots by HPLC. Fractions containing precursor could be screened by incubating the aliquots with Bacteroides thetaiotaomicron with a bile supplement, and monitoring fecapentaene-12 production by TLC and HPLC analysis. The significant fractions were further purified by HPLC. Enough precursor was purified to produce 50-100 μg of fecapentaene-12. The precursor was found to be non-mutagenic by the Ames test. Chromatographic properties, mass spectra, and UV spectra could be obtained.

2.2 Preliminary Structure Determination Experiments

The UV spectrum of purified precursor was virtually identical to that of fecapentaene-12.[53] Absorbance maxima for the precursor were 321, 335 and 353 compared to 320, 335 and 352 for FP-12. However, chromatographic properties of the precursor were very different from that of the fecapentaenes'. Various TLC systems were developed to compare the chromatographic properties. The results are shown in Table 4. In all TLC plates the precursor's spots fluoresced to a lime green color under longwave UV light, which disappeared after 1-2 minutes exposure to air; this is a property identical with the fecapentaenes. However, none of the specific visualization reagents used to detect fecapentaene-12 reacted with the precursor. Only ammonium sulfate charring made precursor visual. *In vitro* incubation of the fluorescent precursor spots removed from semi-preparative TLC plates produced fecapentaene-12. These experiments provided conclusive evidence that the precursor to fecapentaene-12 was isolated.

The TLC data show that the precursor was less polar than the fecapentaenes on reversed phase TLC and more polar in normal phase systems thus indicating its amphipolar nature (i.e.

Table 4. Mobilities of fecapentaene-12 and precursor in TLC.

TLC System	Rf (FP-12)	Rf (Precursor)
C_{18}/MeOH	0.86	0.64
C_{18}/MeOH:H_2O (90:10)	0.78	0.18
C_{18}/IPA	0.83	0.14
C_{18}/THF	0.86	0.83
C_{18}/CH_3CN	0.73	0.03
Sil/$CHCl_3$:IPA (92:8)	0.65	0.05
Sil/$CHCl_3$:MeOH (85:15)	0.75	0.32
Sil/$CHCl_3$:MeOH (92:8)	0.60	0.22

Table 5. 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

[C_{18}-methanol (100), Silica- chloroform:methanol (92:8)]

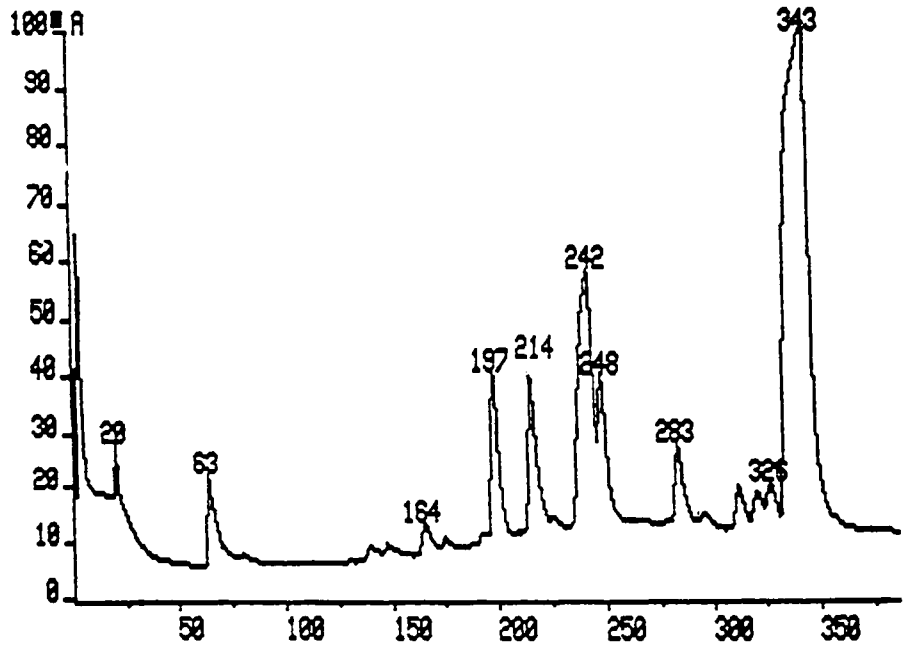


Figure 3. GC chromatogram (EI) of the methylated products of hydrolyzed precursor.

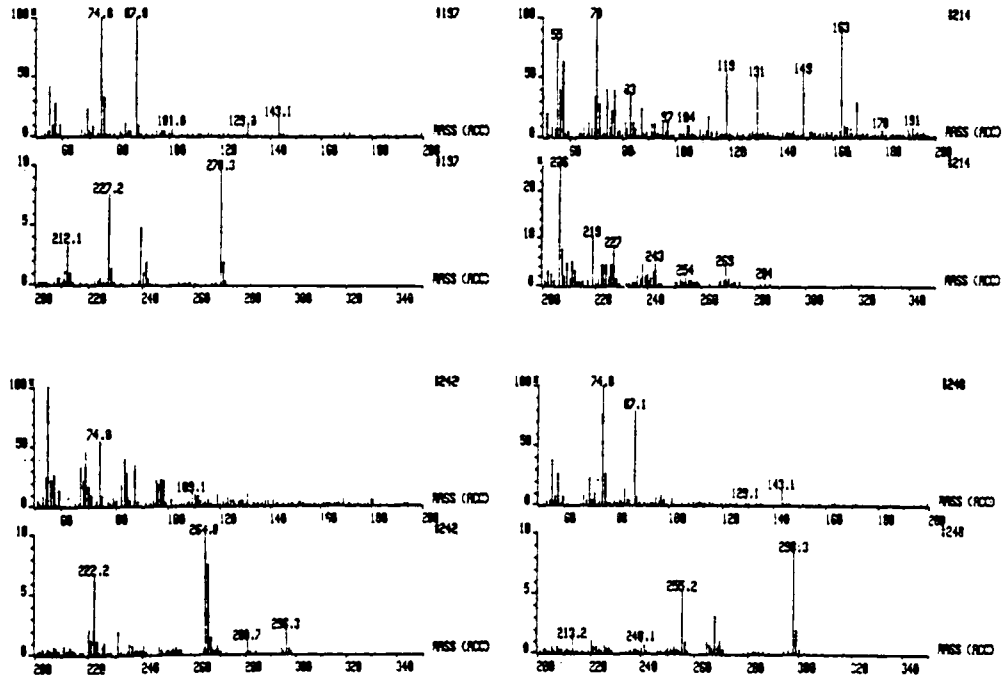


Figure 4. Mass spectrum of peaks 197, 214, 242, and 248 from gas chromatogram (Figure 3.) showing the presence of methyl esters of palmitic (197), heptadecanoic (214), oleic (242) and stearic acids.

displaying polar and non-polar character). The identical UV spectrum of precursor to that of FP-12 indicated the presence of a pentaenyl moiety in the precursor. In addition, the polar nature of the precursor indicates the presence of a highly polar substituent on at least one of the hydroxyl groups.

To compare the extent of derivatization of fecapentaene-12 and the precursor, both compounds were treated with the potent silylating reagent Sylon BTZ. Sylon BTZ, which is available from Supelco, Inc., is a mixture of N,O-bis(trimethylsilyl)acetamide, trimethylchlorosilane and trimethylsilylimidazole and will derivatize all hydroxyl groups in any position. The relative reactivities were tested by TLC analysis. The results shown in Table 5 indicate that the precursor has no free hydroxyl groups.

A mass spectrum was run on the precursor and no molecular ion or fragments could be clearly identified. A MS/MS analysis was then done. Ambiguous information about the precursor's molecular weight was obtained with an M^+ peak at either 589 or 830. The inability to obtain reliable mass data is probably due to the instability of the precursor. However, the MS/MS data uncovered the presence of long chain fatty acid fragments. These fatty acid fragments might arise from an acyl linkage on one of the hydroxyl groups previously discussed. To confirm this hypothesis, precursor was subjected to hydrolysis with potassium hydroxide followed by methylation with diazomethane. The crude reaction mixture was analyzed by GC/MS. The GC analysis is shown in Figure 3 and the mass spectrum of the requisite fractions are shown in Figure 4. These data clearly indicated the presence of methyl esters of four major long chain aliphatic acids, and it was believed that the precursor itself is a mixture of these four fatty acid ester derivatives, the most predominant being a oleoyl derivative.

From the available data the following conclusions could be drawn:

1) The UV and fluorescent characteristic of the precursor are identical to that of

fecapentaene-12 and therefore, an ether linked pentaenyl moiety exists.

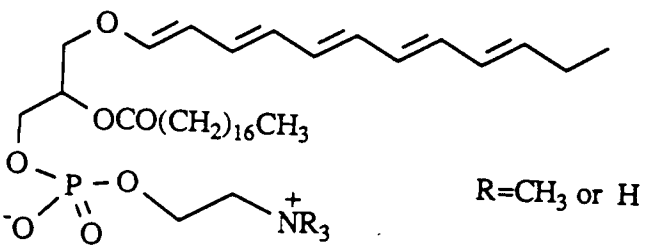
2) The presence of long chain fatty acids in the hydrolyzed medium of precursor, the MS/MS data previously described and the relatively non-polar chromatographic character of the precursor on reverse phase TLC indicates the presence of a fatty acid ester on the precursor.

3) The polar characteristics of the precursor on normal phase TLC systems suggest that the other hydroxyl group is substituted with a polar substituent.

Wilkins and coworkers subjected the precursor to commercial lipase and phospholipase and FP-12 was produced. Thus the sn-2 group was believed to be a fatty acid ester and the sn-3 a phosphate ester. Unfortunately, the phospholipase which gave a positive result is not selective, so details on the phosphate ester could not be determined.

The precursor can roughly be classified as a plasmalogen with extended conjugation. Plasmalogens are more common in anaerobic bacteria[54,55] than in mammalian tissue. The two most common 1-alkenyl phospholipids isolated from bacteria are phosphatidylethanolamine and phosphatidylcholine derivatives.[56] Data on the distribution of alkenyl phospholipids in human tissue is not abundant. Plasmalogens make up about 0.8% of the total amount of phospholipid found in human liver. Table 6 shows the plasmalogen composition in various mammalian tissue; only the ethanolamines and cholines were ever found.[55,56] Since the precursor must arise from bacteria or mammalian tissue the above data indicate that the polar substituent is most likely phosphorylethanolamine or phosphorylcholine. Therefore, the proposed structure of precursor is shown below (**21**).

At the time this work began, the exact nature of the putative phosphate-linked group on the precursor was unknown. We thus elected to begin a synthetic approach to the presumed precursor



21

structure **21** so that the general structure of the natural precursor could be further confirmed by direct comparison with synthetic material. In addition, the development of a viable synthetic approach to **21** would open the way to the preparation of larger amounts of precursor for detailed evaluation of its biological properties and function.

Table 6. 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
Foetal 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

III Synthesis of Perhydroprecursor-A Model Study

3.1 A Statement of the Problem.

In chapter 2 the synthetic proposed precursor, **21**, was shown to be important in order to achieve definitive information on the structure of the natural precursor. One of the important criteria that was important in this regard was evidence that the synthetic precursor possessed a similar amphipolar nature on TLC to that of the natural precursor. (See Table 4.) This particular criterion could be met with a compound with a similar constitution but which did not have the unstable pentaenol ether moiety. This compound would have comparable amphipolar characteristics, would be easily handled (i.e. synthesized), and thus would provide the compelling evidence required to pursue further appropriate syntheses. The importance of this type of compound is best understood by first examining the synthesis of the actual proposed precursor.

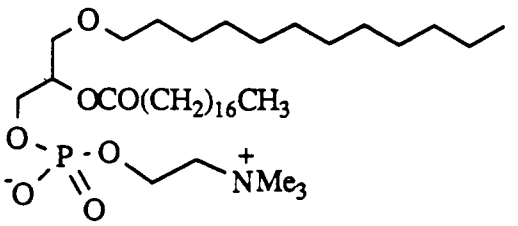
Synthesis of the precursor presents a challenging problem. On first inspection, functionalization of the highly unstable fecapentaene-12 is the apparent route for the synthesis of the precursor. It should be noted that although functionalization of fecapentaene-12 to produce the precursor may not be the ideal synthetic strategy, any compromise we select would almost certainly impart comparable labilities to that of fecapentaene-12. Reviewing the previous syntheses of FP-12 reveals why this is so. All of these syntheses have a highly unstable enol ether derivative as an intermediate (See structures **18** and **13** as examples). This is the type of intermediate that would certainly exist in our synthesis of the precursor. Therefore, we needed a synthetic methodology which avoided oxidative or radical chemistry, acids (including Lewis acids), strong bases, and hydrogenolysis.

This is not to say that these conditions can never be used, but avoidance of these conditions is necessary whenever work is to be done on an enol ether. In addition functionalization must be selective in that a phosphate ester must be in the 3-position and a fatty acid ester must be

in the 2-position.

3.2 Model Study Using Perhydroprecursor

It was felt that the above mentioned constraints would make synthesis of the proposed precursor too challenging without a model system. Therefore, we set out to synthesize a stable form of the precursor to develop optimum conditions that would be compatible with the pentaenyl ether moiety. Our choice was a decahydro derivative (**22**) of the precursor. We selected the phosphatidyl choline function over the phosphatidyl ethanolamine unit because we felt that the former would be simpler to make given the above mentioned constraints.



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The synthesis of ether linked phospholipids is well documented in the literature.[57] The vast majority of these syntheses involve formation of the ether of glycerol followed by protection of the remaining primary hydroxyl group, acylation of the secondary hydroxyl group, deprotection and phosphorylation as shown in Figure 5. Unfortunately the deprotection steps in all of these syntheses utilize conditions which are precluded by the above mentioned constraints. Table 7 demonstrates this point.

Special mention should be made of the last two entries. Silver salts should be considered a Lewis acid in the presence of the highly conjugated system of the pentaenyl ether. The epoxide listed refers to a glycidol ether; the conditions used for epoxide opening might be too basic for an analogous pentaenyl ether to withstand.

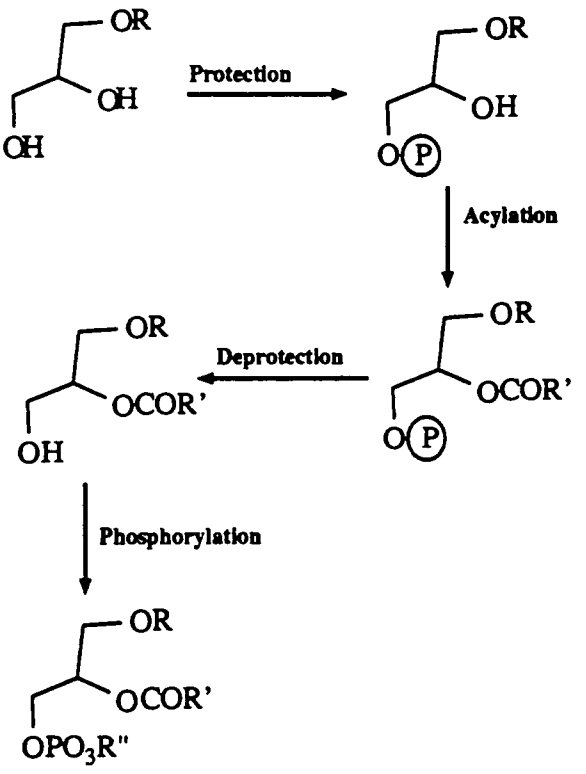


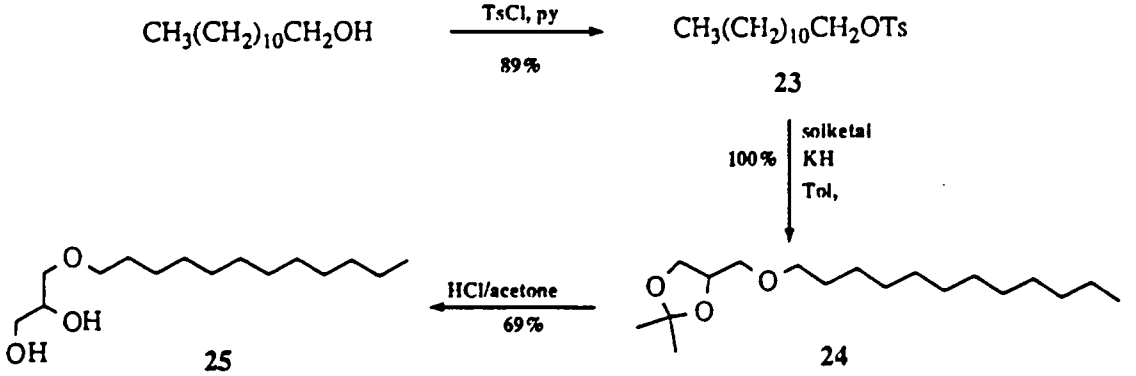
Figure 5. Generic synthesis of phospholids.

Table 7. Protection/Deprotection methods in ether lipid syntheses.

Protecting Group	Deprotection Conditions	Reference
Benzyl ether	H_2/Pt	58
Trityl ether	$(EtO)_3CH/B(OH)_3$	59
Trityl ether	HCl/MeOH	60
Alkyl ester	Pancreatic lipase	61
THP ether	0.1 N HCl	62
TBDMS ether	NBS/THF/DMSO/H_2O	63
Tosylate	NaI, then $Ag^+(^-OPO_3Bn_2)$	64
Epoxide	Phosphorylcholine dianion	65

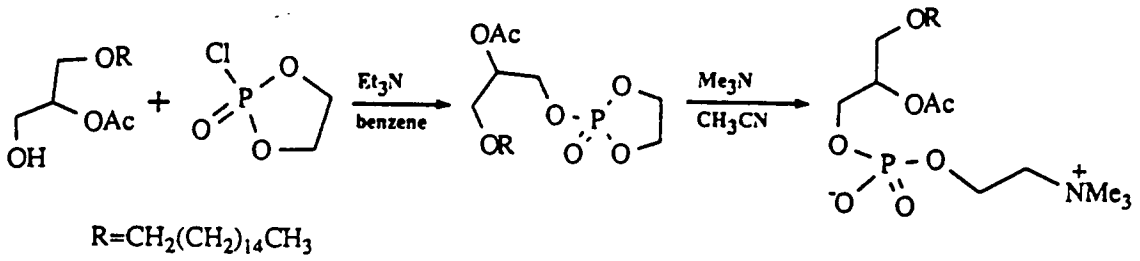
3.2.1 Synthesis of Various Substituted Glycerol Derivatives.

Our synthesis of perhydro-precursor (PPC) starts with the known compound 1-O-lauryl-glycerol whose synthesis was described by Kummerow[85] as shown in Scheme 3. Tosylation of lauryl alcohol with tosyl chloride in pyridine provided a 89% yield of lauryl tosylate (**23**). 1-O-dodecyloxy-2,3-O-isopropylideneglycerol (laurylsolketal) was made by reacting the potassium salt of 2,3-O-isopropylideneglycerol (solketal) with lauryl tosylate in refluxing toluene. A slight modification of the published procedure proved to be more efficient. We replaced potassium metal with potassium hydride to expedite deprotonation; the result was a 100% yield of laurylsolketal (**24**) in less time. Hydrolysis of the isopropylidene ketal with HCl in acetone provided crystalline 1-O-lauryl-glycerol (**25**) in 69% yield.



Scheme 3.

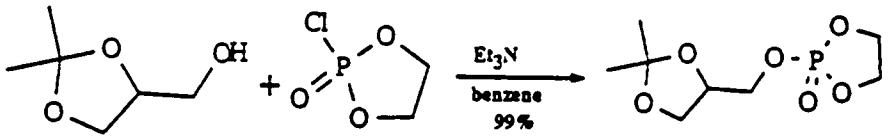
Selecting the appropriate protecting group of the primary hydroxyl group was of critical importance. We initially felt that by introducing the phosphorylcholine group at the 3-position of 1-O-laurylglycerol a viable synthesis of PPC could be realized. We felt that the best synthesis of phosphorylcholines was that described by Hadju, et.al.[62] as shown in Scheme 4. We found,



$R=CH_2(CH_2)_{14}CH_3$

Scheme 4.

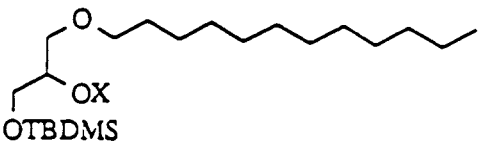
however, that treatment of **25** with 2-chloro-oxo-1,3,2-dioxaphospholane **26** resulted in a complex mixture of products as evidenced by ^{31}P NMR. The presence of the secondary hydroxyl group was believed to be the problem since it is well known that phospholanes are unstable in the presence of mild nucleophiles. To prove this, solketal was reacted with 2-chloro-2-oxo-1,3,2-dioxaphospholane to yield **27** (99%) and only one peak appeared on ^{31}P NMR (Scheme 5).



26 **27**

Scheme 5.

It became apparent that protection of the secondary hydroxyl group was necessary before the phosphate ester could be introduced. The selectivity needed can only be imparted by protecting the primary hydroxyl group with a silyl ether and protecting the secondary hydroxyl group with a fluoride stable system, deprotecting with fluoride and then introducing the phosphate ester. This type of compound is illustrated by structure **28**. Our X group of choice was THP.



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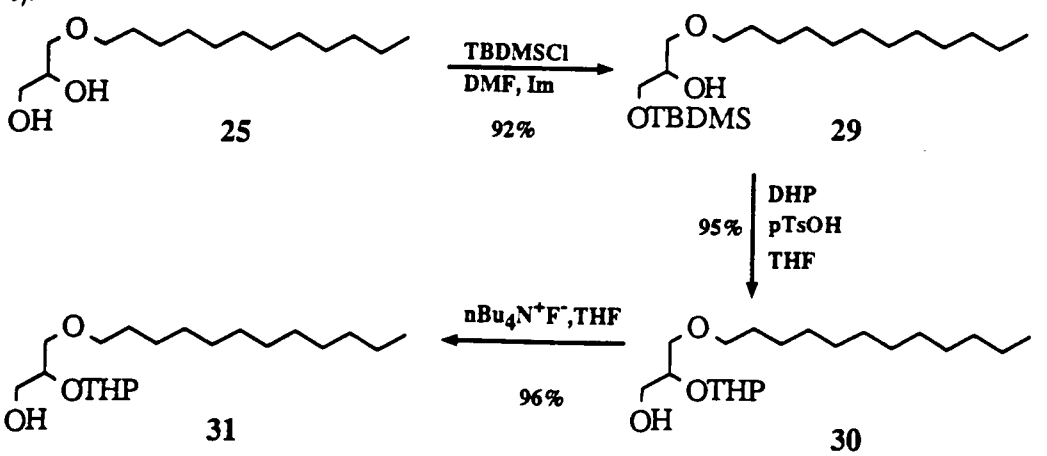
Again starting with 1-O-laurylglycerol, protection of the primary hydroxyl proceeded best with predried tbutyldimethylsilyl chloride in dry DMF and imidazole (Im). We found that by azeotroping off water with benzene from the hygroscopic chlorosilane and making a solution of known concentration in dry DMF provided the best yield of the silyl ether **29** (92%). Protection of the secondary hydroxyl group with a THP ether under standard conditions yielded 1-O-lauryl-2-THP-3-O-(tbutyldimethyl)silylglycerol[67] (**30**) in 95% yield. Deprotection of the silyl ether with tetrabutylammonium fluoride provided a 96% yield of the protected 1-O-laurylglycerol **31** (Scheme 6).



Scheme 6.

Although the reactions shown in Scheme 6 provide a simple route to a viable intermediate toward the synthesis of the model precursor, this pathway would almost certainly not be appropriate for the synthesis of the precursor itself, since deprotection of the THP protecting group requires

acidic conditions. We thus sought an alternate protecting group for the secondary position.

Our first attempt to circumvent the use of the THP ether was to pursue silyl migration. Silyl migration of this type is not unknown and therefore, we attempted several experiments to migrate the silyl ether from the 3-position to the 2-position. We tried reacting **29** with potassium hydride, potassium tert-butoxide and pyridine,[68] all of which resulted in deprotection (with KH) or no reaction.

At this time, it appeared necessary to acylate the secondary hydroxyl group of **29** in lieu of the predescribed THP ether formation. It was hoped that conditions could be worked out where cleavage of the silyl ether could be performed without ester migration occurring.

3.2.2 Intramolecular Transesterification

Intramolecular transesterification is a known reaction, where a variety of bases are used. Most notably, Fife and Benjamin[69] showed that intramolecular tranesterification of ethyl 2-hydroxymethyl benzoate (**32**) to phthalide (**33**) can be catalyzed by imidazole (Figure 6). It is likely that imidazole assists proton removal in the rate determining step. In addition, it was shown that when hydroxide anion was used as the base (pKa=15), the reaction rate was rapid at 30°C with $k_{OH}=10^4\ M^{-1}\ sec^{-1}$. This is in contrast to aqueous alkaline hydrolysis of esters. Beal[70] hydrolyzed the triglyceride, trimyristin, to myristic acid; the reaction required vigorous conditions in which the mixture was heated to 100°C for over two hours.

Therefore, by deprotecting a 2-acyl-3-(trialkyl)silylglycerol derivative, which mimics strongly basic conditions due to the presence of the tetrabutyl ammonium salt of the primary hydroxyl group, ester migration would most likely be rapid at room temperature. In addition, the process would be thermodynamically favored with extra impetus provided by the relief of steric strain as illustrated in Figure 7.

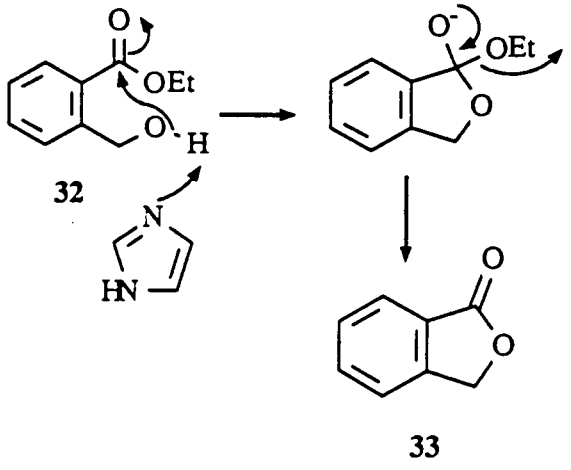


Figure 6. Intramolecular transesterification of ethyl 2-hydroxy benzoate.

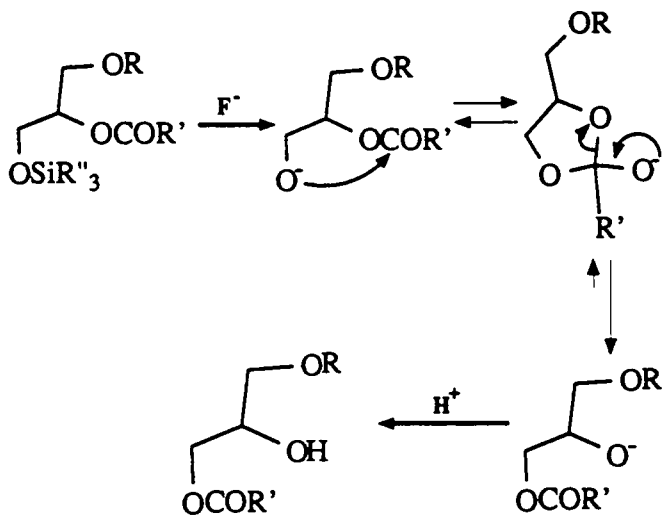
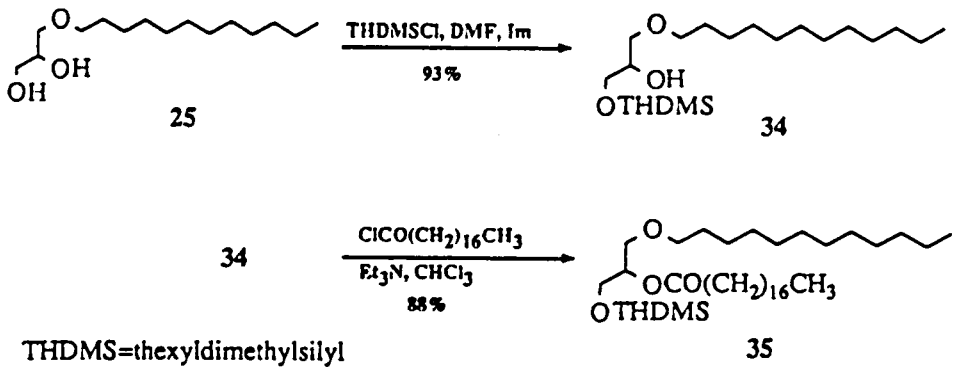


Figure 7. Deprotection of 2-acyl-3-(trialkyl)silylglycerol derivative.

3.2.3 Synthesis and Deprotection of 2-Acyl-3-O-(trialkyl)silylglycerol Derivatives.

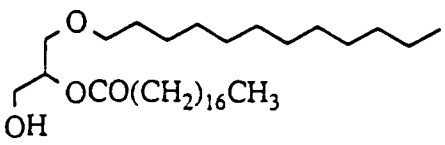
We did not know how effective this ester migration would be relative to silyl ether cleavage lability. To test the relative reactivities, the synthesis of the 2-acyl-3-silyl derivative **36** was performed by the sequence shown in Scheme 7. Protection of 1-O-laurylglycerol (**25**) with thexyldimethylchlorosilane[a] in DMF and imidazole proceeded smoothly in 93% yield to give 1-O-lauryl-3-O-(thexyldimethyl)silylglycerol (**34**). Acylation with stearoyl chloride and triethylamine in refluxing chloroform[62] provided 1-O-lauryl-2-stearoyl-3-O-(thexyldimethyl)silylglycerol (**35**) in 88% yield.



Scheme 7.

Deprotection of **35** was performed using tetrabutylammonium fluoride in THF at temperatures ranging from -40°C to 0°C. It was found that in order for complete silyl ether cleavage to occur, a temperature of -10°C was required which resulted in a 5:1 ratio of regioisomers in favor of the undesirable 3-O-stearoyl-1-O-laurylglycerol (**37**) as opposed to the desired 2-O-stearoyl-1-O-laurylglycerol (**36**).

[a] The liquid thexyl derivative was used instead of the solid tert-butyl derivative because the ease of handling, keeping in mind the hygroscopic nature of trialkylchlorosilanes.



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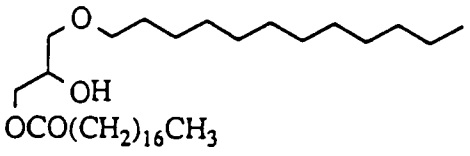

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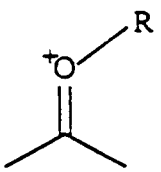
Silyl ketals are known to be much more fluoride labile than silyl ethers.[71] Interestingly, studies conducted toward the relative reactivity of silyl ketals vis-a-vis silyl ethers concluded that silyl ketals are much more stable under acidic conditions. Examination of the electronic effects of the respective silicon compounds reveals why this phenomenon might be so. Considering the two-silyl protecting groups and the two deprotection techniques, it becomes reasonable that the lack of resonance in any silicon oxygen bond and the resultant pure electron withdrawing ability of the oxygen would afford this result (Figure 8).

In equation 1, lability to fluoride treatment is facilitated relative to equation 2 due to the electron withdrawing alkoxy group. With hydrolysis of dialkyl ketals, an intermediate is the alkyl oxonium ketone, **38**. However, the analogous methyl oxonium silane, **39**, shown in equation 4 is not



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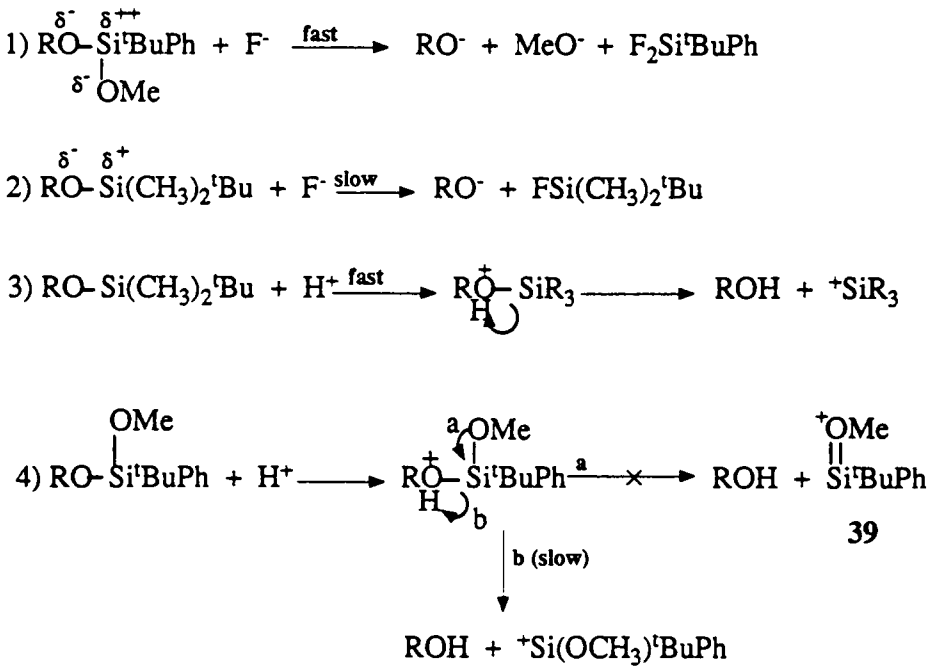
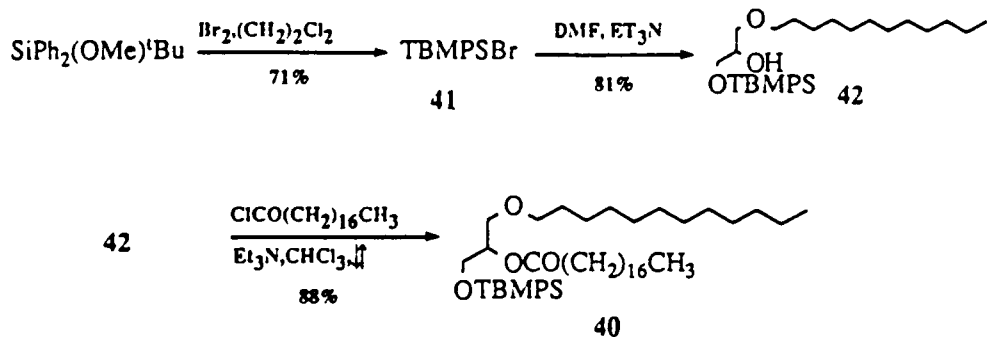


Figure 8. Cleavage of silyl ketals vs. silyl ethers.

a favorable intermediate during the hydrolysis of silyl ketals. Under the presumption that the mechanism in shown Figure 8 is correct, the stability of the silyl cation is, therefore, the key factor in determining relative hydrolytic labilities of silyl ethers and silyl ketals. Since silicon oxygen double bonds are not an important resonance structure, the stability of the silyl cation is based purely upon electron releasing ability of the silyl substituents. Of course, with resonance effects excluded, a trialkyl alkyl silyl cation is much more stable than alkoxy dialkyl silyl cations.

Because of this enhanced lability of silyl ketals to fluoride ion, it appeared that a silyl ketal protecting group might allow deprotection under conditions mild enough to preclude acyl migration. We thus elected to test this hypothesis by the synthesis of 1-O-lauryl-2-stearoyl-3-O-('butylmethoxyphenyl)silylglycerol (**40**) as shown in Scheme 8. Preparation of 'butylmethoxyphenylbromosilane (**41**) was done in 71% yield by brominating 'butylmethoxydiphenylsilane in 1,2-dichloroethane. Protection of 1-O-laurylglycerol (**25**) with 'butylmethoxyphenylbromosilane and triethylamine and DMF proceeded in 81% yield (structure **42**).[71] Acylation as described previously provided **40** in 88% yield.



TBMPS='butylmethoxyphenylsilyl

Scheme 8.

Table 8. Conditions for deprotection of **40** and yields of **37**, **36**, and **40**.

Solvent	$nBuN^+F^-$	Temp.	Time	**40**	**36**	**37**
THF	1M in THF*	-40°C	2 h	58	29	5
THF	predried**	-30°C	0.5 h	-	47	53
THF	predried	-75°C	9 h	66	19	-
$(CH_2)_2Cl_2$	predried	-40°C	5 min	-	-	83
$(CH_2)_2Cl_2$	1M in THF	R.T.	18 h	-	50	50

* contains <5% water

** $nBuN^+F^-.3\ H_2O$ was dried at 0.3 mm Hg and 45°C for 24 hours, dissolved in dry THF and used directly.

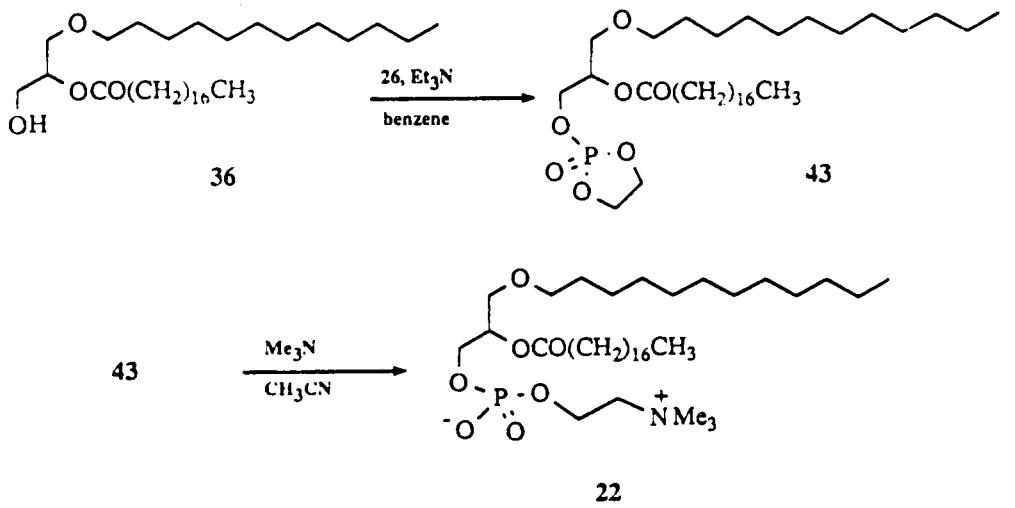
Several conditions were tried to deprotect **40** with minimum ester migration and maximum product formation. Table 8 shows the conditions tried, the yields of unreacted starting material (**40**), yields of 2-stearoyl-1-O-laurylglycerol (**36**) and 3-stearoyl-1-O-laurylglycerol (**37**).

All entries tabulated were experiments where 6 equivalents of fluoride ion were added. The results indicate that completely anhydrous conditions favor rapid deprotection but that a trace of water is needed to avoid transesterification. A balance between the effects can be seen when slightly wet conditions in hydrophobic 1,2-dichloroethane was used. The best yield of the desired product **36** was realized with the conditions shown in entry 5 where a 1:1 ratio of regioisomers was observed.

3.2.4 Completed Synthesis of Perhydroprecursor

Reaction of crystalline **36** with 2-chloro-2-oxo-1,3,2-dioxaphospholane (**26**) and triethylamine in benzene required completely anhydrous conditions and precise stoichiometric amounts of **36**, triethylamine and the phospholane **26**. Such care was required because the phosphate ester product (**43**) was extremely labile to weak nucleophiles (water, methanol, triethylamine, etc.). This high reactivity was observed when methanol was added to the crude product **43** after workup; ^{31}P NMR spectroscopy of the solution showed the presence of a multitude of decomposition products. Workup of the reaction therefore included filtering off the triethylamine hydrochloride and removing the benzene *in vacuo* to yield a yellow oil. The crude oil was immediately dissolved in dry acetonitrile, transferred to a pressure bottle and treated with trimethylamine under argon. The pressure bottle was sealed and heated at 60°C for 12 hours[72] to yield the saturated precursor **22** in 42 % yield from **43** (Scheme 9).

The phospholipid **22** served two purposes. First it provided a model system for the synthesis of the proposed precursor. Second, it could be compared with the natural precursor on



Scheme 9.

TLC to determine if it had similar amphipolar properties. The relative mobilities of FP-12, natural precursor and the phospholipid **22** are shown in Table 9,[53] where it can be seen that the model precursor and natural precursor have very similar chromatographic properties. These data served to confirm that our proposed structure of the FP-12 precursor was approximately correct, and provide a sound basis for further synthetic work in this area.

Table 9. Mobilities of natural precursor, fecapentaene-12 and synthetic phospholipid (**22**) in various TLC systems.

TLC System	Rf (FP-12)	Rf (Precursor)	Rf (22)
C_{18}/MeOH	0.88	0.66	0.70
C_{18}THF	0.84	0.85	0.92
C_{18}/CH_3CN	0.73	0.03	0.05
Si/$CHCl_3$/MeOH/ NH_4OH (84:15:1)	0.75	0.32	0.25

3.3 General Experimental Procedures.

The experimental techniques outlined in this section are valid for the discussions and experimental procedures in all ensuing chapters. Lauryl alcohol, pyridine, tosyl chloride, potassium hydride, solketal, magnesium sulfate, triethylamine, tert-butyldimethylsilyl chloride, dimethylformamide, imidazole, dihydropyran, tetrabutylammonium fluoride, stearoyl chloride, tert-butylmethoxydiphenylsilane, pentane, p-toluenesulfonic acid, benzaldehyde, magnesium bromide etherate, pyridine, 4-dimethylaminopyridine, sodium iodide, 3-bromo-1,2-propanediol, benzyltributylammonium bromide, epichlorohydrin, stearic acid, dicyclohexylcarbodiimde, 4-pyrrolidinopyridine, oxalic acid, tetrabutylammonium hydroxide, glycidol, epibromohydrin, diisopropylamine, n-butyllithium, potassium tert-butoxide, dibenzyl phosphate, guanosine, diisobutylaluminum hydride, manganese dioxide, tributyltin hydride, methoxymethyl phenyl sulfide, sodium hydride, 2,2,2-trifluoroethanol, chloromethyl methyl sulfide, m-chloroperoxybenzoic acid, triethyloxonium tetrafluoborate, 2-penten-1-al, trimethylsilyl chloride, β-methoxystyrene, potassium monopersulfate, sodium, potassium, diethylamine, sodium sulfate, potassium iodide, acrolein and crotonaldehyde were purchased from Aldrich Chemical Company. Diethyl ether, petroleum ether, 35% hydrochloric acid, sodium bicarbonate, methanol, ethyl acetate, benzene, tetrahydrofuran, chloroform, bromine, acetonitrile, glycerol, potassium carbonate, copper sulfate, acetic anhydride, dimethyl sulfoxide, sodium thiosulfate, acetone, ammonium chloride, methylene chloride, silver nitrate, ethanol, pH7 phosphate buffer, hydrazine dihydrochloride, mercuric chloride, calcium oxide, calcium hydride, calcium sulfate, Norit, potassium permanganate and barium oxide were purchased from Fisher Scientific. Phosphorylcholine chloride, calcium salt, 2',3'-O-isopropylideneguanosine, thymidine and cytosine were purchased from Sigma Chemical Company. Thexyldimethylsilyl chloride, 2-chloro-2-oxo-1,3,2-dioxaphospholane and 1-methoxybut-2-en-3-yne were purchased from

Fluka Chemical Corporation. Phosphorus pentoxide was purchased from MCB Reagents. Trimethylamine was puchased from Eastman Kodak Company. Sodium hydroxide was purchased from VWR Scientific. Amberlite IR-120 was purchased from Mallinckrodt Chemical Works. 2,4-heptadienal was purchased form Bedoukian Research. 1,2-Dichloroethane was purchased from J. T. Baker Chemical Company.

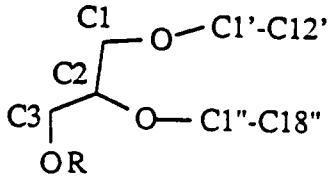
All solvents other than hexanes and pentane were purified before using. Hexanes and pentane were used directly. Pyridine, dimethylsulfoxide and dimethylformamide were distilled from calcium oxide. Acetone was dried over calcium sulfate, distilled, and redistilled from potassium permanganate. Methanol and ethanol were distilled from calcium hydride. Acetonitrile, methylene chloride, chloroform and 1,2-dichloroethane were distilled from phosphorus pentoxide. Benzene, toluene, diethylether and dimethoxyethane were distilled from sodium with benzophenone present as a dryness indicator. Diethylamine and diisopropylamine were distilled from barium oxide. Tetrahydrofuran was distilled from potassium with benzophenone used as a dryness indicator. All reagents purchased from chemical companies were used directly, with the exception of 1-methoxybut-2-en-3-yne, diethylamine, methyl iodide, trimethylsilyl chloride and triethylamine which were distilled prior to use and p-toluenesulfonic acid, p-toluenesulfonyl chloride and p-bromotoluenesulfonyl chloride which were purified prior to use. p-Toluenesulfonic acid was recrystallized from ether and hexanes. p-Toluenesulfonylchloride and p-bromotoluenesulfonylchloride were dissolved in a minimum amount of chloroform and the solution was diluted with 5 volumes of petroleum ether to precipitate impurities. The solution was filtered, clarified with Norit, and concentrated to form white crystals.

Analytical chromatography was carried out on E. Merck aluminum supported silica gel 60 (0.2 mm, F_{254}) plates. Silica gel for flash column chromatography was E. Merck 230-400 mesh. Pretreating silica gel with triethylamine entailed stirring 2 kg of silica gel in 2 L of 10% triethylamine

in hexanes overnight. The mixture was filtered on a Buchner funnel and traces of triethylamine were removed under high vacuum. Basic alumina for flash column chromatography was Woelm Pharma 100-200 mesh super I and was deactivated to grade III. The HPLC system used was a Waters Associates model M-6000A pump and a Waters Associates model 441 UV detector operating at 254 nm. For the preparatory work, a Dynamax macro C-18 column was used, and for analytical studies a Waters Associates Novapak^{R} C-18 column, 5 micron particle size, 8 mm x 10 cm in an RCM radial compression module was used.

Melting points were determined on an Electrothermal point melting apparatus and are uncorrected. Ultraviolet spectra were recorded on a Perkin-Elmer 330 spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer 710B spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 270 MHz on a Bruker WP-270SY spectrometer, and chemical shifts are expressed in terms of parts per million (δ) relative to internal $CHCl_3$ in $CDCl_3$ (δ 7.24), CHD_2OD (δ 3.30) or DMSO (δ 2.49). Carbon-13 NMR spectra (^{13}C NMR) were recorded at 20 MHz on an IBM NR-80 spectrometer, and chemical shifts are expressed in terms of parts per million relative to internal $CDCl_3$ (δ 77.0), CD_3OD (δ 49.0) or DMSO (δ 39.5). Phosphorous NMR (^{31}P NMR) were recorded at 81 MHz on a Bruker WP-200SY spectrometer, and chemical shifts are expressed in parts per million relative to external H_3PO_4 (δ 0.0). Mass spectra analysis was done on a VG 7070-HF mass spectrometer. Elemental analyses were performed by Desert Analytics, Tucson, Arizona.

Proton and carbon NMR spectra assignments of all glycerol derivatives were made according to the structure **A**. The carbon bearing any C12 moiety or precursors thereof (i.e. tosylate, silane, bromide, brosylate or iodide) is designated C1. Similarly, the C2 designation refers to the carbon bearing the stearoyl moiety and any derivatives thereof. The C3 designation refers to precursors to a phosphate ester as shown by R in structure **A**.



R= H, silane, I, or phosphorylcholine.

A

3.4 Experimental

Preparation of 1-dodecyl tosylate (23).

Lauryl alcohol (18.6 g, 0.1 M) was dissolved in 32 mL dry pyridine, cooled to 0°C and 21 g (0.11 M) of tosyl chloride was added in one portion. After 4 hours, 270 mL of 2.5 N HCl (cooled in an ice bath) was poured into the solution. The resultant heterogenous mixture was poured into a 1 L separatory funnel. The aqueous mixture was extracted 3x200 mL with ether. The ether extracts were combined and washed with 2x30 mL of saturated aqueous $NaHCO_3$, 2x30 mL H_2O, 1x20 mL brine, dried over Na_2SO_4, filtered and the ether was removed *in vacuo* to yield a colorless oil. The oil was dissolved in hexanes and crystallized in the freezer for 12 hours. The crystals were filtered to yield pure lauryl tosylate. Yield 30.2 g (89 %). mp 27-28°C (Lit. 26-27°C) ^{1}H NMR ($CDCl_3$) δ 7.77 (d, J=8.1 Hz, 2H, Ph), 7.31 (d, J=8.1 Hz, 2H, Ph), 4.00 (t, J=6.8 Hz, 2H, CH_2OTs), 2.43 (s, 3H, $ArCH_3$), 1.50 (m, 2H, $\underline{CH_2}CH_2OTs$), 1.21 (m, 18H), 0.84 (t, J=6.0 Hz, 3H, CH_3).

Preparation of 1-O-dodecyl-2,3-O-isopropylideneglycerol (24).

Potassium hydride (40 g of 30% in oil dispersion, 26.1 mmoles) was swirled with dry ether three times and the final ether/KH mixture was added to a dry flask. The flask was fitted with a condenser, a drying tube and an addition funnel. Dry toluene was added and stirred for 30 minutes after which the flask was heated to remove the ether. Solketal (2.9 mL, 23.7 mmoles) was then added in one portion and the solution stirred for 6 hours. Lauryl tosylate (23) (8.05 g, 23.7 mmoles) in dry toluene was added dropwise to the solution of the sodium salt and the solution refluxed for 17 hours. Toluene was then removed *in vacuo* and the solution treated with water and extracted with 5x50 mL of ether. The ether extracts were combined and washed with 3x30 mL of H_2O and 1x30 mL brine, dried over $MgSO_4$, filtered, and the ether removed *in vacuo* to yield a

yellow oil. Yield 6.05 g (100%). ^{1}H NMR ($CDCl_3$) δ 4.24 (m, 1H, C2), 4.02 (dd, J=6.3, 8.4 Hz, 1H, C3), 3.69 (dd, J=5.9, 8.4 Hz, 1H, C3), 3.42 (m, 4H, C1 & C1'), 1.54 (m, 2H, C2'), 1.40 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.20 (m, 18H), 0.85 (t, J=6.3 Hz, 3H, C12').

Preparation of 1-O-dodecylglycerol (25).

A mixture of 1-O-laurylsolketal (24) (16.4 g, 0.055 moles) and 0.5 N HCl in 35 mL H_2O/85 mL methanol was heated to 70-80°C for 1 hour. The mixture was cooled and H_2O, ethyl acetate was added, and the aqueous layer was extracted with 5x30 mL of ethyl acetate. The ethyl acetate extracts were combined and washed with 3x30 mL H_2O and 2x30 mL of brine, dried over $MgSO_4$ and filtered. The solvent was removed *in vacuo* to yield white crystals which were recrystallized from ethyl acetate at -10°C. Yield 9.75 g (69%). mp 47-49°C (Lit. 46.5-47.5°C) ^{1}H NMR ($CDCl_3$) δ 3.82 (m, 1H, C2), 3.65 (m, 2H, C1), 3.48 (m, 2H, C3), 3.42 (t, J=6.5 Hz, 2H, C1'), 2.62 (d, J=5.2 Hz, 1H, C2-OH), 2.20 (t, J=6.5 Hz, 1H, C3-OH), 1.55 (m, 2H, C2'), 1.24 (m, 18H), 0.85 (t, J=6.3 Hz, 3H, C12').

Preparation of 1-O-(2-oxo-1,3,2-dioxaphospholanyl)-2,3-O- isopropylideneglycerol (27).

Solketal (1 mL, 8 mmoles) and 1.1 mL (8 mmoles) of triethylamine were dissolved in benzene. The solution was cooled to 7°C, and then 0.75 mL (8 mmoles) of 2-chloro-2-oxo-1,3,2-dioxaphospholane in 5 mL of benzene was added dropwise. After 1 hour the solution was filtered and benzene removed *in vacuo* to yield a beige oil which appeared as a single spot on TLC. Additional proof of the purity of the compound was demonstrated by the observance of one peak on ^{31}P NMR. Yield 1.9g (99%). ^{1}H NMR ($CDCl_3$) δ 4.30 (s, 4H, $(CH_2)_2OP$), 4.03 (m, 4H, C1 & C3), 3.70 (m, 1H, C2), 1.30 (s, 3H, CH_3), 1.25 (s, 3H, CH_3); ^{31}P NMR (CDCl3) δ 16.5; ^{13}C NMR δ 128.0, 73.4, 73.3, 68.1, 68.0, 66.0, 65.2, 26.3, 24.7; IR (neat) 3125, 1300, 1040, 850

cm^{-1}.

Preparation of 1-O-dodecyl-3-0-('butyldimethyl)silylglycerol (**29**).

'Butyldimethylchlorosilane (TBDMSCl) was dissolved in dry benzene in a preweighed flask, and the benzene was distilled off until the boiling point of the silane was reached (125°C). The mixture was cooled to room temperature and more dry benzene was added and again distilled as before. The cooled crystals were weighed under argon and a known volume of dry DMF was added to prepare a solution of 1.4 M TBDMSCl in DMF. In a flame dried flask under argon, 500 mg (1.92 mmoles) of 1-O-laurylglycerol (**25**) in dry DMF was treated with imidazole (288 mg, 4.23 mmoles) followed by 1.40 mL of 1.4 M TBDMSCl in DMF. After 4 hours, 75 mL H_2O was added, and the aqueous solution was extracted with 3x100 mL of ether. The ether extracts were combined and washed with 20 mL brine, dried over $MgSO_4$, filtered, and the ether removed *in vacuo*. The crude product was purified by flash chromatography on silica gel 60 and eluted with 3:2 hexanes:ether; the requisite product appeared as a single spot on TLC. Yield 665 mg (92%). ^1H NMR ($CDCl_3$) δ 3.79 (m, 1H, C2), 3.62 (dd, J=2.6, 5.6 Hz, 2H, C3), 3.42 (m, 4H, C1 & C1'), 2.44 (d, J=5.1 Hz, 1H, C2-OH), 1.55 (m, 2H, C2'), 1.21 (m, 18H), 0.86 (s, 12H, 'Bu & C12'), 0.04 (s, 6H, CH_3Si).

Preparation of 1-O-dodecyl-2-O-tetrahydropyranyl-3-O-('butyldimethyl)silylglycerol (**30**).

1-O-Lauryl-3-O-('butyldimethyl)silylglycerol (**29**) (127 mg, 0.34 mmoles) was dissolved in dry ether, and 0.05 mL (0.54 mmoles) of dihydropyran were added, followed by 19 mg (0.10 mmoles) of p-toluenesulfonic acid in one portion. After 5 hours the solution was added to 50 mL of ether and 50 mL H_2O in separatory funnel. The ether was washed with 2x25 mL H_2O, 1x10 mL brine, dried over $MgSO_4$, filtered and the ether removed *in vacuo*. The crude yellow oil was purified by

flash chromatography using silica gel 60 and eluting with 4:1 hexanes:ether. Yield 148 mg (95%). ^{1}H NMR ($CDCl_3$) δ 4.80 (m, 1H, OCHO), 3.92 (m, 1H), 3.82 (m, 1H), 3.71 (m, 1H), 3.60 (m, 1H), 3.44 (m, 4H), 1.55 (m, 9H), 1.23 (m, 18H), 0.85 (s, 12H, ^{t}Bu & C12'), 0.04 (s, 6H, CH_3Si).

Deprotection of 1-O-dodecyl-2-O-tetrahydropyranyl-3-O-(^{t}butyldimethyl)silylglycerol.

1-O-Lauryl-2-THP-3-O-TBDMS-glycerol (**30**) (148 mg, 0.32 mmoles) was dissolved in dry THF, and 0.47 mL (0.47 mmoles) of 1M tetrabutylammonium fluoride in THF was added in one portion, and the solution was stirred for 2 hours. Fifty mL of ether were added and the organic phase was washed with 3x20 mL H_2O, 1x5 mL brine, dried over $MgSO_4$, and filtered and the ether was then removed *in vacuo*. The residual ^{t}butyldimethylsilyl fluoride (TBDMSF) was removed under high vacuum for 14 hours. Yield 107 mg (96%). ^{1}H NMR ($CDCl_3$) δ 4.73 (m, 0.5H, OCHO), 4.55 (m, 0.5H, OCHO), 3.83 (m, 2H), 3.64 (m, 2H), 3.49 (m, 2H), 3.40 (m, 3H), 2.34 (t, J=6.4 Hz, 1H, OH), 1.79 (m, 2H), 1.52 (m, 6H), 1.23 (m, 18H), 0.85 (t, J=6.2 Hz, 3H, C12'), 0.03 (s, 6H, CH_3Si); ^{13}C NMR ($CDCl_3$) δ 100.7 (C1"), 98.3, 79.9, 75.6, 71.7, 71.4, 70.8, 64.4, 63.7, 62.9, 62.8, 31.8, 31.2, 30.8, 29.5, 29.3, 26.0, 25.3, 25.0, 22.6, 20.7, 19.7, 14.0 (C12'), -4.6 (CSi); IR (neat) 3475, 2950, 2880, 1475, 1125, 1085, 1035 cm^{-1}.

Preparation of 1-O-dodecyl-3-O-(thexyldimethyl)silylglycerol (**34**).

Laurylglycerol (**25**) (5.0 g, 19.2 mmoles) was dissolved in dry DMF. Imidazole (2.6 g, 38.5 mmoles) was added followed by 3.8 mL (19.2 mmoles) of thexyldimethylchlorosilane and the solution was stirred for 7 hours. The solution was poured into 380 mL H_2O/100 mL ether, and the aqueous layer was extracted with 4x100 mL of ether. The ether extracts were combined, washed with 20 mL of H_2O, 20 mL brine , dried over Na_2SO_4, and filtered, and the ether was removed *in vacuo*. The crude product was purified by flash chromatography on silica gel 60 and eluted with

3:2 hexanes:ether; the requisite product appeared as a single spot on TLC. Yield 7.2 g (93%). ^{1}H NMR ($CDCl_3$) 3.77 (m, 1H, C2), 3.58 (m, 2H, C3), 3.42 (m, 4H, C1 & C1'), 2.44 (d, J=4.9 Hz, 1H, OH), 1.55 (m, 3H, C2' & $CHMe_2$), 1.24 (m, 18H), 0.88 (s, 9H, C12' & $\underline{CH_3}CH$), 0.84 (s, 6H, CH_3C), 0.08 (s, 6H, CH_3Si); ^{13}C NMR ($CDCl_3$) δ 71.8 (C2), 70.5 (C1), 63.9 (C1'), 34.0 (C3), 31.8, 29.2, 26.0, 22.9, 20.2, 18.3, 13.9 (C12'), -3.8 (CSi). IR (neat) 3475, 2960, 2880, 1480, 1390, 1262, 1120 cm^{-1}; MS (CI), *m/z* (relative intensity) 403 (M+1, 100), 131 (62).

Preparation of 1-O-dodecyl-2-octadecanoyl-3-O-(thexyldimethyl)silylglycerol. (35).

1-O-Lauryl-3-O-(thexyldimethyl)silylglycerol (34) (1.08 g, 2.69 mmoles) was dissolved in dry $CHCl_3$. Triethylamine (0.75 mL, 5.38 mmoles) was added followed by 1.34 mL (4.03 mmoles) of stearoyl chloride and the solution was refluxed for 18 hours. The $CHCl_3$ was removed *in vacuo*, and the crude mixture was dissolved in ether, filtered, and the ether was removed *in vacuo*. The crude oil was purified on a 3x20 cm flash column packed with silica gel 60 and eluted with 5% ether in hexanes; the requisite product appeared as a single spot on TLC. Yield 1.58 g (88%). ^{1}H NMR ($CDCl_3$) δ 4.97 (m, 1H, C2), 3.68 (dd, J=3.3, 5.1 Hz, 2H, C1), 3.53 (dd, J=1.4, 5.1 Hz, 2H, C3), 3.41 (m, 2H, C1'), 2.28 (t, J=6.84 Hz, 2H, C2"), 1.55 (m, 5H, C2', C3" & $CHMe_2$), 1.24 (m, 46H), 0.87 (m, 6H, C18", C12' & $\underline{CH_3}CH$), 0.83 (s, 6H, $\underline{CH_3}C$), 0.05 (s, 6H, CH_3Si); ^{13}C NMR ($CDCl_3$) δ 173.4 (C1"), 71.7 (C2), 69.0 (C3), 65.9 (C1), 61.5 (C1'), 34.2, 31.8, 29.5, 26.1, 25.0, 22.4, 20.2, 18.4, 15.2, 14.0 (C18"), -3.8 (CSi); IR (neat) 2940, 2860, 1740, 1460, 1380, 1235, 1120 cm^{-1}. MS (CI), *m/z* (relative intensity) 669 (M+1, 50), 584 (25), 427 (100), 341 (39), 285 (42).

Preparation of 1-O-dodecyl-2-octadecanoylglycerol (36).

1-O-Lauryl-2-stearoyl-3-O-(thexyldimethyl)silylglycerol (305 mg, 0.46 mmoles) (35) was dissolved in dry THF. To this solution, 2.74 mL of 1M tetrabutyl ammonium fluoride in THF was

added and the solution was placed in the freezer for 16 hours. The solution was added to 100 mL ether /100 mL H_2O, and the ether layer was washed with 2x100 mL H_2O, 20 mL brine, dried over Na_2SO_4, filtered and the solvents removed *in vacuo*. A 3x20 cm flash column was packed with silica gel 60, loaded with the crude product and eluted with 3:2 hexanes:ether. The requisite fractions were combined and the solvents were removed *in vacuo* to yield a white powder. Yield 159 mg (66%) of 3-stearoyl-1-O-laurylglycerol (**38**) and 38.5 mg (16%) of 2-stearoyl-1-O-lauryl-glycerol (**37**). Both compounds appeared as a single spot on TLC. (Spectral data is of 2-stearoyl-1-O-laurylglycerol.) ^{1}H NMR ($CDCl_3$) δ 5.00 (m, 1H, C2), 3.79 (dd, J=4.6, 6.1 Hz, 2H, C1), 3.60 (t, J=6.1 Hz, 2H, C1'), 3.42 (m, 2H, C3), 2.34 (t, J=7.3 Hz, 2H, C2"), 2.17 (t, J=5.9 Hz, 1H, OH), 1.59 (m, 4H, C2' & C3"), 1.23 (m, 46H), 0.86 (t, J=6.8 Hz, 6H, C18" & C12'); ^{13}C nmr ($CDCl_3$) δ 171.9 (C1"), 73.0 (C2), 71.9 (C1), 70.0 (C3), 63.1 (C1'), 34.4, 32.0, 29.3, 26.1, 25.0, 22.4, 14.0 (C18"); IR (KBr) 3460, 2950, 2870, 1745, 1480 cm^{-1}; MS (CI), *m/z* (relative intensity) 527 (M+1, 2), 285 (6), 267 (19), 131 (30), 57 (100); mp 56-58°C; HRMS, obsd *m/z* 526.5051, $C_{33}H_{66}O_4$ requires *m/z* 526.4961.

Preparation of ^{t}butylmethoxyphenylbromosilane (**41**).

^{t}Butylmethoxydiphenylsilane (15 g, 0.056 moles) was dissolved in 200 mL of dichloroethane and cooled to 0°C. To the cooled solution, 3.4 mL (0.067 moles) of bromine in 10 mL of dichloroethane were added and the solution was stirred for 16 hours. Bromine, dichloroethane and bromobenzene were removed at room temperature by an oil vacuum pump. The residue was distilled at 92-96°C at 1.5 mm Hg to yield a light yellow liquid. Yield 10.75 g (71%). ^{1}H NMR ($CDCl_3$) 7.66 (dd, J=2.0, 8.8 Hz, 2H, Ph), 7.42 (m, 3H, Ph), 3.57 (s, 3H, OCH_3), 1.00 (s, 9H, ^{t}Bu); MS (EI), *m/z* (relative intensity) 274 (M+1, 4), 272 (M-1, 4), 217 (100), 215 (100), 167 (50), 77 (40), 59 (45).

Preparation of 1-O-dodecyl-3-O-('butylmethoxyphenyl)silylglycerol (42).

In a flame dried flask under argon, 1 g (3.85 mmoles) of 1-O-laurylglycerol (25) was dissolved in dry DMF. Dry triethylamine (0.59 mL, 4.24 mmoles) was added followed by 0.81 mL (3.85 mmoles) of 'butylmethoxyphenylbromosilane (42). After 19 hours the solution was added to 100 mL ether/50 mL H_2O. The ether layer was washed with 50 mL H_2O, 3x15 mL .1N aqueous HCl, 50 mL H_2O, 20 mL brine, dried over $MgSO_4$, filtered and the ether removed *in vacuo*. The crude was purified on a flash column packed with silica gel 60 and eluted with 3:2 ether:hexanes to yield a clear colorless oil on evaporation. Yield 1.41 g (81.2%). ^{1}H NMR ($CDCl_3$) δ 7.59 (m, 2H, Ph), 7.37 (m, 3H, Ph), 3.90 (m, 1H, C2), 3.85 (m, 2H, C3), 3.63 (s, 3H, OCH_3), 3.51 (dd, J=5.6, 5.6 Hz, 2H, C1), 3.44 (dd, J=7.0, 7.0 Hz, 2H, C1'), 2.59 (bm, 1H, OH), 1.55 (m, 2H, C2'), 1.24 (m, 18H, C3'-C11'), 0.95 (s, 9H, 'Bu), 0.86 (t, J=6.3 Hz, 3H, C12'); ^{13}C NMR ($CDCl_3$) δ 135.1 (Ph), 129.5 (Ph), 127.6 (Ph), 71.8 (C2), 71.6 (C1), 70.9 (C1'), 64.3 (C3), 51.6 (OMe), 31.8, 29.7, 26.0, 22.9, 18.8, 15.1 ('Bu), 14.0 (C12'); IR (neat) 3370, 2950, 2870, 1470, 1180, 1125, 1100 cm^{-1}; UV (EtOH) 206 nm (1,734); MS (EI), *m/z* (relative intensity) 421 (M^+, 20), 343 (100), 175 (27); Anal. Calcd for $C_{25}H_{48}O_4Si$: C, 68.93; H, 10.69. Found: C, 68.33; H, 10.70.

Preparation of 1-O-dodecyl-2-octadecanoyl-3-O-('butylmethoxyphenyl)silylglycerol (40).

1-O-Lauryl-3-O-('butylmethoxyphenyl)silylglycerol (42) (1.06 g, 2.36 mmoles) was dissolved in dry $CHCl_3$. Triethylamine (0.66 mL, 4.72 mmoles) and stearoyl chloride (1.57 mL, 4.72 mmoles) were added and the solution was refluxed for 12 hours. The $CHCl_3$ was removed *in vacuo* and ether was added, filtered and removed *in vacuo*. The crude product was purified on a flash column packed with silica gel 60 and eluted with 5% ether in hexanes to yield a clear colorless oil. Yield 861 mg (51%). ^{1}H NMR ($CDCl_3$) δ 7.61 (m, 2H, Ph), 7.37 (m, 3H, Ph), 5.13 (m, 1H, C2), 3.94 (m, 2H, C3), 3.62 (s, 3H, OCH_3), 3.60 (m, 2H, C1), 3.40 (t, J=6.8 Hz, 2H, C1'), 2.28 (m, 2H, C2"),

1.57 (m, 4H, C2' & C3"), 1.25 (m, 46H, C3'-C11' & C4"-C17"), 0.91 (s, 9H, ^{t}Bu), 0.86 (t, J=6.2 Hz, 6H, C12' & C18"). ^{13}C NMR ($CDCl_3$) δ 173.8 (C1"), 135.2 (Ph), 131.9 (Ph), 130.0 (Ph), 127.8 (Ph), 75.3 (C2), 72.9 (C1), 69.0 (C1'), 62.1 (C3), 51.7 (CH_3O), 34.9, 32.1, 30.0, 26.2, 25.0, 19.1 (^{t}Bu), 14.2 (C12' & C18"); MS (EI), *m/z* (relative intensity) 661 (M^+, 100), 419 (100), 363 (75), 209 (70), 195 (60), 153 (82), 71 (89), 57 (100).

Deprotection of 1-O-dodecyl-2-octadecanoyl-3-O-(^{t}butylmethoxyphenyl)silylglycerol.

In a typical procedure 1-O-lauryl-2-stearoyl-3-O-(^{t}butylmethoxyphenyl)silylglycerol (**40**) was dissolved in dry THF (or $(CH_2)_2Cl_2$), and the solution was cooled to the prescribed temperature where 6 eq. tetrabutylammonium fluoride in THF was added. After 2 hours the solution was poured into 50 mL ether/50 mL H_2O. The ether was washed 2x50 mL H_2O, 20 mL brine, dried over $MgSO_4$, filtered and the ether removed *in vacuo*. The crude was loaded on 1x20 cm flash column packed with silica gel 60 and eluted with 3:2 hexanes:ether. The requisite fractions were combined and the solvents were removed *in vacuo* to yield white crystals. Yields are given in Table 8.

Preparation of 1-O-dodecyl-2-octadecanoyl-rac-glyceryl-3-phosphorylcholine (**22**).

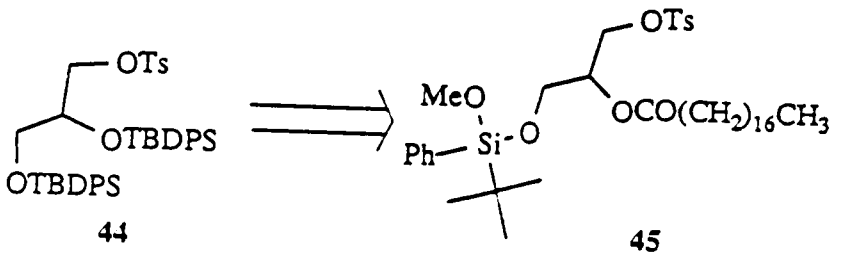
1-O-Lauryl-2-stearoylglycerol (**36**) (116 mg, 0.22 mmol) was dissolved in dry benzene and cooled to 7°C. 2-Chloro-2-oxo-1,3,2-dioxaphospholane (**26**) (0.02 mL, 0.22 mmol) was added followed by triethylamine (0.03 mL, 0.22 mmol) and the solution was stirred for 2 hours. Five mL of pentane were added, the mixture was filtered and the solvents were removed *in vacuo* to yield a beige oil. The crude oil was dissolved in dry acetonitrile, the solution was transferred to a pressure bottle and a few drops of trimethylamine was added. The pressure bottle was sealed and heated to 60°C for 12 hours. The acetonitrile was removed *in vacuo* and the crude was purified by flash chromatography on silica gel 60 with 4:1 chloroform:methanol as the elution solvents. Yield 64 mg

(42%) mp 58-60°C; IR (KBr) 2920, 2840, 1710, 1375, 1170 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.12 (m, 1H, C2), 4.18 (m, 2H, CH_2OPO_3), 4.10 (m, 2H, C3), 3.97 (m, 2H, C1'), 3.72 (m, 1H, C1), 3.55 (m, 1H, C1), 3.40 (m, 2H, CH_2N), 2.79 (s, 9H, CH_3N), 2.29 (m, 2H, C2"), 1.58 (m, 2H, C2'), 1.49 (m, 2H, C3"), 1.23 (m, 46H, C3'-C11' & C4"-C17"), 0.87 (t, J=6.9 Hz, 6H, C12' & C18"); MS (CI), *m/z* (relative intensity) 589 (M^+ -103, 6), 510 (356), 285 (100), 225 (30).

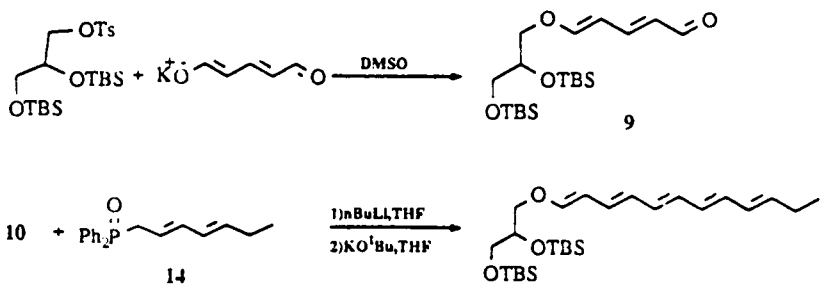
IV Synthesis of Precursor-Primary Approach

4.1 Introduction

In order to approach the synthesis of the unsaturated phospholipid we relied upon the work done on the model phospholipid and the synthesis of FP-12 reported by Nicolaou.[28] We envisioned using Nicolaou's key intermediate (**44**) in his synthesis of FP-12 and substituting appropriate functionalities dictated by our model synthesis to pursue (**45**) as our key intermediate in the synthesis of the biological precursor to FP-12.



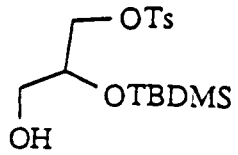
Nicolaou incorporated the pentaenyl moiety by substituting the tosylate with potassium glutaconate followed by a Wittig reaction, as shown in Scheme 10. We felt that the glutaconate substitution reaction alpha to an ester is less likely than the same reaction with an alpha silyl ether. This reasoning lead us to propose a synthesis of the precursor where tosylate displacement by potassium glutaconate would precede the acylation step.



Scheme 10.

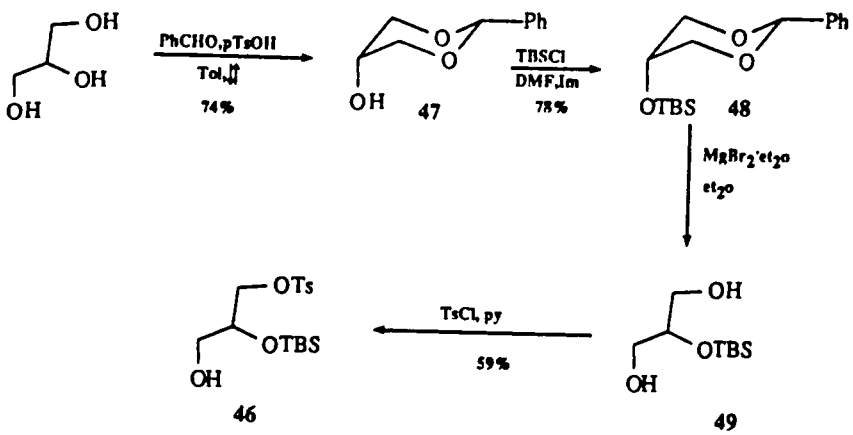
4.2 Synthetic Studies on the Precursor with the Acyl Functionality Introduced Late.

Since potassium glutaconate is stable in aqueous solution, it will not deprotonate an unprotected glycerol derivative such as **46**, and it therefore might be possible to substitute the tosylate with a primary hydroxyl group present. This approach would circumvent the acyl deactivation problem.



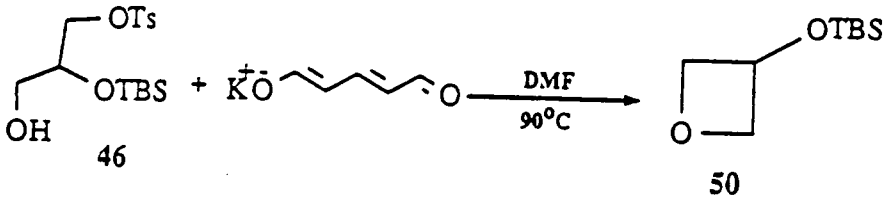
46

The synthesis of compound **46** is shown in Scheme 11. 1,3-benzylideneglycerol (**47**) was synthesized in 74% yield by azeotroping the water by-product of the condensation of benzaldehyde with glycerol in 74% yield.[73] Protection of the free hydroxyl group by reaction of 1,3-benzylidene-glycerol (**47**) with ^{t}butyldimethylsilyl chloride and imidazole in DMF produced (**48**) in 78% yield.



Scheme 11.

Selective hydrolysis of the acetal was achieved by using magnesium bromide etherate,[74] since organic and mineral acids hydrolyzed the silyl ether as well as the acetal. Tosylation of 2-O-(tbutyldimethyl)silylglycerol (**49**) with tosyl chloride in pyridine gave (**46**) in 59% yield. Treatment of (**46**) with potassium glutaconate in DMF yielded the oxetane **50**, as shown in Scheme 12. Since excess potassium glutaconate[75] was used and the oxetane **50** was stable under these conditions, it was clear that this compound represented a stable end product of the reaction. This approach to the precursor was thus aborted.



Scheme 12.

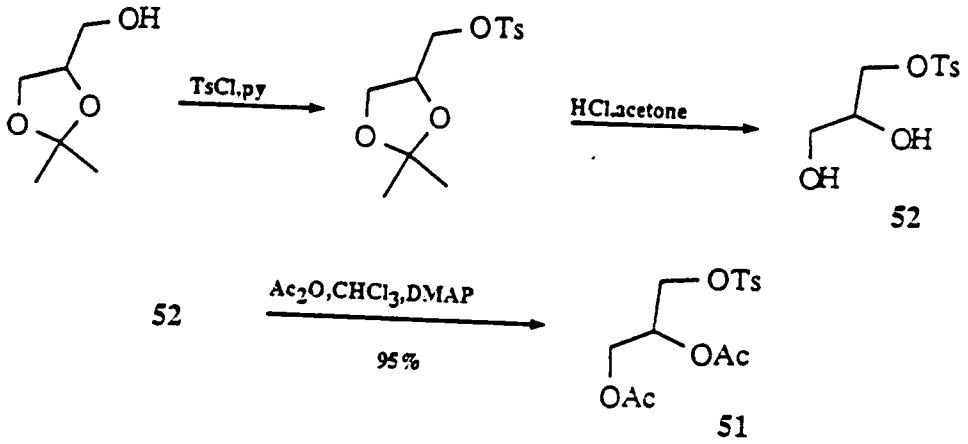
4.3 Synthetic Studies on the Precursor with the Acyl Functionality Present.

The above experiment indicated that glutaconate substitution with an alpha ester functionality present might be a more viable approach to the synthesis of the precursor. We therefore pursued several methodologies to synthesize a substrate with the critical acyl functionality present. This chemistry resulted in a series of compounds being made in which the viability of such an approach could be studied.

4.3.1 Model Study

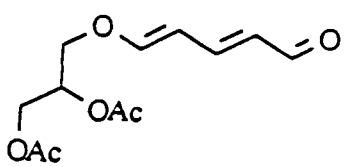
Before any attempts at precursor could be pursued, it was necessary to ascertain the extent to which an alpha acyl functionality would deactivate the primary carbon bearing the leaving group. A model system was devised in which **51** appeared to be the simplest substrate to

synthesize. This compound provided a means to achieve a model system which would mimic the type of substrate, (e.g. **45**), that would ultimately lead to precursor. We synthesized 1-O-tosyl-2,3-diacetylglycerol (**51**) by the route shown in Scheme 13. Tosylation of solketal with tosyl chloride in pyridine and subsequent hydrolysis of the isopropylidene ketal proceeded in similar fashion as that reported in the literature.[27] Diacylation of 1-O-tosylglycerol (**52**) with acetic anhydride and DMAP in



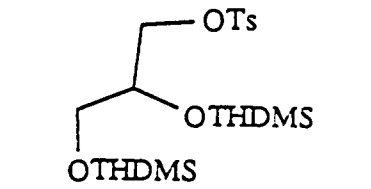
Scheme 13.

chloroform afforded the diacyl tosylglycerol (**51**) in 95% yield. Reaction of (**51**) with potassium glutaconate in DMF at 90°C afforded the requisite compound (**53**) as a crude mixture. However, the compound contained impurities that could not be removed by column chromatography. Since other methods of purification are not useful (i.e. distillation, sublimation or crystallization) the yield of **53** could only be approximated to be 25%.



53

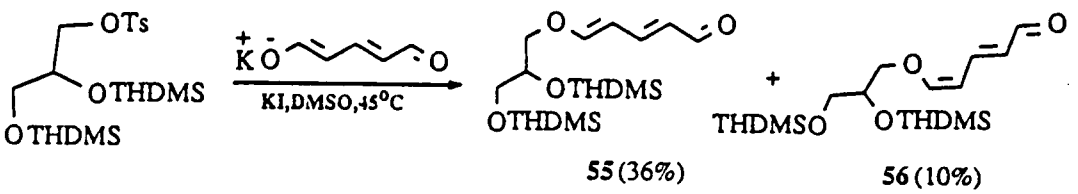
We decided to pursue milder conditions of glutaconate substitution to improve yields and thus obtain purer product. Our model system of choice was a Nicolaou-type intermediate (**54**);



THDMS=thexyldimethylsilyl

54

Nicolaou reported a yield of 37% of the dienal (**12**) from his precursor, **44**. Our synthesis of **54** was exactly the same as that reported in the literature except we replaced tbutyldimethylchlorosilane with thexyldimethylchlorosilane in the protection of 1-O-tosylglycerol because of the greater ease of handling thexyldimethylsilyl chloride as opposed to the tert-butyl analog. Addition of a catalytic amount of potassium iodide in the crucial displacement step enabled us to use milder conditions,[76] and we were pleased to find that the reaction was so clean that both E and Z isomers of the product could be separated (Scheme 14). The yields were improved by 9% (46% vs. 37%) over the analogous reaction in the FP-12 synthesis.

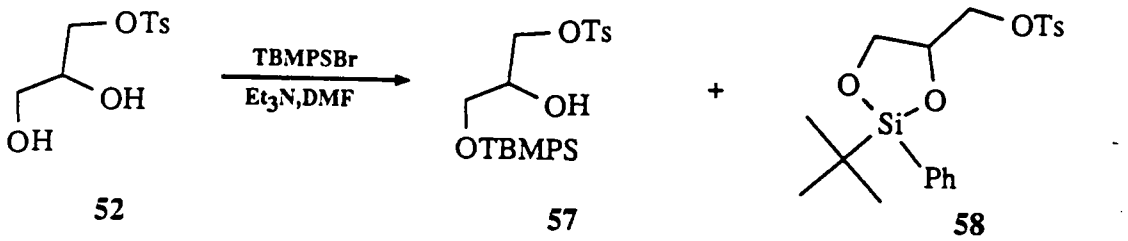


55 (36%) **56** (10%)

Scheme 14.

4.3.2 Acylation of 1-O-Tosyl-3-O-('butylmethoxyphenyl)silylglycerol.

We were now ready to apply our methodologies of the synthesis of the perhydroprecursor (**22**) to the synthesis of the unsaturated derivative. Protection of tosylglycerol with 'butylmethoxyphenylbromosilane and triethylamine in DMF (Scheme 15) provided **57** in 43% yield, together with the cyclized product **58** in 54% yield.* Compound **58** arises through an intramolecular S_N2 reaction which requires no additional base as shown in Figure 9.

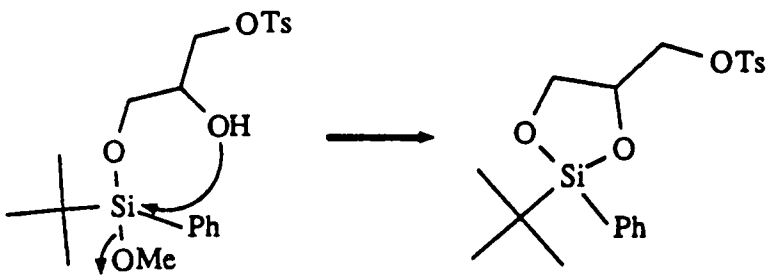


52 57 58

Scheme 15.

Acylation of **57** by an analogous procedure performed on the lauryl derivative, **43**, (i.e. refluxing with Et_3N and $CHCl_3$) caused decomposition of the substrate (**57**). No reaction occurred if the same experiment was run at room temperature instead of in refluxing $CHCl_3$. We did not know the extent of the difference in reactivities between **43** and **57** but we knew that the presence of the tosylate and maybe the silyl ketal may be important. Steric effects or hydrogen bonding between the hydroxyl proton and the methoxy oxygen of the silyl ketal, may have contributed to the stability of **57**. The extent of this contribution, however, could not be determined from our experiments. Rather than pursue this procedure we instead decided to slightly modify our strategy.

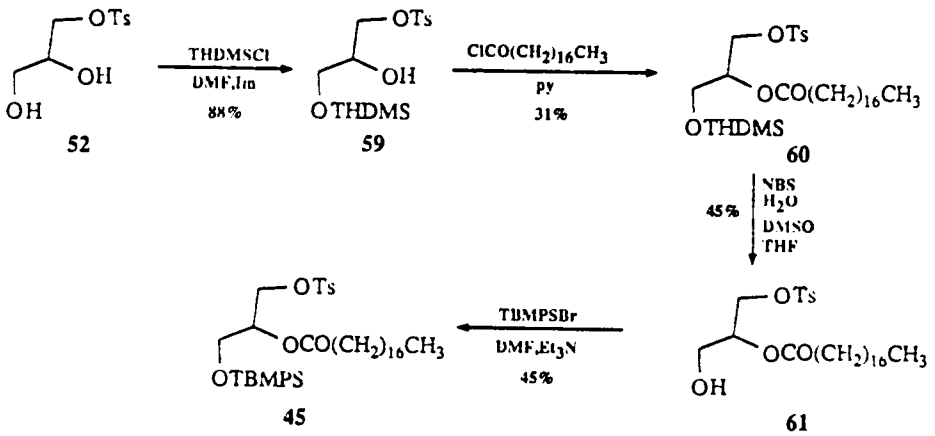
* The same side reaction occurred for 1-O-laurylglycerol in 7.5 % yield.



57 58

Figure 9. Mechanism for formation of **58**.

To ensure that the 2-stearoyl derivative of (**57**) could be made, we approached its synthesis in two ways. The first attempt (Scheme 16) was by monoprotecting 1-O-tosylglycerol (**52**) with one equivalent of thexyldimethylchlorosilane and imidazole in DMF to produce **59** in 88% yield. Acylation of **59** with stearoyl chloride in pyridine was performed as described by Johnson[77] to yield **60** in 31% yield. Deprotection of **60** was done under mildly acidic conditions[63] because there are no mild acid labile functionalities present in **60**, affording **61** in 45% yield with no intramolecular tranesterification. Reprotection of the primary hydroxyl group with the required silyl ketal group was done as usual in 45% yield.

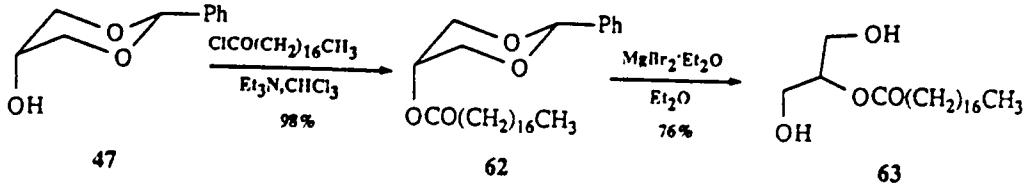


Scheme 16.

4.3.3 Precursor Via 2-Acyl-1,3-benzylideneglycerol

The second approach utilized the strategy realized in the synthesis of 1-O-tosyl-2-('butyldimethyl)silylglycerol (Scheme 17). Starting with 1,3-benzylideneglycerol (**47**), acylation of the free hydroxyl group with stearoyl chloride and triethylamine in chloroform at room temperature

provided the ester **62** in 98% yield. Using magnesium bromide etherate for selective hydrolysis of the acetal in the presence of the ester yielded **63** in 76% yield.[74]

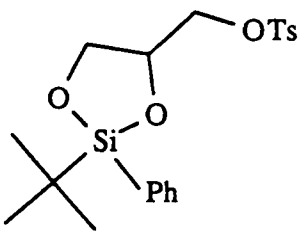


47 **62** **63**

Scheme 17.

With 2-stearoylglycerol in hand, there existed two basic approaches to precursor that could be pursued. Our strategy was to protect one hydroxyl group with the silyl acetal and to tosylate the other hydroxyl group. The problem was to perform these reactions without acyl migration since both derivatization reactions utilized basic conditions which had already been shown to cause transesterification. Simply put, choosing the order with which derivatization is to be done should be the key to an intermediate which could be taken to precursor.

Our experience with protection of 1-O-tosylglycerol (**52**) with methoxy'butylbromo-phenylsilane (**42**) directed us to approach tosylation first due to the high yields of the unwanted cyclization product **58**. Tosylation of 2-stearoylglycerol (**63**) proved to be a slow process. Even

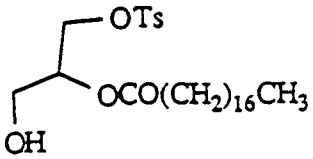


58

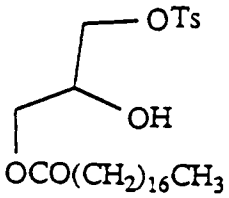
after 12 hours, 2-stearoylglycerol and tosyl chloride in pyridine at room temperature still provided 33% of recovered starting material and unfortunately the product formed was the undesired 1-stearoyl-3-tosylglycerol (64) in 51% yield. In other words, the rate of acyl migration was comparable to that of tosylation under these conditions.

The ester migration problem was addressed first by using one equivalent of pyridine in a suitable organic solvent. The procedure of Wilt[78] in his synthesis of neopentyl p-toluenesulfinate was used, with ether being the solvent of choice. It was found that the solubility of 2-stearoyl-glycerol (63) in ether was minimal at 0°C, and at room temperature no reaction occurred even after 24 hours.

Our second approach to tosylating 2-stearoylglycerol was to set the substrate with 1-equivalent of tosyl chloride in pyridine solution in the freezer for 24 hours. After work up and purification of the reaction components, a 31 % yield of starting material, a 4 % yield of the migrated product 64 and a 60 % yield of the desired product 61 was obtained.



61



64

A summary of the data pertaining to tosylation of 2-stearoylglycerol is tabulated below (Table 10). It was felt that although a 60 % yield was a good one, a secondary approach was attempted to see if overall yields could be improved.

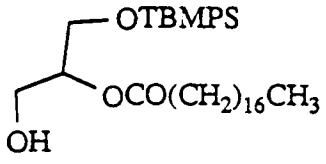
Protecting 2-stearoylglycerol first followed by tosylation was the alternative approach. Adding a large excess of tbutylmethoxyphenylbromosilane (42) to a solution of 2-stearoylglycerol

Table 10. Tosylation of 2-stearoylglycerol (**63**).

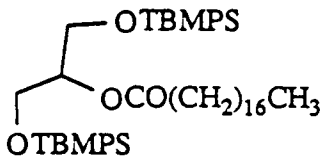
Solvent	Temp.(°C)	time (hrs.)	% Yield		
			64	**61**	**63**
pyridine	25	12	51	0	33
pyridine	0	24	4	60	31
ether/py	25	24	0	0	100[a]

[a] The yield was based upon TLC analysis.

(63) and 1 equivalent of triethylamine in DMF provided a 1:1 ratio of products (65:66) in 50 % and 48 % yields, respectively.

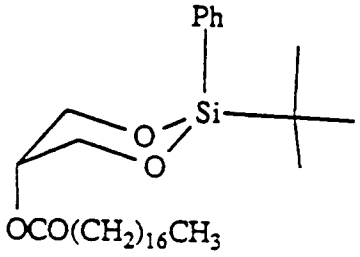


65



66

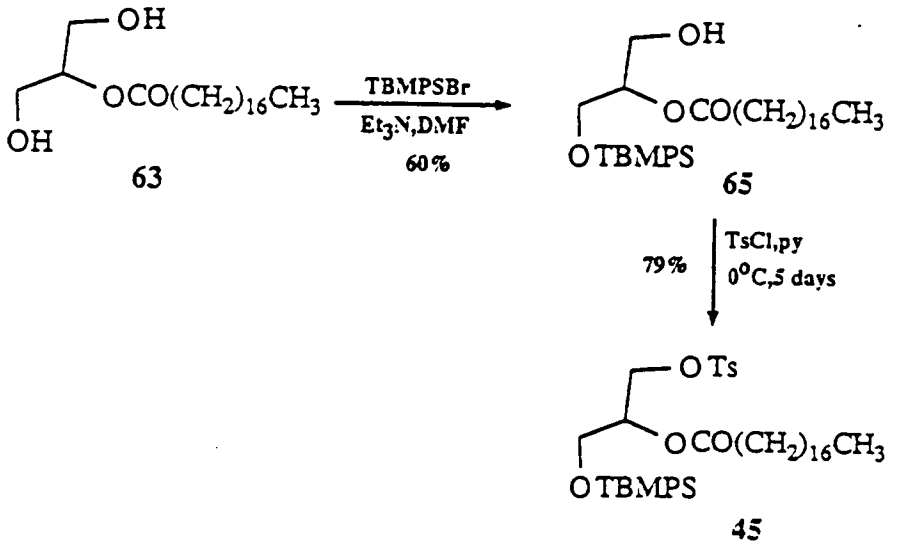
This experiment demonstrated that the reactivity of the bromosilane was much greater than triethylamine-catalyzed intramolecular transesterification, since no acyl migration products were observed and only 1/2 equivalent of triethylamine was needed for diprotection to occur. By adding one equivalent of butylmethoxyphenylbromosilane and one equivalent of triethylamine to a solution of 2-stearoylglycerol in DMF, a 60 % yield of the monoprotected product 65 along with a 19 % yield of the cyclized product 67 was isolated.



67

Protecting the tosylate (61) with the bromosilane proceeded in 84 % yield to give 45. The key intermediate 45 could also be obtained by tosylating the protected stearoylglycerol 65 with tosyl chloride in pyridine for 5 days in 79 % yield. It appeared that both methodologies, protecting first or tosylating first, proceeded in comparable overall yields from 2-stearoylglycerol, however, by

protecting first, no migrated product would be formed and, hence, purification by column chromatography would be simpler and give purer products. We opted for the protection/tosylation method as shown in Scheme 18.



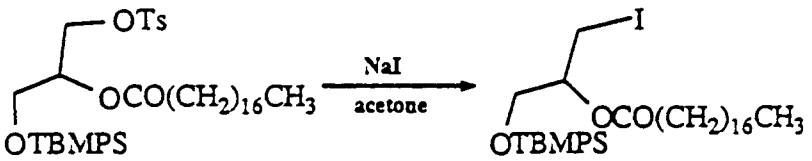
Scheme 18.

Potassium glutaconate displacement of the tosylate was the next synthetic step to perform, albeit a potentially difficult one. This was borne out when the tosylate **45** was treated with potassium glutaconate and a catalytic amount of potassium iodide in DMSO at 50°C. After one hour the starting material was consumed but no dienal ether was formed; instead the reaction generated a black charred mass containing a multitude of unisolable products.

Investigation of leaving group labilities provided us with a range of substrates where hopefully a generous balance could be found between the low reactivity of an α-X ester (where X represents a leaving group) and the high reactivity of the dienal ether product. The leaving (X) groups we used included the bromide, the iodide, the tosylate and the brosylate. The next section will deal with the synthesis of the bromide, the iodide and the brosylate analogs of **45**.

4.3.4 Glutaconate Displacement of Various Leaving Groups.

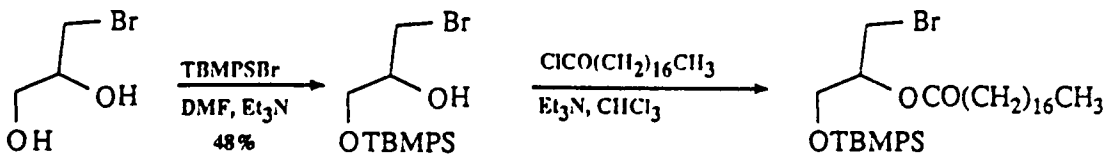
While it is believed that KI displaces the tosylate in the aforementioned reaction and thus the synthesis of the iodide might appear superfluous, it was synthesized anyway to ensure that the proposed mechanism in Figure 10 was in reality the true one. The iodide was made by simply refuxing the tosylate (**45**) with 2 equivalents of sodium iodide in acetone for 1 day to give **68** in 60 % yield (Scheme 19).



45 **68**

Scheme 19.

The synthesis of the bromide, shown in Scheme 20, started with 1-bromo-2,3-propanediol. Protection of the primary hydroxyl group with tbutylmethoxybromophenylsilane (**42**) under standard conditions gave the protected bromopropanediol **69** in 48 % yield. Acylation of **69** with stearoyl chloride and triethylamine in refluxing chloroform provided a 9 % yield of **70**, admittedly a very poor



69 **70**

Scheme 20.

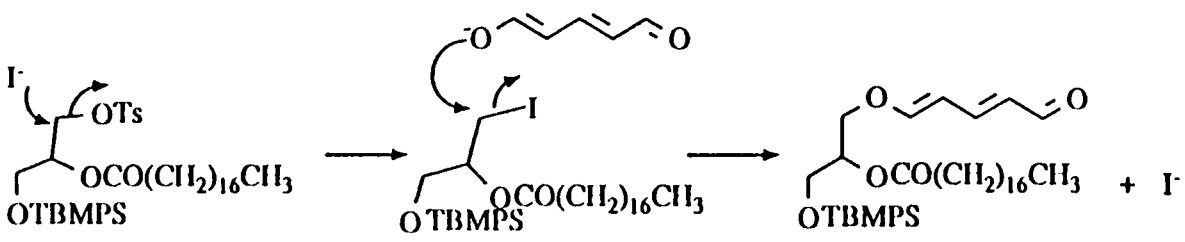
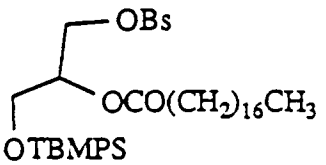


Figure 10. Mechanism for the iodide catalyzed displacement of a tosylate.

yield but adequate for the reactivity study. The brosylate was made by replacing tosyl chloride with brosyl chloride in the sulfonate esterification reaction to make the brosylate **71**. The reaction proceeded in 45 % yield.



71

With all four analogs ready to use the study could now proceed. Table 11 presents the data giving compound number, leaving group (X), catalyst used, solvent, yield of product, yields of starting material (where applicable), and reaction time.

Table 11 demonstrates that the iodide is a labile compound where a reaction occurs under mild conditions. It could not be determined for certain that the desired product was ever formed. However, if any of the desired dienal ether was present in solution, the conditions required to form the dienal ether were too harsh for this compound to withstand. The last entry was a reaction to determine if the silyl ketal moiety was responsible for the high lability of the dienal ether. As can be seen, it can be concluded that the presence of the stearoyl ester causes instability of the product **75**. Examination of the electronic effects of the substrate **68** and product **75** provided insight into why the reactivities were observed. Comparing **72** with **68** (Figure 11) under the reaction conditions might explain the low reactivity of **68** vis-a-vis **72**. The presence of the ester functionality destabilizes the transition state **73** relative to **74**, hence, requiring more rigorous conditions for substitution to occur. Similarly, compound **75** is more reactive due to potential ester hydrolysis leaving a naked alkoxide anion alpha to a dienal ether; this intermediate is therefore, set up for a wide variety of side reactions initialized by the one shown in Scheme 21.

Table 11. Conditions and results from reaction of **45, 60, 68, 70** and **71** with potassium glutoconate.

S.M.[a]	X	solvent	catalyst	%yield (S.M.[a])	%yield product	T(°C)	t(hrs.)
70	Br	DME	18-C-6[b]	N/A	NR[c]	25	18
70	Br	DME	18-C-6	N/A	NR	80	2
68	I	MeOH	none	0	0	65	17
68	I	DMPU[d]	none	0	0	25	2
68	I	DME	18-C-6	N/A	NR	80	5
45	OTs	DMSO	KI	0	0	50	1
45	OTs	DMSO	KI	0	0	40	16
45	OTs	DMSO	KI	49	0	25	16
71	OBs	DMSO	none	43	0	25	2
60	OTs	DMSO	KI	22	0	45	17
60	OTs	DME	18-C-6	36	0	40	48

[a] S.M. refers to starting material or the substrate.
[b] 18-crown-6 was used to enhance the solubility of potassium glutoconate in DME.
[c] No reaction observed.
[d] DMPU is an acronym for dimethylpropyleneurea, a noncarcinogenic substitute for HMPA.

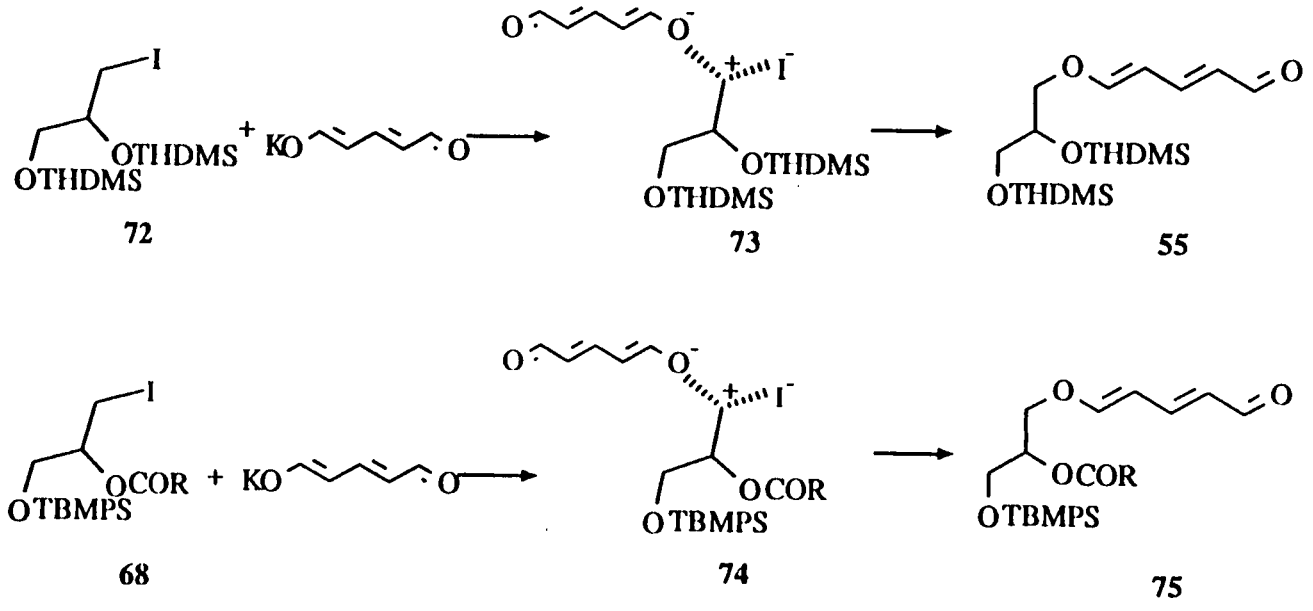
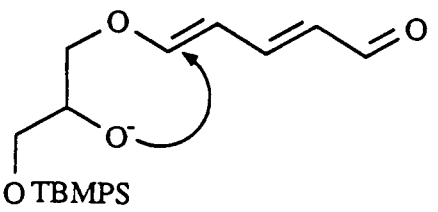


Figure 11. Comparison of transition states between **73** and **74**.



Scheme 21.

In conclusion, this type of reactivity found in compounds **45**, **60**, **68**, **70** and **71**, representing a successive range of reactivities, did not provide the product originally sought and therefore, precluded any attempts at precursor by methodologies outlined in this chapter.

4.4 Experimental

Preparation of 1,3-O-benzylideneglycerol (47).

Glycerol (36 mL, 0.5 mol), 51 mL (0.5 mol) and 1.6 g (8 mmol) of p-toluenesulfonic acid were dissolved in 150 mL of toluene. The flask was fitted with a 40 cm Vigreaux column, a Dean-Stark adapter, and a condenser at which the solution was stirred, heated to 100°C until 9 (0.5 mol) mL of water was liberated (ca. 4 hrs.). The solution was cooled to room temperature and the toluene removed *in vacuo*. The oil was crystallized from toluene and hexanes and washed with 5x20 mL of 2% K_2CO_3 and dried under a high vacuum. The material was recrystallized from toluene and hexanes to a constant melting point of 33-34°C (Lit. 33-34°C). Yield 67 g (74%). ^{1}H NMR ($CDCl_3$) δ 7.49 (m, 2H, Ph), 7.35 (m, 3H, Ph), 5.52 (s, 1H, CHPh), 4.16 (dd, J=1.6. 10.5 Hz, 2H), 4.07 (dd, J=1.6, 10.5 Hz, 2H), 3.59 (m, 1H, CHOH), 3.19 (bd, 1H, OH); IR (KBr) 3475, 1420, 1195 cm^{-1}; UV (EtOH) 206 nm (9.7).

Preparation of 2-O-(^{t}butyldimethyl)silyl-1,3-benzylideneglycerol (48).

In a dry flask, 5g (27.8 mmoles) 1,3-benzylideneglycerol (47) was dissolved in dry DMF and 4.2 g (27.8 mmoles) of TBDMSCl was added and stirred at room temperature for 2 hours. Fifty milliliters of ether was added and washed with 5x50 mL of H_2O, 1x20 mL brine, dried over $MgSO_4$, filtered and the ether removed *in vacuo* to yield a clear oil which appeared as a single spot on TLC. Yield 6.4g (78%). ^{1}H NMR ($CDCl_3$) δ 7.5 (m, 2H, Ph), 7.32 (m, 3H, Ph), 5.51 (s, 0.5H, CHPh), 5.38 (s, 0.5H, CHPh), 4.20 (dd, J=5.8, 9.6 Hz, 1H, CHOSi), 4.01 (d, J=2.6 Hz, 2H), 3.55 (m, 2H), 0.86 (m, 9H, ^{t}Bu), 0.04 (s, 6H, CH_3Si); ^{13}C NMR ($CDCl_3$) δ 129.7 (Ph), 129.2 (Ph), 128.3 (Ph), 126.6 (Ph), 126.2 (CPh), 72.4, 72.0, 25.8 (^{t}Bu), -5.4 (CSi); IR (neat) 2975, 2900, 1740, 1476, 1265, 1110 cm^{-1}.

Preparation of 2-O-('butyldimethyl)silylglycerol (49).

2-O-TBDMS-1,3-O-benzylideneglycerol (48) (2.65 g, 9 mmoles) of benzaldehyde was dissolved in dry ether and 2.32 g (9 mmoles) of magnesium bromide etherate was added and the mixture stirred for 7 hours where another 2.32 g of $MgBr_2Et_2O$ was added. After 21 hours an additional 2.32 g of $MgBr_2Et_2O$ was added and the mixture stirred for 1 hour. Saturated $NaHCO_3$ aqueous solution (50 mL) was added. The heterogenous mixture was filtered and the aqueous layer drawn off and extracted with 2x100 mL of ether. All ether extracts were combined and washed with 1x10 mL H_2O and 1x20 mL brine, dried over $MgSO_4$, filtered and the ether was removed *in vacuo* to yield crystals. The crystals were dissolved in warm hexanes and cooled in freezer to yield pure crystals. Yield 1.06 g (57%). ^1H NMR ($CDCl_3$) δ 3.80 (m, 1H, C2), 3.63 (d, J=5.4 Hz, 4H, C1 & C3), 2.20 (bm, 2H, OH), 0.88 (s, 9H, 'Bu), 0.09 (s, 6H, CH_3Si); ^{13}C NMR ($CDCl_3$) δ 72.5 (C2), 63.8 (C1 & C3), 25.5 ('Bu), -4.6 (CSi); IR (KBr) 3350, 3000, 1480, 1260, 1042 cm^{-1}; MS (CI) *m/z* (relative intensity) 207 (M^+, 100), 131 (82), 89 (40); mp 56-57°C.

Preparation of 1-tosyl-2-O-('butyldimethyl)silylglycerol (46).

2-O-TBDMS-glycerol (49) (1 g, 4.85 mmoles) was dissolved in dry pyridine and tosyl chloride (0.93 g, 4.58) was added in one portion and the solution stirred for 24 hours. The solution was poured into 200 mL ether and the ether was washed with 6x30 mL 25% aqueous $CuSO_4$, 1x30 mL H_2O, 1x10 mL brine, dried over $MgSO_4$, filtered and the ether removed *in vacuo*. Starting material was removed by recrystallizing the crude product from warm hexanes and the mother liquor was stripped of hexanes to yield a clear colorless oil which appeared as a single spot on TLC. Yield 1.03 g (59%). ^1H NMR ($CDCl_3$) 7.75 δ (d, J=7.5 Hz, 2H, Ph), 7.30 (d, J=7.5 Hz, 2H, Ph), 3.95 (m, 3H, C1 & C2), 3.61 (t, J=6.0 Hz, 1H, OH), 3.53 (m, 2H, C3), 2.40 (s, 3H, CH_3Ph), 0.81 (s, 9H, 'Bu), 0.01 (s, 6H, CH_3Si).

Preparation of 3-[('butyldimethyl)silyloxy]oxetane (50).

1-Tosyl-2-O-('butyldimethyl)silylglycerol (46) (149 mg, 0.41 mmol) was dissolved in dry DMF and 288 mg (2.07 mmol) of potassium glutoconate was added. The solution was heated to 90°C for 1 hour and then cooled to room temperature. Ether (100 mL) was added and the ether layer was washed with 3x50 mL water, 20 mL brine, dried over $MgSO_4$, filtered and the ether removed *in vacuo* to yield a yellow oil, which was purified by flash chromatography (Et_3N pretreated silica gel 60) eluting with ether. The compound was pure by TLC. Yield 40 mg (51%). ^1H NMR δ 4.70 (m, 1H, CHOSi), 3.86 (dd, J=2.3, 8.4 Hz, 2H), 3.67 (dd, J=2.0, 8.4 Hz, 2H), 0.83 (s, 9H, 'Bu), 0.02 (s, 6H, CH_3Si).

Preparation of 2,3-O-diacetyl-1-tosylglycerol (51).

1-Tosylglycerol (52) (716 mg, 2.91 mmoles) and 781 mg (6.4 mmoles) of DMAP were dissolved in dry $CHCl_3$. Acetic anhydride (0.60 mL, 6.4 mmoles) was added and the solution stirred at room temperature for 4 hours at which time 50 mL of ether and 50 mL H_2O was added. The ether layer was washed with 2x50 mL H_2O, 1x25 mL 25% aqueous $CuSO_4$, 1x50 mL H_2O, 20 mL brine, dried over $MgSO_4$, filtered and the ether removed *in vacuo*. The crude product was purified on a silica gel 60 flash column and eluted with 4:1 ether:hexanes to yield a light beige oil which appeared as a single spot on TLC. Yield 872 mg (95%). ^1H NMR ($CDCl_3$) δ 7.74 (d, J=9.1 Hz, 2H, Ph), 7.33 (d, J=9.1 Hz, 2H, Ph), 4.10 (m, 1H, C2), 3.12 (m, 4H, C1 & C3), 2.40 (s, 3H, CH_3Ph), 1.95 (2s, 6H, CH_3OCO).

Preparation of 5-[1-O-[2,3-(thexyldimethyl)silyl]glyceryl]pentadienal (55 & 56).

1-Tosyl-2,3-O-di(thexyldimethyl)silylglycerol (54) (22.4 g, 41 mmoles) , 13.9 g (102 mmoles) of potassium glutoconate and 0.68 g (4.1 mmoles) of potassium iodide was dissolved in

200 mL of dry DMSO under argon. The mixture was heated to 50-60°C for 12 hours at which time the reaction mixture was poured into 500 mL of brine. The aqueous layer was extracted with 4x500 mL of ether. The ether extracts were washed with 5x100 mL of H_2O, 2x125 mL of brine, dried over Na_2SO_4, filtered and the ether was removed *in vacuo*. The crude oil was purified on a flash column packed with triethylamine pretreated silica gel 60 and eluted with 5% ether in hexanes to yield a red oil which appeared as a single spot on TLC. Yield 6.7 g (36%) trans and 1.8 g (10%) 1-cis product. ^{1}H NMR (trans) ($CDCl_3$) δ 9.43 (d, J=8.3 Hz, 1H, C5'), 7.02 (dd, J=11.4, 15.2 Hz, 1H, C3'), 6.96 (d, J=11.4 Hz, 1H, C1'), 6.03 (dd, J=8.3, 15.2 Hz, 1H, C4'), 5.79 (dd, J=11.4, 11.4 Hz, 1H, C2'), 4.01 (dd, J=5.3, 15.1 Hz, 1H, C1), 3.85 (m, 2H, C2 & C1), 3.50 (m, 2H, C3), 1.58 (m, 2H, $CHMe_2$), 0.81 (m, 24H, CH_3C & CH_3CH), 0.09 (2s, 12H, CH_3Si); ^{13}C NMR ($CDCl_3$) δ 193.2 (C5'), 160.1 (C1'), 151.5 (C3'), 127.4 (C4'), 105.6 (C2'), 74.0 (C1), 71.6 (C2), 63.8 (C3), 32.3, 25.1, 20.3, 18.8, -1.2, -2.2, -3.5; IR (neat) 2970, 2850, 1690, 1620, 1460, 1255 cm^{-1}; MS (CI) m/z (relative intensity) 457 (M+1, 90), 377 (92), 131 (30), 81 (100). ^{1}H NMR (cis) ($CDCl_3$) δ 9.46 (d, J=8.9 Hz, 1H, C5'), 7.49 (dd, J=14.9, 11.2 Hz, 1H, C3') 6.39 (d, J=5.6 Hz, 1H, C1'), 6.00 (dd, J=8.9, 14.9 Hz, 1H, C4'), 5.27 (dd, J=11.2, 5.6 Hz, 1H, C2'), 4.07 (dd, J=3.7, 11.6 Hz, 1H, C1), 3.92 (dd, J=11.6, 6.0 Hz, 1H, C1), 3.85 (m, 1H, C2), 3.53 (m, 1H, C3), 3.46 (dd, J=8.2, 10.8 Hz, 1H, C3), 1.58 (m, 2H, $CHMe_2$), 0.78 (m, 24H, CH_3C & CH_3CH), 0.01 (2s, 12H, CH_3Si).

Preparation of 1-tosyl-3-O-(tbutylmethoxyphenyl)silylglycerol (57).

In a flame dried flask under argon, 1.76 g (7.14 mmoles) of 1-tosylglycerol (52) was dissolved in dry DMF. Triethylamine (1.09 mL, 7.86 mmoles) followed by 1.65 mL (7.86 mmoles) of tbutylmethoxyphenylbromosilane (42) was added. After 24 hours the solution was added to 500 mL of ether and the ether was washed with 50 mL H_2O, 3x15 mL .1N aqueous HCl, 1x50 mL H_2O, 20 mL brine, dried over $MgSO_4$, filtered and the ether removed *in vacuo*. The crude oil was purified

on a flash column packed with silica gel 60 and eluted with 3:1 ether:hexanes to yield a clear colorless oil which appeared as a single spot on TLC. Yield 1.34 g (43%). ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J=9.1 Hz, 2H, $PhSO_3$), 7.54 (d, J=9.1 Hz, 2H, $PhSO_3$), 7.37 (m, 5H, PhSi), 4.12 (m, 2H, C1), 3.94 (m, 1H, C2), 3.83 (m, 2H, C3), 3.60 (s, 3H, CH_3OSi), 2.43 (s, 3H, CH_3Ph), 0.91 (s, 9H, ^{t}Bu); ^{13}C NMR ($CDCl_3$) δ 135.5 (Ph), 130.3 (Ph), 128.1 (Ph), 70.1 (C1), 69.4, 63.5, 51.7, 26.0, 21.6, 19.2; IR (neat) 3580, 3120, 3000, 2975, 2940, 2900, 1619, 1490, 1444, 1378, 1205, 1190, 1125, 1105 cm^{-1}; UV (EtOH) 219 nm (14,501); MS (EI) *m/z* (relative intensity) 438 (M^+, 2), 381 (5), 349 (73), 209 (81), 91 (100).

Preparation of 1-tosyl-3-O-(thexyldimethyl)silylglycerol (59).

Tosylglycerol (52) (2.7 g, 10.3 mmol) was dissolved in 30 mL dry DMF under nitrogen, and 1.54 g of imidazole (26.7 mmol) was added followed by 2.24 mL of thexyldimethylchlorosilane (11.3 mmol) dropwise and the solution was allowed to stir for 1 hour. The solution was added to 200 mL of ether and the ether layer was washed with 4x25 mL of water, 25 mL of brine, dried over $MgSO_4$, filtered and the ether removed *in vacuo*. The crude was purified on flash column packed with silica gel 60 and eluted with 25% ether in hexanes to a clear, colorless oil which appeared as a single spot on TLC. Yield 4.9 g (88%). ^{1}H NMR ($CDCl_3$) δ 7.75 (d, J=8.3 Hz, 2H, $PhSO_3$), 7.30 (d, 2H, J=8.3 Hz, $PhSO_3$), 3.98 (m, 2H, C1), 3.79 (m, 1H, C2), 3.59 (d, J=7.1 Hz, 2H, C3), 2.47 (bs, 1H, OH), 2.37 (s, 3H, CH_3Ph), 1.52 (m, 1H, $CHMe_2$), 0.87 (d, J=2.5 Hz, 6H), 0.80 (s, 6H), -0.03 (s, 6H, CH_3Si); ^{13}C NMR ($CDCl_3$) δ 144.7 ($PhSO_3$), 132.6 (Ph), 129.6 (Ph), 127.7 (Ph), 70.2 (C1), 69.0, 62.1, 33.9, 25.9, 21.2, 19.9, 18.3, -3.8 (CSi); IR (neat) 3625, 2970, 2870, 1480, 1370, 1255, 1180, 1105 cm^{-1}; MS (CI) *m/z* (relative intensity) 389 (M+1, 100), 235 (70), 157 (79), 131 (53), 85 (71).

Preparation of 1-tosyl-2-stearoyl-3-O-(thexyldimethyl)silylglycerol (60).

1-Tosyl-3-O-(thexyldimethyl)silylglycerol (59) (1 g, 2.6 mmol) was dissolved in dry pyridine. Stearoyl chloride (1.7 mL, 5.2 mmol) was added and the was solution was stirred at room temperature for 15 hours and then methanol (5 mL) was added and the solution stirred for an additional 1 hour. The solution was added to 100 mL of hexane and the hexanes were washed with 50 mL of water, 2x25 mL 25% aqueous $CuSO_4$, 50 mL water, 25 mL of brine, dried over $MgSO_4$, filtered, and the hexanes were removed *in vacuo*. The crude product was purified on a flash column packed with silica gel 60 which appeared as a single spot on TLC. Yield 515 mg (31%). ^{1}H NMR ($CDCl_3$) δ 7.75 (d, J=7.9 Hz, 2H, $PhSO_3$), 7.31 (d, J=7.9 Hz, 2H, $PhSO_3$), 4.92 (m, 1H, C2), 4.17 (m, 2H, C1), 3.62 (d, J=5.3 Hz, 2H, C3), 2.41 (s, 3H, CH_3Ph), 2.20 (t, J=7.9 Hz, 2H, C2"), 1.54 (m, 3H, C3" & $CHMe_2$), 1.25 (m, 28H, C4"-C17"), 0.84 (t, 3H, C18"), 0.80 (d, J=6.6 Hz, 6H, CH_3CH), 0.74 (s, 6H, CH_3C), 0.02 (s, 6H, CH_3Si).

Preparation of 1-tosyl-2-stearoylglycerol (61) by deprotection of 60.

1-Tosyl-2-stearoyl-3-O-(thexyldimethyl)silylglycerol (60) (515 mg, 0.79 mmol) was dissolved in dry THF in a flame dried flask. To this mixture, 1 mL (56 mmol) of water, 10 mL (14.1 mmol) of DMSO and 660 mg of NBS was added and the solution was stirred for 48 hours in the dark, and 20 mL of 1% aqueous $Na_2S_2O_3$ was added and the solution was stirred for 10 minutes. The solution was extracted 3x50 mL of ether, the ether extracts were combined, washed with 20 mL of brine, dried over $MgSO_4$, filtered and ether was removed *in vacuo*. The compound was purified by flash column chromatography using silica gel 60 and eluting with 3:2 hexanes:ether to isolate a clear, colorless oil which appeared as a single spot on TLC. Yield 180 mg (45%). ^{1}H NMR ($CDCl_3$) δ 7.75 (d, J=7.7 Hz, 2H, $PhSO_3$), 7.31 (d, J=7.7 Hz, 2H, $PhSO_3$), 4.98 (m, 1H, C2), 4.18 (d, J=5.5 Hz, 2H, C1), 3.69 (d, J=6.1 Hz, 2H, C3), 2.41 (s, 3H, CH_3Ph), 2.22 (t, J=7.7 Hz, 2H, C2"), 1.52 (m,

2H, C3"), 1.24 (m, 28H, C4"-C17"), 0.85 (t, J=6.4 Hz, 3H, C18").

Preparation of 1-tosyl-2-stearoylglycerol (61) by tosylation of 2-stearoylglycerol (63).

2-Stearoylglycerol (63) (100 mg, 0.28 mmol) and 53.4 mg (0.28 mmol) of p-toluenesulfonyl chloride was dissolved in dry pyridine at -10°C and the solution set in the freezer for 24 hours. The solution was poured into 100 mL of ether and immediately washed with 50 mL of water, 2x50 mL of ice cold 3N HCl and 50 mL of water, 50 mL saturated $NaHCO_3$, 50 mL of water and 20 mL brine. The ether layer was dried over $MgSO_4$, filtered and the ether was removed *in vacuo*. Purification was done by flash column chromatography using silica gel 60 and eluting with 25% hexanes in ether. The purified product appeared as a single spot on TLC. Yield 85.4 mg (59.7%).

Preparation of 2-stearoyl-1,3-O-benzylideneglycerol (62).

1,3-O-Benzylideneglycerol (47) (3.0 g, 16.7 mmol) was dissolved in dry chloroform, and 11.1 mL (33.3 mmol) of stearoyl chloride was added followed by 4.6 mL (33.3 mmol) of triethylamine and the solution was stirred at room temperature for 14 hours. At that time, 1 mL (56 mmol) of water was added and the solution was stirred for 1 hour. The chloroform was removed *in vacuo*, ether was added, filtered and the ether removed *in vacuo*. The white crystals were recrystallized from methanol to a constant melting point. Yield 7.3 g (98%). mp 60-62°C ^{1}H NMR ($CDCl_3$) δ 7.48 (m, 2H, Ph), 7.36 (m, 3H, Ph), 5.55 (s, 1H, CHPh), 4.70 (m, 1H, CHOCOR), 4.23 (dd, J=1.1, 11.0 Hz, 2H), 4.17 (dd, J=1.1, 11.0 Hz, 2H), 2.41 (t, J=8.0 Hz, 2H, CH_2COO), 1.64 (m, 2H, CH_2CH_2COO), 1.27 (m, 28H), 0.86 (t, J=6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.7 (COO), 137.9 (Ph), 129.0 (Ph), 128.2 (Ph), 127.4 (Ph), 126.0 (Ph), 101.2 (CPh), 69.1, 65.7, 34.4, 31.9, 29.6, 29.4, 29.3, 29.1, 24.9, 22.6, 14.2, 14.0; IR (KBr) 3040, 2950, 1750, 1430, 1375 cm^{-1}; MS (EI) *m/z* (relative intensity) 446 (M^+, 41), 324 (40), 267 (37), 105 (100), 57 (63).

Preparation of 2-stearoylglycerol (63).

2-Stearoyl-1,3-O-benzylideneglycerol (62) (2.14 g, 4.8 mmol) was dissolved in 250 mL dry ether followed by 3.7 g of (14.4 mmol) $MgBr_2Et_2O$ and the mixture was stirred until the solution became homogenous. The solution was stirred for an additional 24 hours, and 80 mL of 5% aqueous $NaHCO_3$ was added, and refluxed for 2 hours. The solution was filtered into a separatory funnel, the water drawn off, the ether was dried over Na_2SO_4, filtered and the ether removed *in vacuo* to yield white crystals which were recrystallized from ether and hexanes. Yield 1.32 g (76%). mp 72-73°C ^{1}H NMR ($CDCl_3$) δ 4.91 (m, 1H, C2), 3.81 (dd, J=8.4, 8.4 Hz, 4H, C1 & C3), 2.35 (t, J=12.8 Hz, 2H, C2"), 1.94 (t, J=8.4 Hz, 2H, OH), 1.61 (m, 2H, C3"), 1.24 (m, 28H, C4"-C17"), 0.87 (t, J=8.8 Hz, 3H, C18"); ^{13}C NMR ($CDCl_3$) δ 174.1 (C1"), 75.0 (C2"), 62.3 (C1 & C3), 34.3 (C3"), 31.8, 30.0, 29.2, 29.0, 24.9, 22.6, 13.9 (C18"); MS (CI) *m/z* (relative intensity) 359 (M+1, 100), 341 (23), 285 (36), 85 (19).

Preparation of 3-O-(^{t}butylmethoxyphenyl)silyl-2-stearoylglycerol (65).

2-Stearoylglycerol (63) (215 mg, 0.60 mmol) was dissolved in dry DMF and then 0.05 mL (0.60 mmol) ^{t}butylmethoxyphenylbromosilane (42) was added followed by 0.083 mL of (0.60 mmol) dry triethylamine. The solution was stirred at room temperature for 12 hours. The solution was added to 50 mL ether/50 mL water, the water layer was removed and extracted 2x50 mL of ether, the ether extracts were combined, washed with 2x50 mL of water, 20 mL of brine, dried over $MgSO_4$, filtered and the ether was removed *in vacuo*. The crude was loaded on flash column packed with silica gel 60 and eluted with 3:1 hexanes:ether to yield a clear, colorless oil. Yield 198 mg (60%). ^{1}H NMR ($CDCl_3$) δ 7.60 (m, 2H, Ph), 7.38 (m, 3H, Ph), 5.00 (m, 1H, C2), 3.98 (d, J=6.5 Hz, 2H, C3), 3.85 (dd, J=5.7, 5.7 Hz, 2H, C1), 3.63 (s, 1.5H, CH_3OSi), 3.56 (s, 1.5H, CH_3OSi), 2.42 (t, J=6.4 Hz, OH), 2.31 (m, 2H, C2"), 1.71 (m, 2H, C3"), 1.25 (m, 28H, C4"-C17"), 0.95 (s, 9H, ^{t}Bu),

0.87 (t, J=6.4 Hz, 3H, C18"); ^{13}C NMR ($CDCl_3$) δ 173.7 (C1"), 135.1 (Ph), 134.9 (Ph), 130.0 (Ph), 129.8 (Ph), 127.7 (Ph), 127.6 (Ph), 74.6 (C2), 68.7, 65.8, 61.9, 51.5, 50.5, 34.3 (C2"), 31.8, 29.6, 29.5, 29.3, 29.2, 29.1, 29.0, 25.9, 25.8, 22.5, 18.6, 18.1, 13.9 (C18"); IR (neat) 3460, 2955, 2880, 1750, 1480, 1125 cm^{-1}; UV (EtOH) 262 nm (3,887); MS (EI) *m/z* (relative intensity) 550 (M^+, 2), 493 (18), 461 (100), 405 (32), 267 (33), 195 (65), 57 (100).

Preparation of 1-tosyl-2-stearoyl-3-O-('butylmethoxyphenyl)silylglycerol (45) by tosylation of (65).

A solution of 177 mg (0.32 mmol) of 1-O-('butylmethoxyphenyl)silyl-2-stearoylglycerol (65) and 67 mg (0.35 mmol) of p-toluenesulfonic chloride in dry pyridine was prepared at -10°C. The solution was placed in the freezer for 4 days at which time the cold solution was added to 100 mL of ether. The ether was washed with 2x50 mL of ice cold 3N aqueous HCl, 50 mL of water, 50 mL of saturated aqueous $NaHCO_3$, 50 mL of water, 20 mL brine, dried over $MgSO_4$, filtered and the ether removed *in vacuo*. The crude was loaded on a flash column packed with silica gel 60 and eluted with 4:1 hexanes:ether to yield a clear, colorless oil. Yield 178 mg (79%). ^1H NMR ($CDCl_3$) δ 7.79 (d, J=8.7 Hz, 2H, $PhSO_3$), 7.50 (m, 2H, PhSi), 7.37 (m, 3H, PhSi), 7.31 (d, J=8.7 Hz, 2H, $PhSO_3$), 5.06 (m, 1H, C2), 4.25 (m, 2H, C1), 3.86 (m, 2H, C3), 3.59 (s, 1.5H, CH_3OSi), 3.58 (s, 1.5H, CH_3OSi), 2.43 (s, 3H, CH_3Ar), 2.19 (m, 2H, C2"), 1.52 (m, 2H, C3"), 1.26 (m, 28H, C4"-C17"), 0.88 (m, 12H, C18" & 'Bu); IR (neat) 2910, 2850, 1740, 1480, 1180 cm^{-1}; UV (EtOH) 300 nm (6,280).

Preparation of 1-tosyl-2-stearoyl-3-O-('butylmethoxyphenyl)silylglycerol (45) by protection of 61.

Compound 61 (180 mg, 0.35 mmol) was dissolved in dry DMF. Triethylamine (0.05 mL, 0.39 mmol) and 0.08 mL (0.97 mmol) of 'butylmethoxyphenylbromosilane was added and the solution was stirred at room temperature for 12 hours. The solution was added to 50 mL ether/50

water. The ether layer was washed with 2x50 mL of water, 20 mL of brine, dried over $MgSO_4$, filtered and the ether was removed *in vacuo*. The compound was purified on flash column packed with silica gel 60 and eluted with 4:1 hexanes:ether to yield a clear, colorless oil. Yield 112 mg (45%).

Preparation of 2-[-1-('butylmethoxyphenyl)silyloxy-3-iodo]propyl stearate (68).

1-Tosyl-2-stearoyl-3-O-('butylmethoxy-phenyl)silylglycerol (45) (43 mg, 0.06 mmol) was dissolved in dry acetone, 18 mg (0.12 mmol) of sodium iodide was added and the solution was refluxed 24 hours. The solution was cooled to room temperature, filtered, and the acetone was removed *in vacuo*, hexanes was added, refiltered and hexanes removed *in vacuo*. The compound was purified by flash column chromatography using silica gel 60 and eluting with 4:1 hexanes:ether to yield a light yellow oil which appeared as a single spot on TLC. Yield 24 mg (60%). ^1H NMR ($CDCl_3$) δ 7.59 (m, 2H, Ph), 7.37 (m, 3H, Ph), 4.87 (m, 1H, C2), 3.94 (m, 2H, C1), 3.63 (s, 3H, CH_3OSi), 3.44 (m, 2H, C3), 2.60 (m, 2H, C2"), 1.29 (m, 2H, C3"), 1.25 (m, 28H, C4"-C17"), 0.95 (s, 9H, 'Bu), 0.87 (t, J=6.5 Hz, 3H, C18").

Preparation of 2-[-1-('butylmethoxyphenyl)silyloxy-3-bromo]propanol (69).

Bromopropanediol (1 mL, 9.71 mmol) was dissolved in dry DMF in a flame dried flask and 1.98 mL (23.9 mmol) of 'butylmethoxybromophenylsilane (42) and 1.35 mL (9.71 mmol) of triethylamine was added simultaneously and the solution was stirred for 2 hours at room temperature. The solution was added to 100 mL ether and 50 mL of water, the ether layer was washed with 3x50 mL of water, 20 mL brine, dried over Na_2SO_4, filtered and the ether removed *in vacuo*. The compound was loaded on a flash column packed with silica gel 60 and the column eluted with 3:2 ether:hexanes to isolate a clear, colorless oil which appeared as a single spot on

TLC. Yield 1.62 g (48%). ^{1}H NMR ($CDCl_3$) δ 7.58 (m, 2H, PhSi), 7.39 (m, 3H, PhSi), 3.90 (m, 2H, C3), 3.65 (m, 3H, C2 & C1), 3.64 (s, 3H, CH_3OSi), 2.63 (d, J=8.3 Hz, 1H, OH), 0.95 (s, 9H, ^{t}Bu).

Preparation of 2-[-1-(^{t}butylmethoxyphenyl)silyloxy-3-bromo]propyl stearate (70).

2-[-1-(^{t}Butylmethoxyphenyl)silyloxy-3-bromo]propanol (69) (1.62 g, 4.67 mmol) was dissolved in dry chloroform in flame dried flask. Stearoyl chloride (1.73 mL, 4.67 mmol) and 0.65 mL (4.67 mmol) of triethylamine was added and the solution was refluxed for 12 hours. The chloroform was removed *in vacuo*, ether was added, filtered and the ether was removed *in vacuo*. The crude product was loaded on a flash column packed with silica gel 60. The column was eluted with 5% ether in hexanes to yield a light yellow oil which appeared as a single spot on TLC. Yield 262 mg (9%). ^{1}H NMR ($CDCl_3$) δ 7.57 (m, 2H, PhSi), 7.38 (m, 3H, PhSi), 5.12 (m, 1H, C2), 3.98 (m, 2H, C3), 3.64 (m, 2H, C1), 3.63 (s, 3H, CH_3OSi), 2.30 (m, 2H, C2"), 1.60 (m, 2H, C3"), 1.25 (m, 28H, C4"-C17"), 0.94 (s, 9H, ^{t}Bu), 0.86 (t, J=6.4 Hz, 3H, C18").

Preparation of 1-brosyl-2-stearoyl-3-O-(^{t}butylmethoxyphenyl)silylglycerol (71).

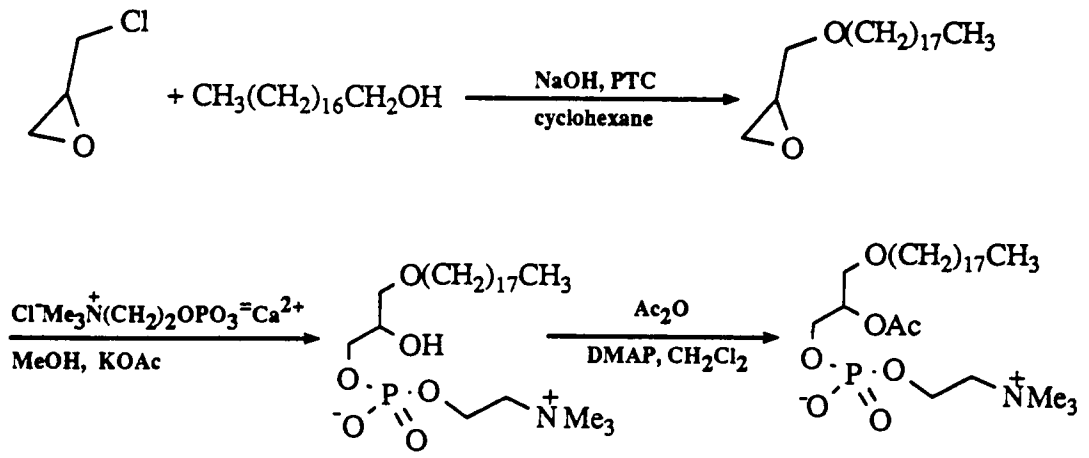
1-O-(^{t}Butylmethoxyphenyl)silyl-2-stearoylglycerol (65) (72.5 mg, 0.13 mmol) and 37 mg (0.14 mmol) of p-bromotoluenesulfonyl chloride was dissolved in dry pyridine at -10°C and the solution was placed in the freezer for 5 days. The solution was poured into 100 mL of ether, the ether was washed with 2x50 mL ice cold 3N aqueous HCl, 50 mL of water, 50 mL of saturated aqueous sodium bicarbonate, 50 mL water, 20 mL of brine, dried over $MgSO_4$, filtered and the ether removed *in vacuo*. The compound was purified on a flash column packed with silica gel 60 and eluted with 10% ether in hexanes to yield a clear, colorless oil which appeared as a single spot on TLC. Yield 46 mg (45%). ^{1}H NMR ($CDCl_3$) δ 7.77 (d, J=8.8 Hz, 2H, $PhSO_3$), 7.67 (d, 2H, J=8.8 Hz, $PhSO_3$), 7.51 (m, 2H, PhSi), 7.38 (m, 3H, PhSi), 5.09 (m, 1H, C2), 4.31 (m, 2H, C1), 3.86 (m, 2H,

C3), 3.59 (s, 1.5H, CH_3OSi), 3.58 (s, 1.5H, CH_3Si), 2.20 (t, J=5.8 Hz, 2H, C2"), 1.53 (m, 2H, C3"), 1.27 (m, 28H, C4"-C17"), 0.89 (s, 9H, ^{t}Bu), 0.88 (t, J=6.1 Hz, 3H, C18").

V Precursor Synthesis-Secondary approach

5.1 Introduction

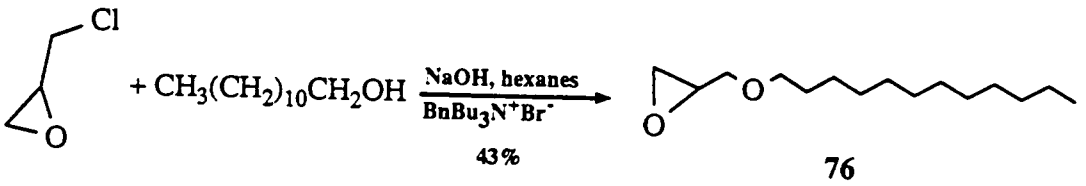
In chapter 3 there was a list of protecting groups of 1-O-alkyl glycerols used in the synthesis of phospholipids (Table 7). One of those listed was the epoxide, namely 1-O-stearoyl-glycidol. Julia, et. al.[65] performed epoxide opening and coincidental deprotection using phosphoryl choline. Their synthesis is illustrated in Scheme 22, which incidentally could be applied to the synthesis of perhydroprecursor.



Scheme 22.

5.2 Model Study Using Perhydroprecursor

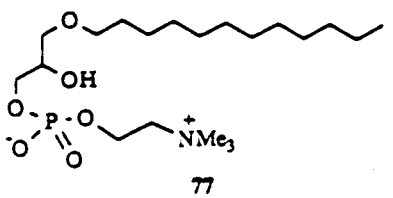
To ensure that this synthetic strategy would work we applied it to a compound that was very similar to Julia's compound, namely perhydroprecusor. Starting with epichlorohydrin, Julia's methodology was used to synthesize 1-O-laurylglycidol (76) in 43% yield (Scheme 23). We were not able to reproduce Julia's result in the epoxide opening step when using their reported conditions.



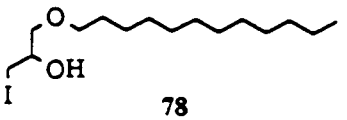
Scheme 23.

5.2.1 Epoxide Opening with Phosphorylcholine

Excluding Julia's report, epoxide opening by phosphate anions performed by other workers all used the monobasic phosphate anion. It was felt that epoxide opening in the vast majority of these type of reactions was assisted by the presence of a weak acid.[79] The monobasic phosphate ion therefore contributed both acid and nucleophilic characteristics. With this in mind, it was felt that the presence of a weak acid along with phosphorylcholine would assist epoxide opening. However, it was found that when one equivalent of ammonium chloride was added, the reaction still did not produce the needed intermediate **77** even though the epoxide was consumed. It is not known what happened. When phosphorylcholine was replaced with sodium iodide, a 77% yield of the desired iodo alcohol **78** was isolated.



77



78

This reaction indicated that an equilibrium may have existed and that the presence of the slightly soluble ammonium chloride drove the equilibrium to the right as shown in Figure 12.

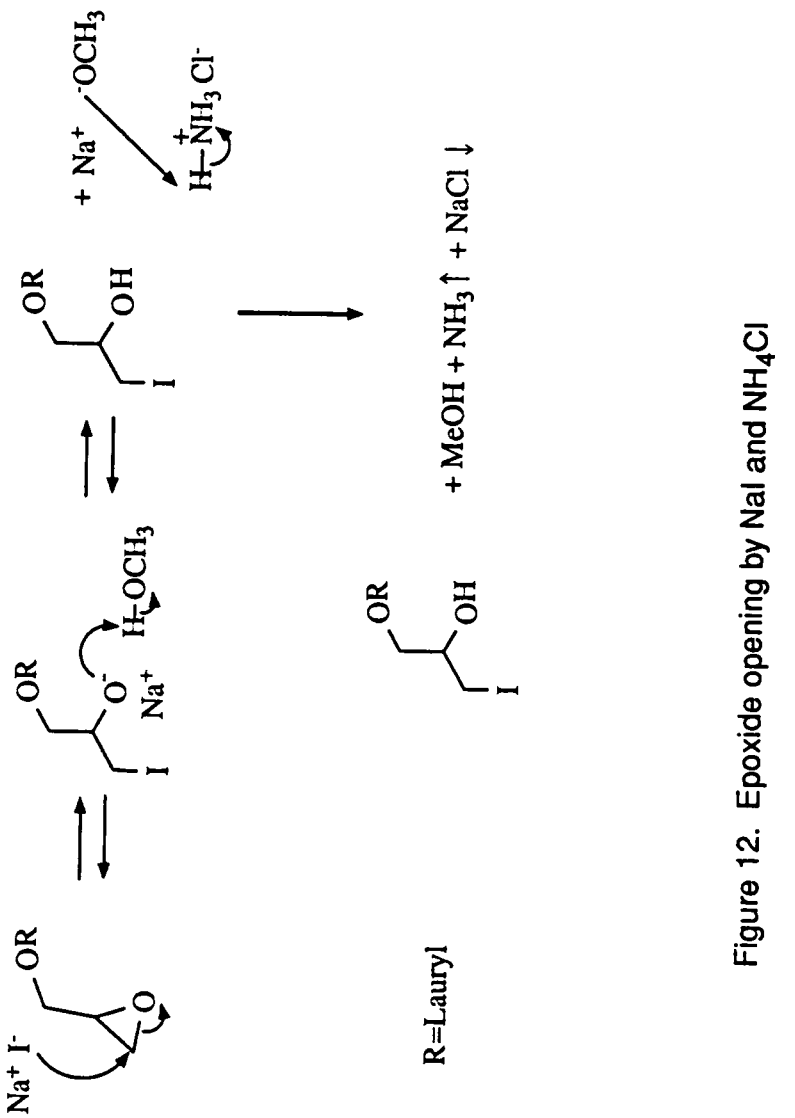
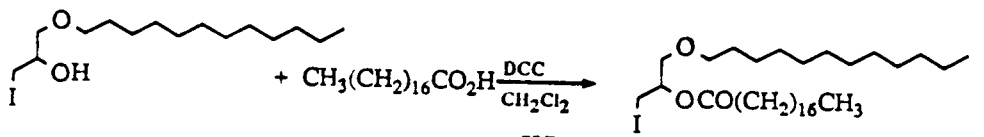


Figure 12. Epoxide opening by NaI and NH_4Cl

Alternatively, nucleophilic attack by the iodide may be concurrent with proton transfer from ammonium chloride.

This is an important result because previously epoxide opening by iodide required strongly acidic conditions (TBDMSI[80] or HI[81] for example). Herein, we report epoxide opening by iodide ion using weakly acidic conditions which more closely preserves the constraints inherent in the synthesis of the precursor discussed in chapter 3. Acylation of **78** proceeded in 75% yield using stearic acid and DCC in methylene chloride (Scheme 24).[82]

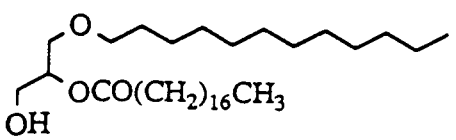


78 79

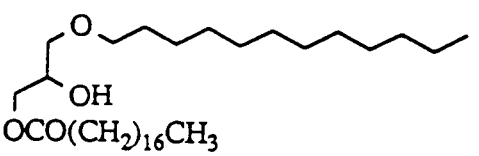
Scheme 24.

5.2.2 Attempted Iodide Displacement with Phosphorylcholine

It was found that iodide displacement by phosphorylcholine in refluxing methanol did not yield the desired phospholipid. To our surprise, a mixture of compounds **36** and **37**, previously synthesized, was isolated in a ratio of 5:1 (**36**:**37**).

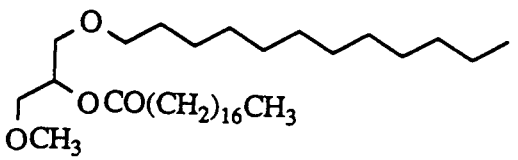


36



37

Maintaining extreme dry conditions did not change the reaction products significantly. We excluded hydrolysis of the iodide as a possible explanation because no methyl ether **80** was detected. If simple hydrolysis of the iodide were the mode of operation, then the methyl ether, **80**,

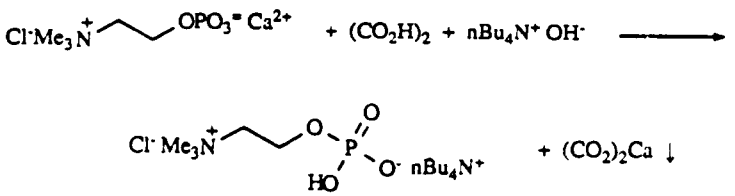


80

should also be formed, since the reaction was done in methanol. We thus propose that a phosphate ester intermediate is formed, but then is hydrolyzed by methanol as shown in Figure 13.

Phosphorylcholine, which is available as the calcium chloride salt, is only soluble in water and methanol. Therefore, in order to circumvent the hydrolysis problem a non-nucleophilic solvent must be used and phosphorylcholine must be made soluble in this solvent. The use of tetrabutylammonium salts is very popular in this regard.

The problem is to selectively remove the calcium and chloride ion from the phosphate dianion. Removal of the calcium ion was best performed by adding one equivalent of oxalic acid and one equivalent of tetrabutylammonium hydroxide to an aqueous solution of phosphoryl choline chloride, calcium salt; calcium oxalate immediately precipitated out of solution, and could be filtered off to give an aqueous solution of **81** and tetrabutylammonium chloride (Scheme 25).



81

Scheme 25.

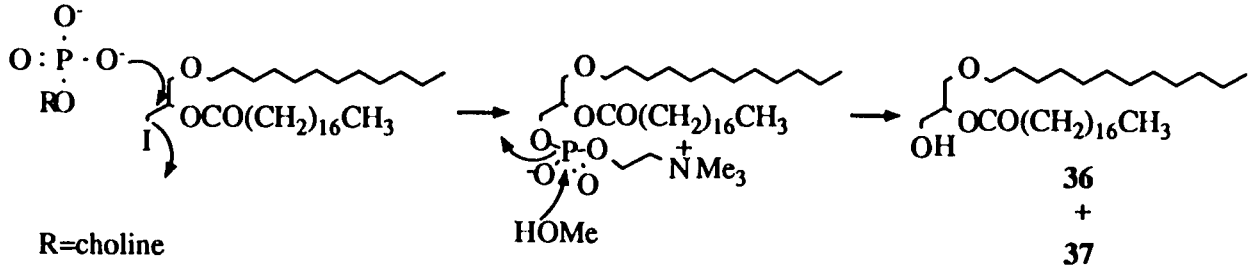
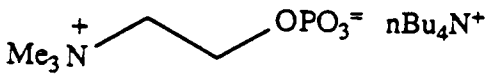


Figure 13. Methanolysis of phosphate ester.

Removal of the chloride ion proved to be a little more difficult. Lyophilization of the aqueous mixture, which provided a thick oil, was the first step. A suitable solvent needed to be found in order to separate tetrabutylammonium chloride from the free acid of phosphorylcholine. When the mixture was triturated with acetone, crystals slowly began to form. The crystals were filtered and proved to be monoprotic phosphorylcholine upon inspection by ^{1}H NMR. Redissolving the crystals in water and titrating with aqueous tetrabutylammonium hydroxide[83] to an end point (pH=7.3) and again removing the water *in vacuo* gave a 74% overall yield of the tetrabutylammonium salt of phosphorylcholine (**82**). Care was taken to ensure that the phosphate

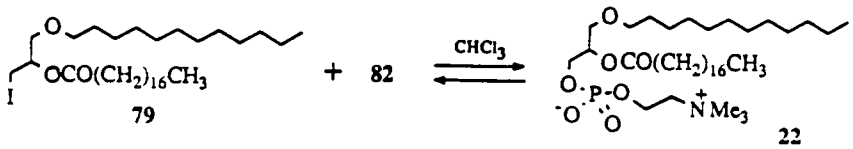


82

ion never came in contact with protic electrophilic solvents, such as acetone, because base catalyzed aldol condensation occurred, regenerating monoprotic phosphorylcholine and tetrabutylammonium salts, which mixture was useless to us.

Reacting the iodide, **79**, with one equivalent of tetrabutylammonium phosphorylcholine in chloroform did not consume any of the starting materials. However, when ten equivalents of tetrabutylammonium phosphorylcholine (**82**) were used, the iodide was consumed. It was believed that an equilibrium between the phosphatidylcholine **22** and the iodide existed (Scheme 26) and that a large excess of the phosphate salt was needed to drive the equilibrium to the right. Unfortunately, no pure product could be isolated due to the fact that all separation techniques could not separate the excess phosphorylcholine **82** from other products of similar constitution (i.e. containing a phosphorylcholine moiety). Since perhydroprecursor had already been synthesized by another method, it was therefore decided to use what was learned about perhydroprecursor to

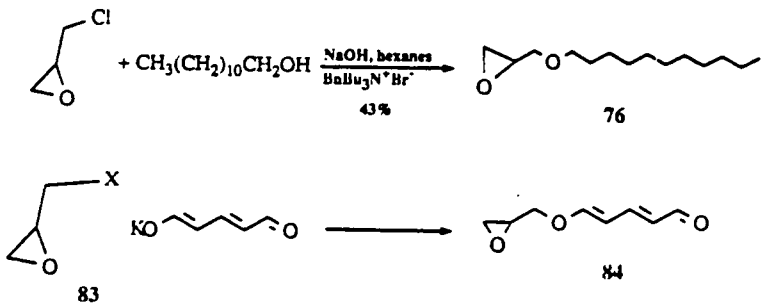
pursue the synthesis of the precursor.



Scheme 26.

5.3 Epoxide in Precursor Synthesis

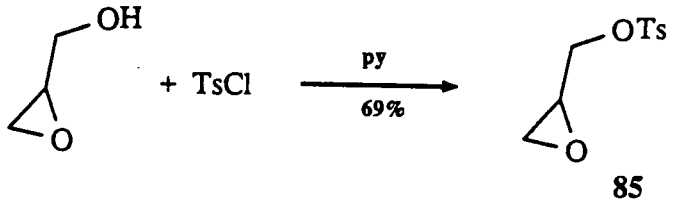
The first obstacle to overcome, in applying the previously described synthesis to the actual precursor, was to devise conditions where potassium glutaconate would perform in a similar way to sodium laurylate. This comparative analysis is best illustrated in Scheme 27. As can be seen in the bottom reaction several questions arise. We needed to determine what the X functionality in **83** should be, and what solvent, temperature, and catalyst should be used in order to achieve a clean reaction.



Scheme 27.

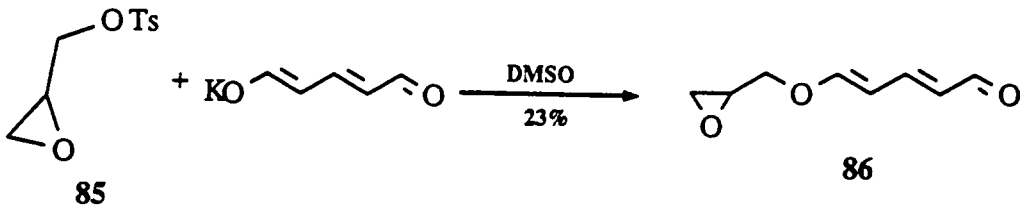
5.3.1 Synthesis of Glycidyl 5-O-Penta-2,4-dien-1-al Ether.

The tosylate group was the first X group tried and tosylglycidol (**85**) was synthesized in 69% yield as shown in Scheme 28. Reacting the tosylate, **85**, with potassium glutaconate in



Scheme 28.

DMSO provided a 23% yield of the epoxy dienyl ether **86** (Scheme 29). It was felt that this reaction went well for this type of reaction in view of the low yields reported with other reactions using glutaconate salts as the nucleophile. Several experiments were performed to determine the conditions which would give optimum yield. The results are tabulated below (Table 12), where all entries use potassium glutaconate as the nucleophile and the X group refers to a generic structure.



Scheme 29.

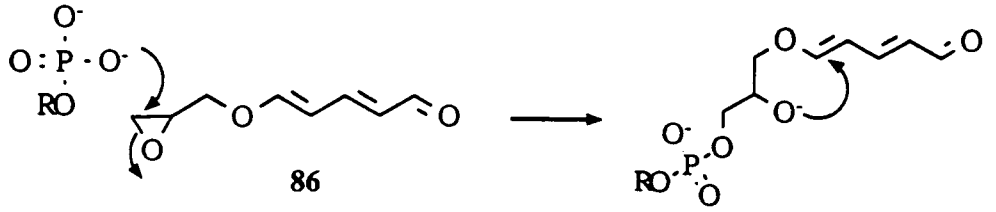
83, or in other words, a 1-X-2,3-propylene oxide analog. We found that the most polar solvent, DMSO, could be easily removed by a single aqueous wash in the workup and subsequent chromatography. Loss of the polar dienal ether product would be minimized by this purification procedure. The bromo ether, epibromohydrin, gave the best overall yield in this study.

The epoxy dienyl ether **86** was reacted with phosphorylcholine chloride, calcium salt, in methanol. The reaction produced several highly polar products, none of which contained an aldehyde or vinyl functionality as observed by ^{1}H NMR. A reasonable explanation for this

Table 12. Optimum conditions for glutoconate substitution on epibromohydrin.

X	solvent	T (°C)	catalyst	time (hrs)	% yield 84
OTs	DMSO	25	none	3	23
OTs	DME	40	18-C-6	24	19
Br	DMF	25	18-C-6	3	12
Br	DMSO	25	18-C-6	18	57

phenomenon is shown in Scheme 30 where epoxide opening by the phosphate anion would generate an alkoxide anion poised for a homo-Michael attack on the dienyl ether functionality.

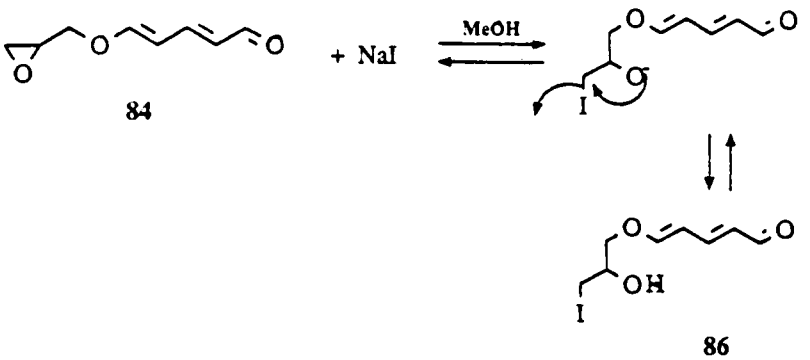


R=choline

Scheme 30.

5.3.2 Synthesis of Key Intermediate to Precursor.

In order to achieve needed derivatization of the 2 and 3 positions of the dienyl ether **84**, it was decided to open the epoxide with sodium iodide. It was observed by TLC that, treatment of the epoxy dienyl ether **84** with sodium iodide in methanol gave a clean conversion to a product assumed to be **86**. However, upon workup, which included removal of the methanol *in vacuo*, the



Scheme 31.

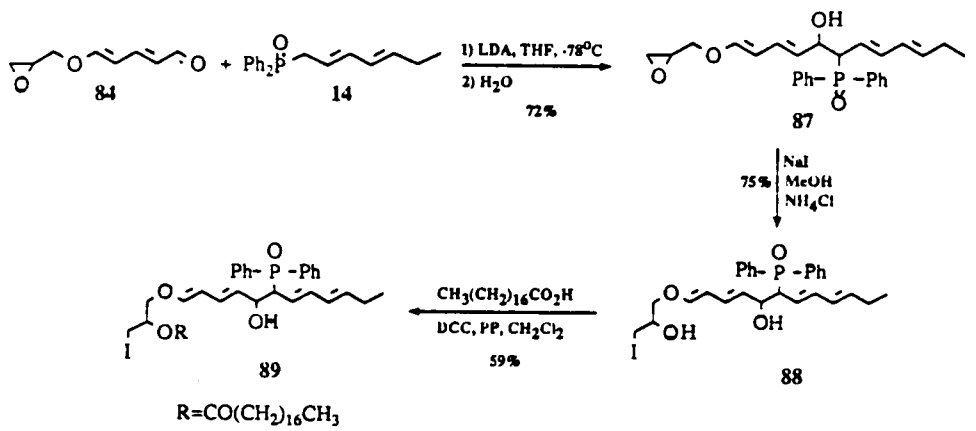
TLC of the crude mixture showed that the starting material was the only species present. The fact that the reaction was so clean and that the starting material was regenerated indicated that two things must be happening. Apparently, the iodide opening of the epoxide did not generate an

alkoxide ion reactive enough to denature the dienyl ether moiety. This is probably because the alkoxide anion formed is protonated by methanol before intramolecular homo-Michael can occur (Scheme 31). In addition, reforming the starting material in the evaporated crude mixture suggests that epoxide formation by nucleophilic attack on the carbon bearing the iodide is more favorable than homo-Michael attack on the dienyl ether. This notion suggests that the mechanism shown in Figure 12 may be correct.

It was decided that the use of mild acid was required for the formation of the iodoalcohol. Adding Amberlite IR-120 to the reaction did not change the result of the reaction; epoxide reformation again was observed after the solvent was removed. When ammonium chloride was used as the mild acid, applying the conditions used in making 1-dodecyloxy-2-hydroxy-3-iodo-propane (**78**), complete decomposition of the dienyl ether was observed.

Protection of the aldehyde therefore, became necessary. We applied a unique aldehyde protecting technique; the compound, **87**, which resembled the "Wittig Adduct", **13**, also served as an intermediate that could eventually be taken to the pentaenyl ether moiety present in the target molecule. By reacting the dienal ether **86** with the lithium salt of heptadienyldiphenyl phosphine-oxide **14** in THF and trapping the Wittig adduct at -78°C with water,[32] a 72% yield of the alcohol **87** was achieved with no evidence of epoxide opening. An attempt at protecting the allylic alcohol with thexyldimethylchlorosilane and imidazole in DMF, however, did not consume any of the alcohol. Epoxide opening with sodium iodide and ammonium chloride in methanol gave a 75% yield of the iodo alcohol (**88**). Since protection of the allylic alcohol was not successful, it was believed that selectivity of the secondary alcohol over the allylic alcohol could be achieved during the acylation step. Indeed, when a methylene chloride solution of the iodo diol (**88**) was added to a mixture of 1.5 equivalents of stearic acid, dicyclohexylcarbodiimide (DCC) and 4-pyrrolidinopyridine in methylene chloride, a 59% yield of the ester **89** was isolated with no evidence of acylation of the

allylic alcohol (Scheme 32).



Scheme 32.

5.3.3 Attempted Iodide Displacement with Phosphate Anions.

With the key intermediate **89** made, our next task was to devise a way to substitute the iodide with a phosphate ester. This is not a trivial task since we found ourselves dealing with an alpha halo ester; this is the type of compound which has a stability toward nucleophilic substitution already examined in the previous chapter.

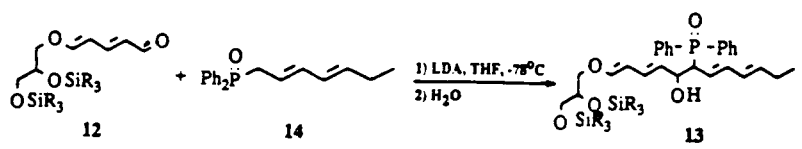
Although the iodide, **89**, would be difficult to convert to the required phosphate ester, we regarded this compound as one with realizable potential. We felt that the compound, **89**, would allow several reaction conditions to be tried. It was hoped that the type of barrier observed with the potassium glutaconate substitution of the tosylate, **45**, could be avoided here. The first attempt was to react the iodide **89** with the calcium chloride salt of phosphorylcholine in methanol. After refluxing for 24 hours, no reaction occurred. However, when silver nitrate was added to the cooled solution, complete decomposition of the intermediate **89** was observed within an hour. It was presumed that the silver salt complexed with the double bonds of **89** and did not complex with the

iodide.

Andrews[84] demonstrated that competitive silver ion complexation existed between the double bond and the iodide of cis-1,2-diiodoethylene. The silver-olefin complex was believed to be favored; the relative concentration of the two complexes could not be determined, however, the equilibrium constant of the complex was determined to be K=17.8 mol^{-1}, where K=[Ag-S^-]/[Ag^+][substrate] and Ag-S^+ refers to the silver-substrate complex. Brandt[85] calculated the K value for the silver-ethylene complex to be 94.0 mol^{-1}. These data indicate that there is competition between silver-olefin complexation and silver-iodide complexation. In our system it seems that silver has a greater affinity for the double bonds than it does for the iodide.

Perhaps the most interesting reaction was the one where the substrate **89** was stirred with ten equivalents of tetrabutylammonium phosphorylcholine in chloroform. After 24 hours, the colorless solution had developed a deep red color. This was somewhat of a surprise, manifested by the fact that upon inspection of the crude reaction by ^1H NMR, a retro-Wittig seemed to have occurred. Figure 14 shows how tetrabutylammonium phosphorylcholine must have behaved as a base rather than a nucleophile.

This is a reasonable mechanism based upon what is known about the chemistry of these Wittig adducts. In the synthesis of FP-12 reported by Nicolau, a key step is the formation of the lithium salt of the phosphine oxide **14** with LDA and reaction of this salt with the dienal ether **12** at -78°C in THF (Scheme 33). If this solution is allowed to warm to room temperature, a retro-



Scheme 33.

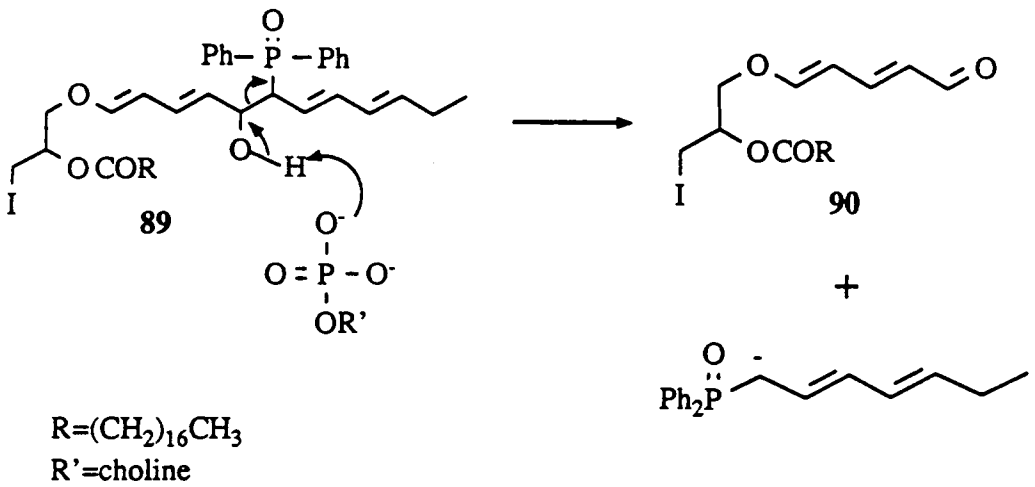
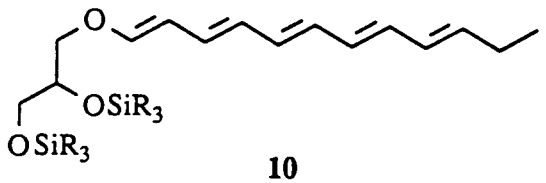


Figure 14. Retro-Wittig with phosphorylcholine as the base.

Wittig will occur, regenerating the starting materials. However, the Wittig adduct can be trapped at -78°C with water as discussed earlier. When the Wittig adduct is treated with potassium tert-butoxide in THF at -20°C, elimination occurs producing protected FP-12 (**10**). This difference in reactivity between LDA and potassium tert-butoxide is due to the strength of the lithium oxygen



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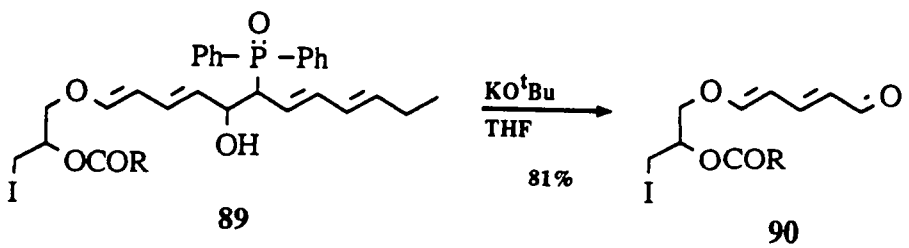
ionic bond formed in the unprotonated Wittig adduct relative to the analogous potassium oxygen bond. The former salt favors aldehyde formation and the phosphine oxide anion (i.e. retro-Wittig products). The latter salt favors a 4-membered cyclic intermediate, an oxaphospetane, required for elimination to occur.[32]

We felt that the tetrabutylammonium salt formed in Figure 14 would favor oxaphosphetane formation more readily than the potassium salt, by virtue of a weaker cation-oxygen interaction due to the larger tetrabutylammonium cation. The tetrabutylammonium salt did not eliminate and therefore, it was suspected that the potassium salt of **89** would also lead to retro-Wittig products. In fact, when the Wittig adduct **89** was treated with potassium tert-butoxide in THF, a smooth

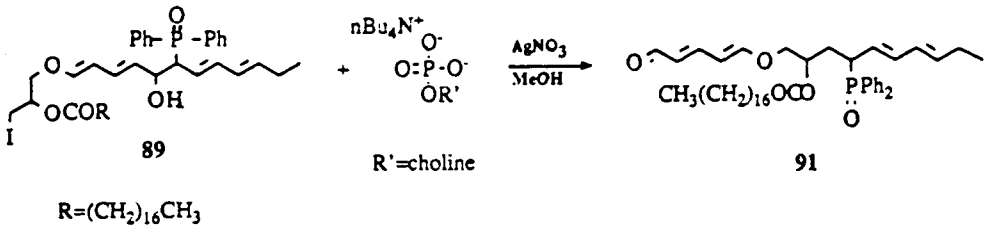


89 **90**

R=$(CH_2)_{16}CH_3$

Scheme 34.

conversion to the aldehyde **90** (81% yield) occurred (Scheme 34). When the Wittig adduct, **89**, was treated with one equivalent of tetrabutylammonium phosphorylcholine and silver nitrate in methanol, the unusual aldehyde, **91**, was isolated (Scheme 35). Apparently, a retro-Wittig occurred followed by cation exchange between the tetrabutylammonium heptadienyldiphenylphosphine oxide and silver



Scheme 35.

nitrate. Subsequent silver ion promoted iodide substitution by the phosphine oxide anion yielded **91** as shown in Figure 15. In addition tetrabutylammonium phosphorylcholine was not present in the mixture and the monoprotic phosphorylcholine was isolated.

It was already mentioned that phosphorylcholine behaves as a base and therefore, attempts were made to reduce the basicity of the dibasic phosphate anion. A review of the literature pertaining to the synthesis of phospholipids revealed a synthesis of the plasmalogen **95** using a monobasic dibenzyl phosphate. Serebrennikova[64] displaced the iodide of the enol ether **92** with silver dibenzyl phosphate (**93**). Deprotection of the phosphate ester **94** was performed by using sodium iodide (Scheme 36).

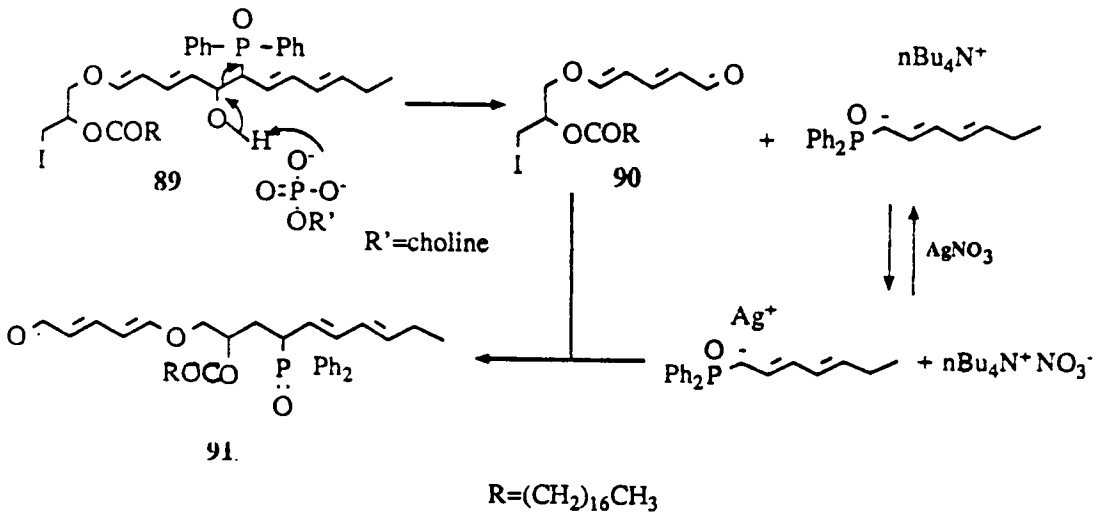
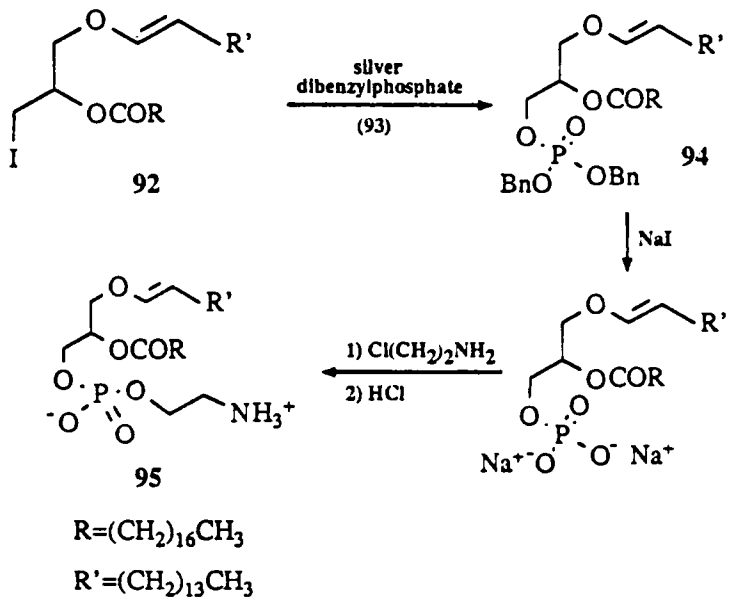


Figure 15. Rearrangement of 89.



Scheme 36.

We decided to borrow this methodology because it was believed that the monobasic dibenzyl phosphate anion would be a nucleophile rather than a base. If iodide substitution by dibenzyl phosphate was successful than deprotection and functionalization of the phosphate ester should be relatively simple.

In order to pursue all the options available, two derivatives of dibenzyl phosphate were made and used as well as dibenzyl phosphate which is available from Aldrich. Potassium dibenzyl phosphate was made by deprotonating dibenzyl phosphate with potassium tert-butoxide in THF and using the salt *in situ*. The methodology involved in the synthesis of silver salts of carboxylic acids was relied upon to generate silver dibenzyl phosphate. The silver salts of various carboxylic acids were generated by mixing the free acid with sodium bicarbonate and silver nitrate in water where the silver carboxylate precipitated out of the aqueous mixture.[86] It was discovered that potassium tert-butoxide was a more suitable base than sodium bicarbonate because contamination by silver

carbonate was eliminated. A 56% yield of silver dibenzyl phosphate (**93**) was achieved by stirring stoichiometric amounts of dibenzyl phosphate, potassium tert-butoxide and silver nitrate in water precipitating the requisite product (Scheme 37). Characterization was done by melting point,[86] ^{1}H

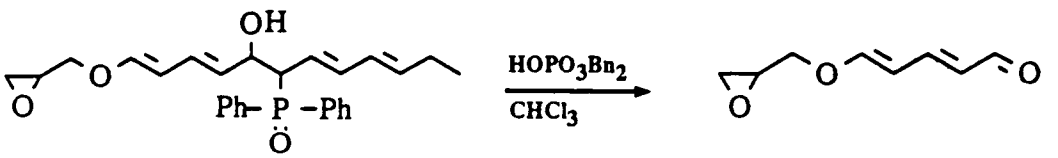
$$HOPO_3Bn_2 + KO^tBu + AgNO_3 \xrightarrow{H_2O} AgOPO_3Bn_2 \downarrow$$

93

Scheme 37.

NMR, (which did not reveal the presence of any tert-butanol contaminants), and by adding potassium iodide to a methanolic solution of the compound isolated and observing rapid formation of the methanol-insoluble silver iodide. This indicated that the silver salt of dibenzyl phosphate had been formed. A series of dibenzyl phosphate derivatives was than available for the subsequent study.

Earlier in this chapter it was mentioned that phosphate induced epoxide opening is most favorable if the phosphate nucleophile was monoprotic. Therefore, the epoxide **87** was treated with the monoprotic dibenzyl phosphate in chloroform. The crude reaction was analyzed by ^{1}H NMR and it was found that a retro-Wittig had occurred. This is the first example of an acid promoted retro-Wittig (Scheme 38).



87 **84**

Scheme 38.

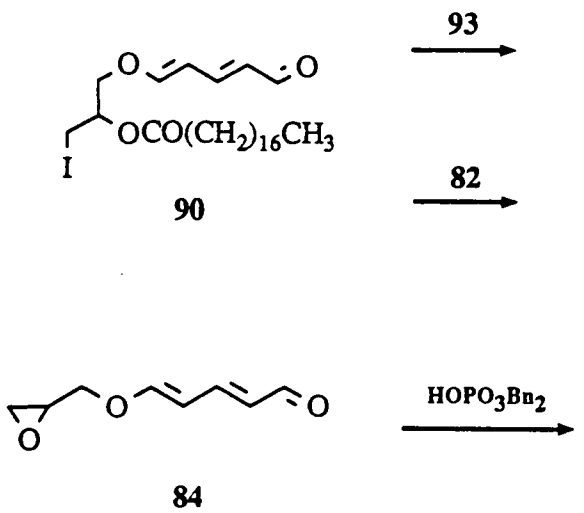


Figure 16. Approaches to synthesis of precursor.

Table 13. Reactions toward phosphate substitution on glycerol derivatives.

Substrate	Phosphate ion	solvent	results
89	**82**	$CHCl_3$	retro-Wittig (**90**)
89	**82** + $AgNO_3$	MeOH	**91**
89	$AgOPO_3Bn_2$	MeOH	decomposition
89	$KOPO_3Bn_2$	THF	decomposition
87	$HOPO_3Bn_2$	$CHCl_3$	retro-Wittig (**84**)
84	HOPO3Bn_2	$CHCl_3$	decomposition
90	**82**	$CHCl_3$	decomposition
90	$AgOPO_3Bn_2$	MeOH	decomposition

We next reacted the iodide **89** with silver dibenzyl phosphate in methanol. For the second time it was observed that the silver salt was a better olefinophile than an iodophile in this system because decomposition of the iodide **89** occurred immediately.

Finally, it was decided that removal of silver salts and reduced basicity of the phosphate nucleophile would impart the required result. Reacting the iodide **89** with potassium dibenzyl phosphate, however, required refluxing THF, which proved to be too harsh for the components of the mixture and complete decomposition was observed.

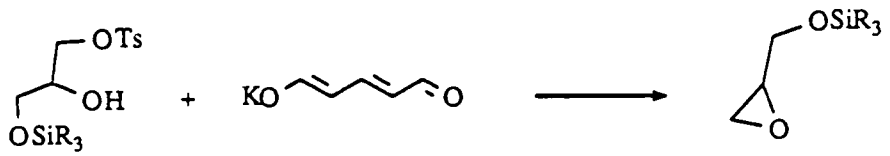
An analogous set of experiments were also run on the iodo-dienal ether **89** and the epoxy dienal ether **84** as shown in Figure 16; all of these reaction conditions caused decomposition of the respective dienyl ether. A review of the experiments involving phosphate substitution are shown in Table 13 listing the substrates, the solvents, the phosphate and results.

5.4 Conclusion

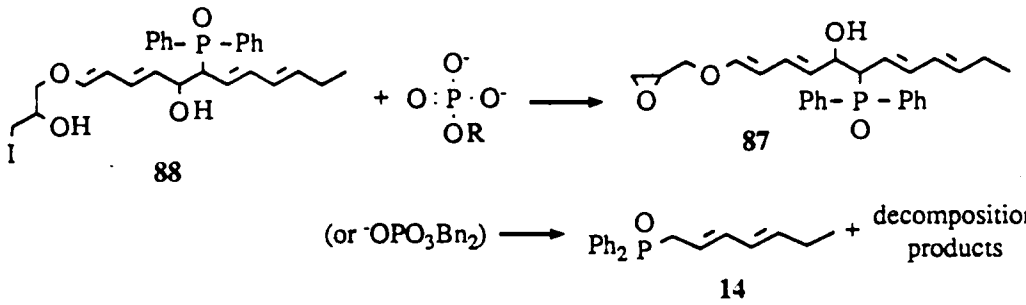
It is not known whether or not this secondary approach to precursor can be modified in order to achieve the desired result or whether a tertiary approach can be devised. The major obstacle to overcome seems to be that of displacement of a leaving group alpha to an ester, be it a glutaconate nucleophile (as in the first approach) or a phosphate nucleophile (as in the second approach). Unfortunately, the ester needs to be present during substitution. The reason for this is best understood by realizing that a free secondary hydroxyl can not be present in the key reactions involved in both approaches. Scheme 39 shows the most likely products to be formed in such systems.

Reasoning for these predictions arises from previous experience with closely related reactions. In the case for approach 1, it should be remembered that mixing potassium glutaconate and 1-tosyl-2-('butyldimethyl)silylglycerol (**46**) in hot DMSO produced the oxetane **50** as shown in

Approach 1.

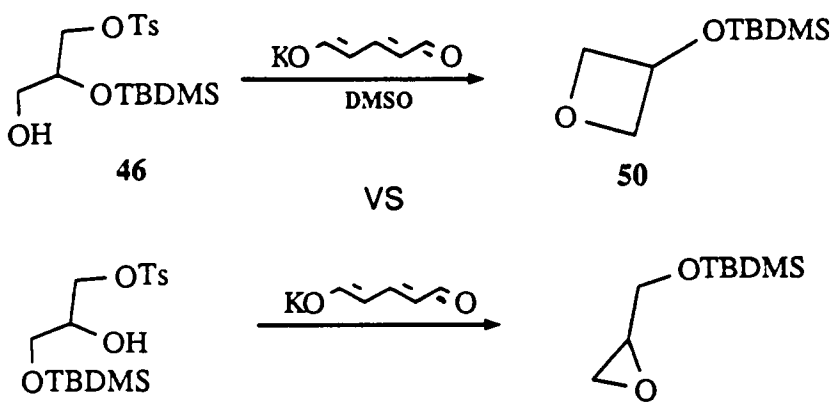


Approach 2.



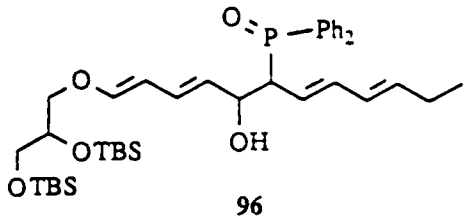
Scheme 39.

Scheme 40. Certainly one would expect that formation of a three membered ring would be of comparable probability to formation of a four membered ring.

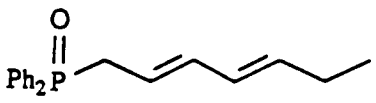


Scheme 40.

In the case of approach 2, a previously ignored reaction must now be addressed. When the Wittig adduct **96** was treated with tetrabutylammonium fluoride the only isolable product was the diphenylphosphine oxide **14**. It was presumed that the mechanism involved followed the pathway depicted in Figure 17.



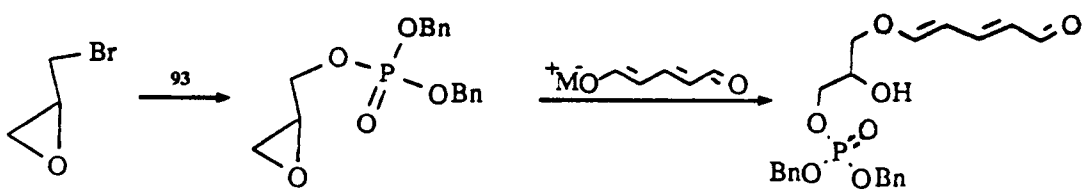
96



14

Although the precursor has yet to made, the preliminary results pertaining to the data generated thus far warrants the continued pursuit. Perhaps another metal salt of dibenzyl phosphate would displace the iodide and still maintain the integrity of the Wittig adduct part of the molecule. Such a metal may be copper.

A possible tertiary approach would be to introduce the phosphate ester and the glutaconate moiety before acylation of the secondary hydroxyl. This strategy is shown in Scheme 41. The problem that may arise from this strategy is in the second step where an intramolecular homo-Michael reaction may occur *in situ*. This reaction was shown in Scheme 21.



Scheme 41.

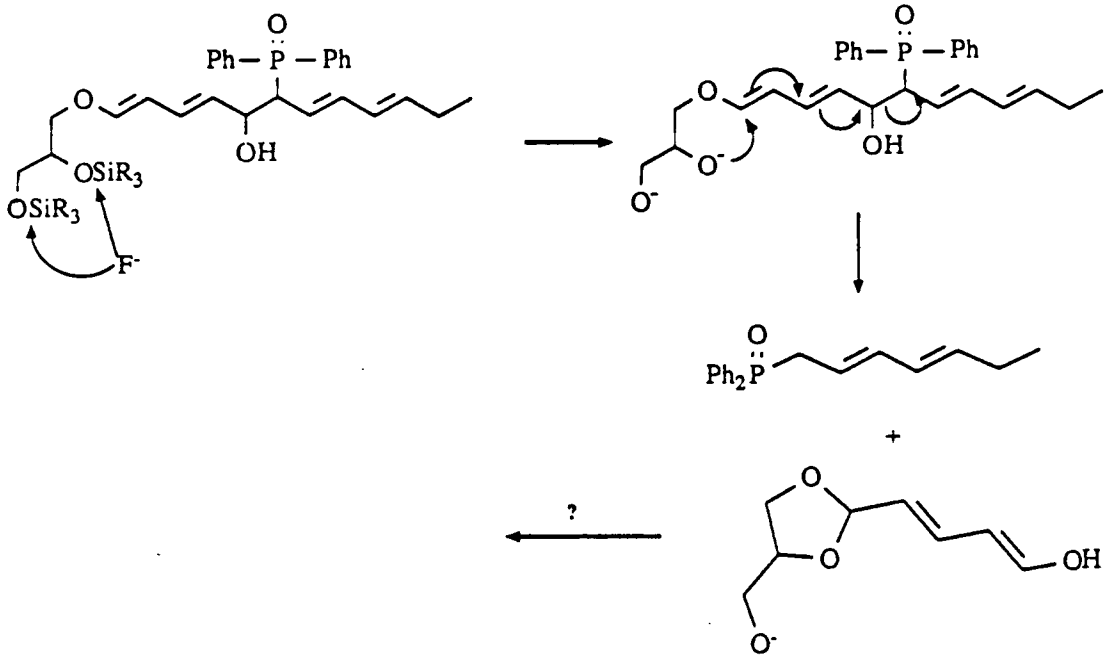


Figure 17. Fluoride induced decomposition of "Wittig Adduct"

Any additional approaches proposed would most likely meet with similar obstacles already discussed. However, the chemistry observed during the synthesis of perhydroprecursor and the attempted synthesis of precursor will prove to be invaluable for future endeavors in plasmalogen synthesis.

5.5 Experimental

Preparation of 1-O-dodecylglycidol (76).

Sodium hydroxide (1 g, 25 mmol) was dissolved in 2 mL of water and 1.9 mL (8.3 mmol) of dodecanol and 89 mg (0.25 mmol) of tributylbenzylammonium bromide was added. Epichlorohydrin (2.6 mL, 33.3 mmol) was dissolved in hexanes. The hexanes solution was added to the aqueous mixture and the biphasic mixture was heated to 50°C for 4 hours. The hexanes were washed with 3x5 mL of water and the hexanes and unreacted epichlorohydrin were removed *in vacuo* at 40°C. The crude product was loaded on a flash column packed with silica gel 60 and eluted with 1:1 ether:hexanes to yield a clear colorless oil, which appeared as a single spot on TLC. Yield 867 mg (43%). ^{1}H NMR ($CDCl_3$) δ 3.67 (dd, J=3.3, 11.1 Hz, 1H, C1), 3.49 (dd, J=7.0, 7.6 Hz, 1H, C1'), 3.42 (dd, J=2.8, 7.6 Hz, 1H, C1'), 3.35 (dd, J=5.6 ,11.1 Hz, 1H, C1), 3.12 (m, 1H, C2), 2.77 (dd, J=3.5, 3.9 Hz, 1H, C3), 2.58 (dd, J=3.1, 3.9 Hz, 1H, C3), 1.56 (m, 2H, C2'), 1.24 (m, 18H, C3'-C11'), 0.85 (t, J=6.7 Hz, 3H, C12'); ^{13}C NMR ($CDCl_3$) δ 71.7, 71.4, 50.8 (C2), 44.2 (C3), 31.9, 30.0, 29.3, 26.0, 22.6, 14.0 (C12'); IR (neat) 2940, 2860, 1475, 1110 cm^{-1}; MS (CI) *m/z* (relative intensity) 243 (M+1, 65), 185 (15), 169 (60), 127 (34), 110 (100).

Preparation of 1-dodecyloxy-2-hydroxy-3-iodopropane (78).

1-O-Dodecylglycidol (82 mg, 0.34 mmol) was dissolved in dry methanol. Sodium iodide (254 mg, 1.69 mmol) and 18 mg (.34 mmol) of ammonium chloride was added and the solution was stirred at room temperature for 22 hours. The methanol was removed *in vacuo*, ether was added and filtered. The ether was removed *in vacuo* to yield a colorless oil, which appeared as a single spot on TLC. Yield 97 mg (77%). ^{1}H NMR ($CDCl_3$) δ 3.73 (m, 1H, C2), 3.49 (d, J=6.1 Hz, 2H, C1), 3.45 (t, J=6.4 Hz, 2H, C1'), 3.31 (dd, J=7.0, 10.6 Hz, 1H, C3), 3.24 (dd, J=6.2, 10.6 Hz,

1H, C3), 2.52 (d, J=5.6 Hz, 1H, OH), 1.55 (m, 2H, C2'), 1.25 (m, 18H, C3'-C11'), 0.86 (t, J=6.2 Hz, 3H, C12'); ^{13}C NMR ($CDCl_3$) δ 73.1 (C2), 71.7 (C1), 69.9 (C1'), 31.8, 29.5, 29.4, 29.2, 26.0, 22.6, 14.0 (C12'), 9.2 (C3); IR (neat) 3440, 2940, 2840, 1475, 1115 cm^{-1}; MS (CI) *m/z* (relative intensity) 371 (M+1, 19), 353 (81), 243 (23), 169 (100).

Preparation of 1-dodecyloxy-2-stearoyl-3-iodopropane (79).

1-Dodecyloxy-2-hydroxy-3-iodopropane (78) (158 mg, 0.43 mmol) was dissolved in dry methylene chloride. In a separate flask 269 mg (0.95 mmol) of stearic acid, 97 mg (0.47 mmol) of dicyclohexylcarbodiimide (DCC) and 5 mg (0.34 mmol) of 4-pyrrolidinopyridine (PP) were stirred in methylene chloride for 30 minutes. The first solution was added to the stearic acid, DCC and PP solution and the mixture was stirred at room temperature for 1 hour. The solution was filtered, the methylene chloride was removed *in vacuo* and the crude oil was loaded on a flash column packed with silica gel 60 and eluted with 20% ether in hexanes to yield a colorless oil. Yield 203 mg (75%). ^{1}H NMR ($CDCl_3$) δ 4.84 (m, 1H, C2), 3.59 (dd, J=5.0, 10.6 Hz, 1H, C1), 3.49 (dd, J=5.4, 10.6 Hz, 1H, C1), 3.43 (m, 2H, C1'), 3.40 (dd, J=4.5, 12.1 Hz, 1H, C3), 3.29 (dd, J=6.0 Hz, 1H, C3), 2.32 (t, J=8.0 Hz, 2H, C2"), 1.61 (m, 2H, C2'), 1.52 (m, 2H, C3"), 1.27 (m, 46H, C3'-C11', C4"-C17"), 0.86 (t, J=6.5 Hz, 6H, C12'& C18"); ^{13}C NMR ($CDCl_3$) δ 172.9 (C1"), 71.8 (C2), 71.2 (C1), 70.9 (C1'), 34.4 (C2"), 31.9, 29.6, 29.3, 26.1, 25.0, 22.6, 14.0 (C12'& C18"), 4.3 (C3); IR (neat) 2950, 2860, 1750, 1470, 1170, 1030 cm^{-1}; MS (CI) *m/z* (relative intensity) 637 (M+1, 20), 512 (100), 353 (27), 325 (83), 285 (40); Elemental anal. Calcd for $C_{33}H_{65}IO_3$: C, 62.24; H, 10.29. Found: C, 62.72; H, 10.22.

Preparation of tetrabutylammonium phosphorylcholine (82).

Oxalic acid (393 mg, 4.37 mmol) was added to 3 mL of 40% by weight aqueous

tetrabutylammonium hydroxide (4.59 mmol). Phosphorylcholine chloride, calcium salt (1.13 g, 4.37 mmol) was added and the mixture was filtered. The solution was freeze dried and the oil was triturated with dry acetone until crystals formed. The crystals were suction filtered, dissolved in water and titrated with 40% by weight tetrabutylammonium hydroxide to a pH of 7.3 (ca. 1.5 mL). The solution was freeze dried to yield a colorless oil. Yield 1.37 g (74%). ^{1}H NMR ($CDCl_3$) δ 4.19 (m, 2H, OCH_2), 3.67 (m, 2H, NCH_2), 3.33 (m, 8H, NCH_2Pr), 3.27 (s, 9H, NCH_3), 1.65 (m, 8H, CH_2Et), 1.42 (m, 8H, CH_2Me), 0.98 (t, 12H, CH_3).

Preparation of 1-tosylglycidol (85).

Glycidol (1 mL, 15.8 mmol) was dissolved in dry pyridine and cooled to -5°C. p-Toluenesulfonic chloride (3.32 g, 17.4 mmol) was added and the solution was stirred for 2 hours at which time the solution was added to 100 mL of ether and the ether was washed with 3x25 mL of 3N aqueous HCl, 25 mL of saturated aqueous sodium bicarbonate, dried over sodium sulfate, filtered and the ether removed *in vacuo*. The crude oil was loaded on a flash column packed with silica gel 60 and eluted with 3:2 ether:hexanes to yield a colorless oil which appeared as a single spot on TLC. Yield 2.37 g (69%). ^{1}H NMR ($CDCl_3$) δ 7.77 (d, J=8.3 Hz, 2H, Ph), 7.31 (d, J=8.3 Hz, 2H, Ph), 4.24 (dd, J=3.6, 11.6 Hz, 1H, C1), 3.98 (dd, J=6.1, 11.6 Hz, 1H, C1), 3.14 (m, 1H, C2), 2.77 (dd, J=3.9, 4.9 Hz, 1H, C3), 2.54 (dd, J=2.5, 4.9 Hz, 1H, C3), 2.40 (s, 3H, CH_3).

Preparation of 5-[(2,3-epoxy)-propyl-1-oxy]-penta-5,3-dien-1-al (84).

Potassium glutaconate (100 mg, 0.74 mmol) was dissolved in dry DMSO and 0.25 mL (2.94 mmol) of epibromohydrin, and 6 mg (0.02 mmol) of 18-C-6 was added. The solution was stirred at room temperature for 18 hours. The solution was poured into 25 mL of brine and the mixture was extracted with 3x100 mL of ether. The ether extracts were dried over sodium sulfate,

filtered and the ether was removed *in vacuo*. The crude oil was loaded on a flash column packed with triethylamine pretreated silica gel 60 and eluted with ether to yield a red oil, which appeared as a single spot on TLC. Yield 65 mg (57%). ^{1}H NMR ($CDCl_3$) δ 9.34 (d, J=7.8 Hz, 1H, C5'), 7.10 (d, J=11.1 Hz, 1H, C1'), 6.92 (dd, J=9.7, 16.3 Hz, 1H, C3'), 5.96 (dd, J=16.3, 7.8 Hz, 1H, C4'), 5.77 (dd, J=9.7, 11.1 Hz, 1H, C2'), 4.16 (dd, J=2.9, 11.9 Hz, 1H, C1), 3.72 (dd, J=5.5, 11.9 Hz, 1H, C1), 3.18 (m, 1H, C2), 2.79 (dd, J=4.1, 5.5 Hz, 1H, C3), 2.62 (dd, J=2.7, 5.5 Hz, 1H, C3); ^{13}C NMR ($CDCl_3$) δ 192.8 (C5'), 158.6 (C1'), 150.7 (C3'), 127.7 (C4'), 106.2 (C2'), 71.5 (C1), 49.4 (C2), 43.7 (C3); IR (neat) 3110, 3050, 2975, 2860, 2750, 2400, 1675, 1625, 1280, 1240, 1170, 1125, 1000 cm^{-1}; MS (EI) *m/z* (relative intensity) 154 (M^+, 20), 97 (74), 81 (58), 57 (100); UV (EtOH) 296 nm (21,741).

Preparation of 1-[(2,3-epoxy)-propyl-1-oxy]-5-hydroxy-6-(diphenyloxophosphinyl)-dodeca-1,3,7,9-tetraene (87).

Preparation of 1.48 mL of 0.48 M LDA (0.71 mmol) was done by dissolving 1 mL diisopropylamine in 1 mL of dry THF in a flame dried flask under argon, cooled to -20°C, 0.38 mL of 1.86 mL nBuLi in hexanes was added and the solution was stirred at -20°C for 1 hour under argon. In a separate flame dried flask under argon, 98 mg (0.33 mmol) of 1-hepta-2,4-dienyl-diphenylphosphine oxide was dissolved in dry THF and cooled to -78°C. LDA was added and the solution was stirred for 2 minutes, where 51.3 mg (0.33 mmol) of 5-[(2,3-epoxy)-propyl-1-oxy]-penta-2,4-dien-1-al in dry THF was added and stirred at -78°C for 45 minutes. Brine (5 mL) was added and the solution was added to 25 mL of water. The aqueous layer was extracted with 4x50 mL of ether, the ether extracts were combined, washed with 20 mL of brine, dried over potassium carbonate, filtered and the ether was removed *in vacuo*. The crude oil was loaded on a flash column packed with triethylamine pretreated silica gel 60 and eluted with 4% methanol in ether to

yield a light yellow oil, which appeared as a single spot on TLC. Yield 107 mg (72%). ^{1}H NMR ($CDCl_3$) δ 7.81 (m, 2H, Ph), 7.68 (m, 2H, Ph), 7.49 (m, 6H, Ph), 6.48 (d, J=11.4 Hz, 1H, C1'), 6.00 (dd, J=11.0, 14.1 Hz, 1H, C7'), 5.89 (m, 1H, C8'), 5.87 (dd, J=14.5, 15.2 Hz, 1H, C3'), 5.74 (m, 1H, C4'), 5.52 (m, 1H, C9'), 5.42 (m, 2H, C2'& C10'), 5.19 (m, 0.5H), 4.99 (m, 0.5H), 4.51 (m, 1H, C5'), 3.95 (m, 1H, C1), 3.61 (m, 1H, C1), 3.29 (m, 0.5H, C6'), 3.18 (m, 1H, C2), 3.07 (m, 0.5H, C6'), 2.81 (dd, J=2.8, 4.1 Hz, 1H, C3), 2.63 (dd, J=2.1, 4.1 Hz, 1H, C3), 2.02 (dd, J=6.9, 15.9 Hz, 1H, C11'), 0.96 (m, 3H, C12,); ^{13}C NMR ($CDCl_3$) δ 150.3 (C1'), 137.1, 132.5, 132.1, 131.9, 131.6, 131.2, 131.1, 128.8, 128.6, 128.4, 128.3, 128.1, 127.6, 122.5, 122.2, 106.9 (C2'), 70.2 (C1), 49.9 (C6'), 49.5 (C2), 44.3 (C3), 25.4 (C11'), 13.2 (C12'), 13.1 (C12'); IR (neat) 3320, 2960, 2930, 2900, 1660, 1620, 1175, 1105, 1030, 1005 cm^{-1}; MS (CI) *m/z* (relative intensity) 451 (M+1, 6), 378 (16), 313 (15), 297 (100), 203 (83), 155 (60); UV (EtOH) 230 nm (37,058).

Preparation of 1-[(3-iodo-2-hydroxy)-propyl-1-oxy]-5-hydroxy-6-(diphenyloxophosphinyl)-dodeca-1,3,7,9-tetraene (88).

A solution of 630 mg (1.4 mmol) of 1-[(2,3-epoxy)-propyl-1-oxy]-5-hydroxy-6-(diphenyloxophosphinyl)-dodeca-1,3,7,9-tetraene in dry methanol was prepared. Sodium iodide (1.05 g, 7.0 mmol) and 74.9 mg (1.4 mmol) of ammonium chloride was added and the solution was stirred at room temperature for 24 hours. The methanol was removed *in vacuo* and the crude oil was loaded on a flash column packed with triethylamine pretreated silica gel 60 and eluted with 2% methanol in methylene chloride to yield a light yellow oil. Yield 607 mg (75%). ^{1}H NMR ($CDCl_3$) δ 7.80 (m, 2H, Ph), 7.66 (m, 2H, Ph), 7.47 (m, 6H, Ph), 6.46 (d, J=12.5 Hz, 1H, C1'), 5.98 (dd, J=10.4, 15.7 Hz, 1H, C7'), 5.85 (m, 2H, C8'& C3'), 5.70 (m, 1H, C4'), 5.52 (m, 1H, C10'), 5.44 (dd, J=9.5, 3.7 Hz, 1H, C9'), 5.39 (dd, J=5.5, 12.5 Hz, 1H, C2'), 5.13 (m, 0.5H), 4.98 (m, 0.5H), 4.51 (m, 1H, C5'), 3.78 (m, 2H, C2), 3.31 (m, 2H, C3 & C6'), 3.23 (dd, J=3.4, 9.5 Hz, 1H, C3), 2.00 (m,

2H, C11'), 0.94 (m, 3H, C12'); ^{13}C NMR ($CDCl_3$) δ 150.4 (C1'), 150.2 (C1'), 137.3, 137.1, 137.0, 136.4, 132.3, 132.0, 131.9, 131.6, 131.5, 131.2, 131.0, 130.7, 129.7, 129.0, 128.8, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.6, 122.1, 121.8, 106.7 (C2'), 106.6 (C2'), 72.1 (C1), 72.0 (C1), 69.1 (C2), 52.6, 49.3 (C6'), 46.4, 46.3, 46.2, 25.3 (C11'), 13.2 (C12'), 13.1(C12'), 9.5 (C3), 9.0 (C3); IR (neat) 3340, 2980, 1670, 1620, 1440, 1175, 1125, 1030, 1011 cm^{-1}; MS (CI) *m/z* (relative intensity) 560 (M-18, 17), 435 (79), 376 (12), 279 (100), 203 (32); UV (EtOH) 227 nm (49,130).

Preparation of 1-[(3-iodo-2-octadecanoyl)-propyl-1-oxy]-5-hydroxy-6-(diphenyloxophosphinyl)-dodeca-1,3,7,9-tetraene (89).

Stearic acid (296 mg, 1.04 mmol) was dissolved in dry methylene chloride and 214 mg (1.04 mmol) of dicyclohexylcarbodiimide was added and the solution was stirred at room temperature for 30 minutes. 4-Pyrrolidinopyridine (6.4 mg, 0.043 mmol) was added followed by 501 mg (.867 mmol) of 1-[(3-iodo-2-hydroxy)-propyl-1-oxy]-5-hydroxy-6-(diphenyloxophosphinyl)-dodeca-1,3,7,9-tetraene. The solution was stirred at room temperature for 15 hours. A few drops of methanol was added and the solution was stirred for an additional hour. The solvents were removed *in vacuo* and the crude oil was loaded on a flash column packed with triethylamine pretreated silica gel 60 and eluted with 4% methanol in methylene chloride to yield a light yellow oil, which appeared as a single spot on TLC. Yield 432 mg (59%). ^1H NMR ($CDCl_3$) δ 7.79 (m, 2H, Ph), 7.65 (m, 2H, Ph), 7.48 (m, 6H, Ph), 6.44 (d, J=12.9 Hz, 1H, C1'), 6.03 (dd, J=10.7, 15.6 Hz, 1H, C7'), 5.87 (m, 2H, C8'& C3'), 5.72 (m, 1H, C4'), 5.53 (m, 1H, C9'), 5.46 (dd, J=5.5, 12.9 Hz, 1H, C2'), 5.40 (m, 1H, C10'), 5.20 (d, J=2.8 Hz, 0.5H), 5.01 (m, 0.5H), 4.90 (m, 1H, C2), 4.52 (m, 1H, C5'), 4.11 (m, 1H, C1), 3.85 (m, 1H, C1), 3.46 (m, 1H, C6'), 3.37 (m, 1H, C3), 3.27 (dd, J=4.3, 9.5 Hz, 1H, C3), 2.31 (t, J=5.8 Hz, 2H, C2"), 2.02 (m, 2H, C11'), 1.91 (m, 2H, C3"), 1.64 (m, 6H, C4"-C6"), 1.24 (m, 22H, C7"-C17"), 1.08 (m, 1.5H, C12'), 0.95 (m, 1.5H, C12'), 0.87 (t, J=6.4 Hz,

3H, C18"); ^{13}C NMR ($CDCl_3$) δ 172.5 (C1"), 149.9 (C1'), 149.7 (C1'), 137.2, 137.0, 136.6, 136.4, 132.1, 132.0, 131.8, 131.6, 131.3, 131.2, 131.1, 131.0, 130.8, 129.5, 129.0, 128.7, 128.5, 128.3, 128.1, 127.3, 127.1, 122.1, 122.0, 120.3, 120.2, 106.9 (C2'), 71.9, 71.7, 70.3, 69.6, 51.5, 50.6, 50.4, 49.4 (C6'), 48.6, 46.0, 34.1, 29.5, 29.3, 29.2, 29.0, 28.9, 25.6, 25.4, 24.7, 22.5, 13.9(C12'), 13.2 (C12'), 2.8 (C3); IR (neat) 3340, 2940, 2850, 1740, 1665, 1630, 1465, 1430, 1170, 1020, 1005 cm^{-1}; MS (CI) 469 (M-376, 22), 451 (40), 377 (100), 341 (52), 285 (100), 267 (53), 203 (50); UV (EtOH) 226 nm (42,200).

Preparation of 1-[(3-iodo-2-octadecanoyl)-propyl-1-oxy]-penta-1,3-dien-1-al (90).

In a flame dried flask under argon, 5.7 mg (0.051 mmol) of potassium tbutoxide was dissolved in dry THF and cooled to -20°C. After 10 minutes, 36 mg (0.043 mmol) of 1-[(3-iodo-2-octadecanoyl)-propyl-1-oxy]-5-hydroxy-6-(diphenyloxophosphinyl)-dodeca-1,3,7,9-tetraene (90) was added and the solution was stirred at -20°C for 1 hour. The solution was warmed to room temperature, 50 mL of ether was added, the ether was washed with 3x50 mL of water, 20 mL of brine, dried over magnesium sulfate, filtered, and the ether was removed *in vacuo*. The crude solid was loaded on a flash column packed with triethylamine pretreated silica gel 60 and eluted with ether to yield a yellow solid, which appeared as a single spot on TLC. Yield 19 mg (81%). ^1H NMR ($CDCl_3$) δ 9.44 (d, J=8.0 Hz, 1H, C5'), 7.02 (dd, J=11.3, 14.7 Hz, 1H, C1'), 6.96 (d, J=12.1 Hz, 1H, C3'), 6.05 (dd, J=14.7, 8.0 Hz, 1H, C4'), 5.82 (dd, J=11.3, 12.1 Hz, 1H, C2'), 4.99 (m, 1H, C2), 4.10 (dd, J=4.0, 11.5 Hz, 1H, C1), 4.03 (dd, J=3.6, 11.5 Hz, 1H, C1), 3.39 (dd, J=4.7, 11.4 Hz, 1H, C3), 3.30 (dd, J=5.9, 11.4, 1H, C3), 2.33 (t, J=7.9 Hz, 2H, C2"), 1.62 (m, 4H, C3"-C4"), 1.28 (m, 26H, C5"-C17"), 0.86 (t, J=6.7 Hz, 3H, C18").

Preparation of silver dibenzyl phosphate (93).

Dibenzyl phosphate (278 mg, 1 mmol) was added to water followed by 110 mg (1 mmol) of potassium ^{t}butoxide and the mixture was stirred at room temperature until the solution became homogenous. Silver nitrate (170 mg, 1 mmol) was added where a white precipitant immediately formed. The crystals were suction filtered to yield pure silver dibenzyl phosphate. Yield 216 mg (56%). mp 223-225°C (Lit. mp 229-231°C[87]); ^{1}H NMR ($CDCl_3$) δ 7.29 (m, 10H, Ph), 4.91 (s, 2H, CH_2Ph), 4.82 (s, 2H, CH_2Ph).

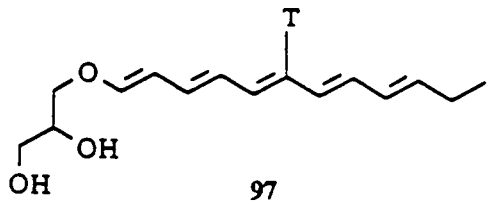
VI DNA Interaction

6.1 Introduction

The mode of action a carcinogen has on DNA may be covalent binding, intercalation, chromosomal protein binding or altering the DNA precursor pool. Where the last two examples are difficult to demonstrate directly through *in vitro* experimentation, the first two examples might be observed by various experimental techniques.

In order to determine the extent to which FP-12 interacts with DNA, whether covalent binding, intercalation, or hydrogen binding, direct incubation of FP-12 with DNA must be performed. A convenient way to detect any interaction between FP-12 and DNA during incubation would be to monitor the mixture using UV spectroscopy. FP-12 shows a characteristic UV spectrum and therefore, any covalent binding or intercalation should change this spectrum. Kingston, Duh and Kassaee[88] mixed synthetic FP-12 with calf thymus DNA in ethanol and monitored the mixture with UV light. The only change in the UV spectrum of FP-12 observed was a slight hypsochromic shift; the characteristic "triplet" remained unaltered.

Although the above result tends to indicate that no covalent binding or intercalation occurred, it must be remembered that any change in the FP-12 UV spectrum resulting from such interaction will be related to the extent of binding. In other words, if FP-12 binds to DNA in low concentrations relative to the concentration of FP-12 used in the incubation study, such a binding would not be detected by UV spectroscopy.



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In order to test this hypothesis, a more sensitive study needed to be performed. One way to do this would be to detect adduct formation between radioactive FP-12 and DNA by measuring the level of radioactivity of DNA fragments. The synthesis of 6-[^{3}H]-FP-12 (97) was performed by Kingston and Kassaee.[89]

Kingston and Duh[88] incubated tritiated FP-12 with calf thymus DNA at pH's of 7, 8 and 9 in methanol, acetone, and THF under aerobic and anaerobic conditions; a total of 18 experiments was performed. Each experiment was conducted as outlined below:

1. DNA and 6-[^{3}H]-FP-12 were combined in a buffer solution.
2. Modified DNA was precipitated to a constant activity of supernatant.
3. The DNA was degraded with magnesium chloride, DNAase, snake venom phosphodiesterase I and alkaline phosphatase.
4. The modified nucleosides were separated on a Sephadex LH-20 column.
5. The fractions were checked for radioactivity and fractions which contained radioactivity were pooled.
6. The fractions were analyzed on a C-8 reversed phase HPLC column.
7. The fractions containing modified nucleosides were counted for radioactivity.
8. The number of FP-12 molecules bound per number of nucleoside bases was calculated according to the formula:

$$\frac{mci_{total} \times mmols_{FP\text{-}12} \times 10^{20}}{\#\ bases\ added \times mci_{FP\text{-}12}}$$

The results from these experiments showed low level binding with numbers ranging from the low of 6/10^6 (methanol, pH=9, anaerobic) to a high of 20/10^6 (THF, pH=7, aerobic) with an average of 10/10^6. A value indicative of significant binding would be approximately 50/10^6. Table 14 shows the values under various conditions.

Table 14. Results from the incubation of 6-[^{3}H]-FP-12 with calf thymus DNA. (Values represent # FP-12 molecules/10^6 nucleoside bases.)

pH (conditions)	Solvent Methanol	THF	Acetone
pH 7			
(anaerobic)	9	10	12
(aerobic)	9	20	8
pH 8			
(anaerobic)	9	8	9
(aerobic)	8	7	16
pH 9			
(anaerobic)	6	7	13
(aerobic)	9	9	8

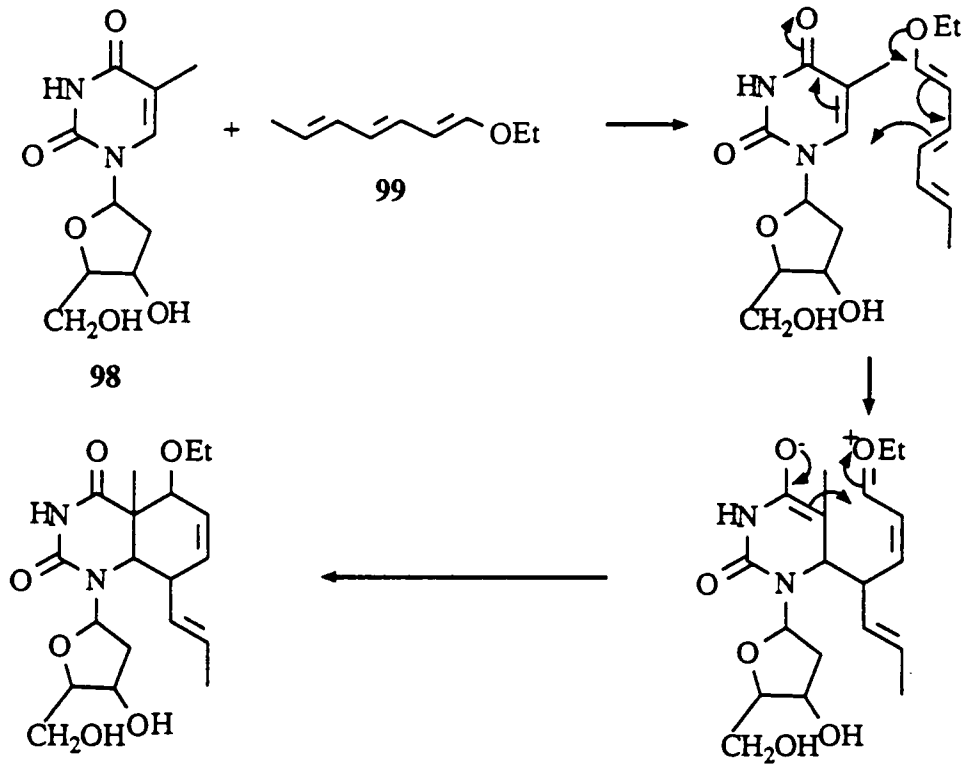


Figure 18. Cycloaddition of thymidine with trienol ether.

6.2 Nucleoside Adduct Study.

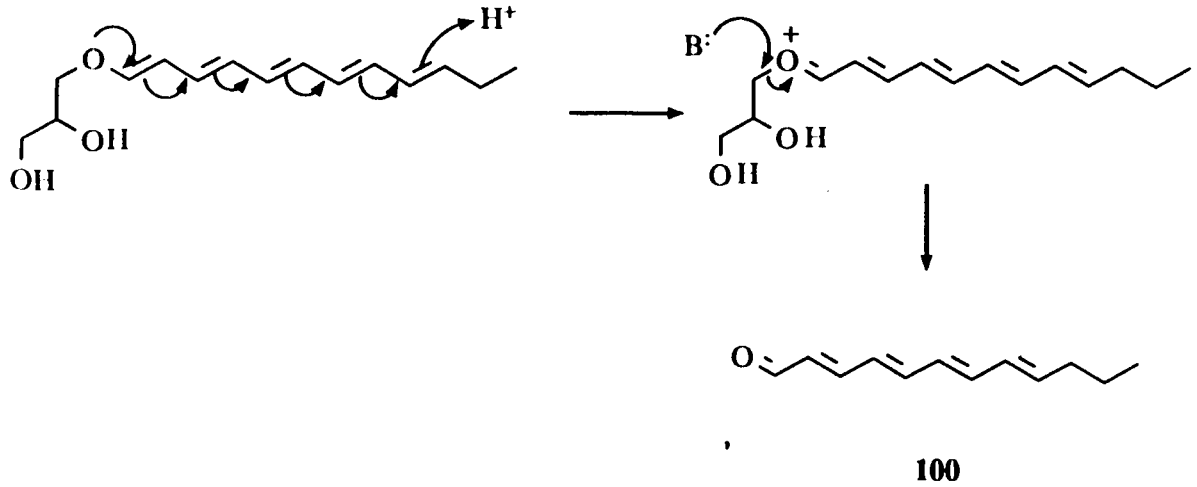
The above tabulated results tend to indicate that no significant covalent bonding has occurred between FP-12 and DNA. To help confirm this reasoning, synthetic FP-12 was reacted with guanosine in DMSO where no reaction occurred after 2 days. Guanosine was the nucleoside chosen because the mechanism of action was believed to be nucleophilic attack by the nucleoside base on FP-12 and guanosine is the most nucleophilic of the nucleoside bases by virtue of its primary amine function.

Another mechanism was proposed in which a cycloaddition could occur as shown in Figure 18. To test the possibility of this mechanism, thymidine, **98**, was reacted with heptatrienyl ethyl ether, **99**. It was discovered that thymidine did not react with the trienol ether, **99**, even in refluxing methanol, thus suggesting that the mechanism of Figure 18 is unlikely to occur between DNA and FP-12.

6.3 FP-12 as a Precursor.

Because of these negative results with intact FP-12, we decided to re-evaluate possible mechanisms of genotoxicity. Since FP-12 showed low level binding with DNA but did not react directly with guanosine, it was felt that FP-12 might undergo a reaction *in vivo*, converting it to a species which then could bind to DNA. Two general processes were considered that would convert FP-12 into a compound that would show DNA binding ability.

Treatment of FP-12 with acid leads to decomposition into a complex mixture of presumably monomeric and polymeric products. However, if a controlled hydrolysis of the enol ether of FP-12 were to occur in a biological system, a tetraenal (**100**) would result (Figure 19). Likewise, oxidation of FP-12 by cyctochrome oxidase or other systems could yield a reactive epoxy ether (Figure 20). The oxidative process will be further discussed in the next chapter.



100

Figure 19. Hydrolysis of FP-12.

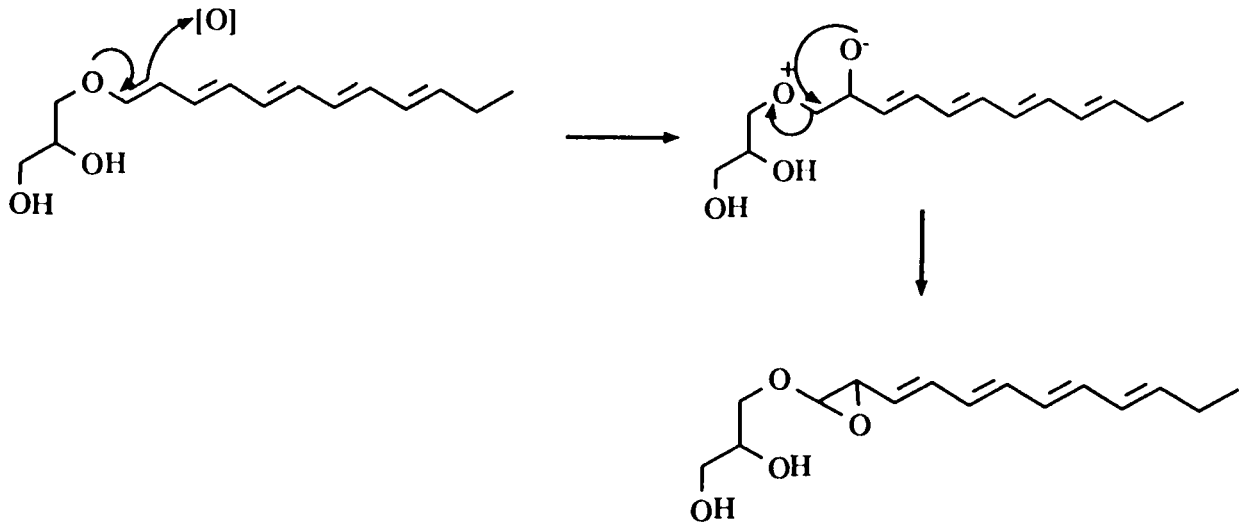


Figure 20. Oxidation of FP-12.

If indeed FP-12 undergoes some sort of biochemical transformation *in vivo*, then FP-12 can be considered as a biological precursor to an ultimate mutagen or carcinogen. Further studies are clearly needed to prove or disprove this hypothesis.

Mutagenicity studies were performed on undecatetraenal, **101**, (a homolog of dodecatetraenal **100**) by Kingston, Van der Gen and Wilkins.[41] The results shown in Table 15

101

indicated that undecatetraenal is far less mutagenic than FP-12. This result does not however exclude the possibility that the mutagenic effect of FP-12 is mediated through a dodecatetraenal, since FP-12 may be functioning as a protected tetraenal where its lipid character allows it to pass through the cell membrane, and it is then hydrolyzed in close proximity to the cell nucleus and immediately reacts with DNA there.

6.3.1 Reaction of Guanosine with Enals.

Support for the above stated theory was supplied by published data. Many workers have reported spectroscopic observations of reactions of acrolein with nucleoside base derivatives, but only three groups have actually isolated and identified any adducts formed. Shapiro[90] demonstrated possible genotoxic factors of acrolein by reacting acrolein with cytosine and adenine derivatives to form the adducts **102** and **103**. Galliani[91] reacted acrolein with deoxyguanosine in DMSO to form **104**. Chung[92] performed a similar reaction by mixing crotonaldehyde with deoxyguanosine in aqueous phosphate pH 7 buffer solution at 90°C to form **106**. The last two reactions were repeated

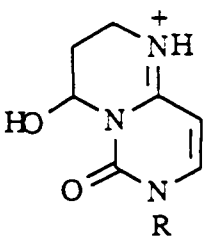
Table 15. Mutagenacity of Natural Fecapentaene-12 (**4**) and undecatetraenal (**101**).

Concentration (ng/plate)	Mutation Ratio[a]			
	TA 98		TA 100	
	4	**101**	**4**	**101**
250,000	NT[b]	< 1.2	NT	1.8
100,000	NT	< 1.2	NT	1.3
50,000	NT	< 1.2	NT	< 1.2
25,000	NT	< 1.2	NT	< 1.2
10,000	TOX[c]	< 1.2	TOX	< 1.2
5,000	TOX	NT	TOX	< 1.2
2,500	10.9	NT	TOX	NT
1,250	8.6	NT	9.2	NT
625	4.8	NT	5.8	NT
313	2.6	NT	3.6	NT
156	1.8	NT	2.7	NT
78	1.3	NT	1.8	NT

[a] Mutation Ratio = number of revertant colonies on treated plates/ number of spontaneous revertants on control plates. Salmonella typhimurium tester strains spontaneous reversions: TA 98=19, TA 100=172.

[b] NT = not tested

[c] TOX = Full or partial toxicity to background lawn observed.



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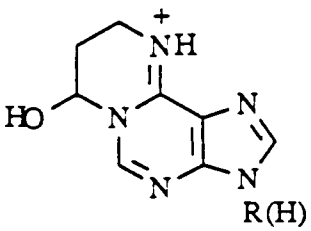
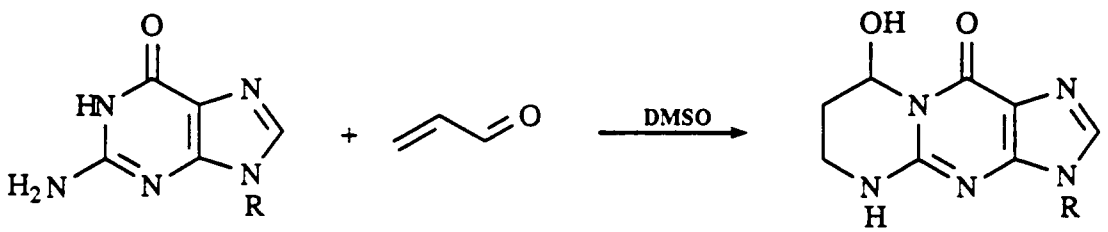


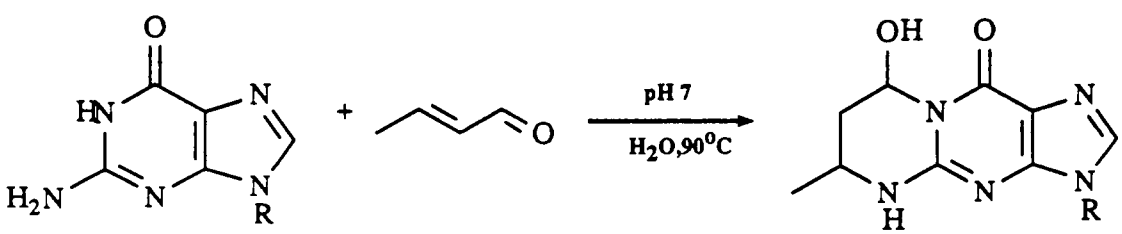
103

R= CH_3, ribosyl, or deoxyribosyl

in our laboratory, except that we used guanosine to make **105** and **107** (Scheme 42). These results indicated that the genotoxicity of acrolein and crotonaldehyde may arise from their ability to react with the deoxyguanosine unit of DNA, thus disrupting hydrogen bonding and causing other effects.



R=deoxyribosyl, **104**
R=ribosyl, **105**



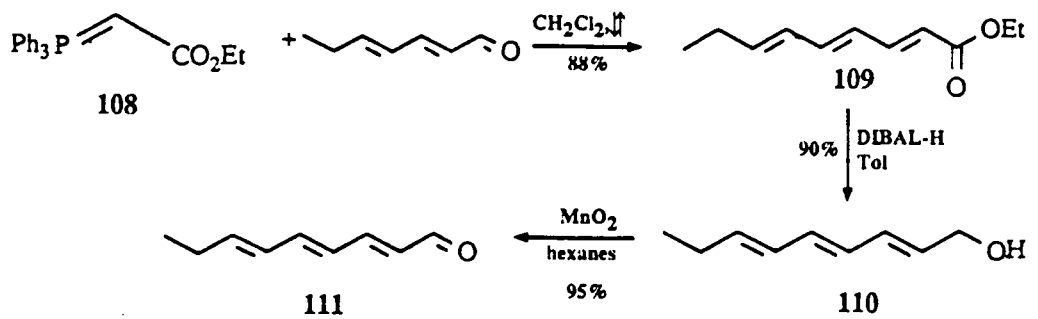
R=deoxyribosyl, **106**
R=ribosyl, **107**

Scheme 42.

6.3.2 Synthesis of Nonatrienal and Undecatetraenal

These data encouraged us to try analogous experiments with heptadienal, nonatrienal and undecatetraenal. In order to demonstrate the role polyenals may have as genotoxic agents it was felt that the formation of covalent bonds between the polyenals and a nucleoside base would provide the most convincing evidence for that role. Naturally, it was necessary to acquire the three polyenals. While heptadienal was available from Bedoukian Research Laboratories, the other two aldehydes needed to be synthesized from heptadienal.

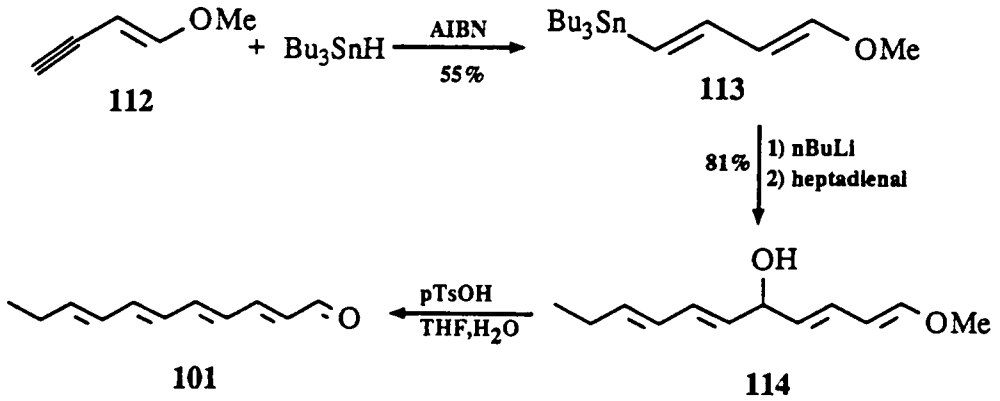
The synthesis of nonatrienal could be achieved by homologation of heptadienal by a Wittig reaction, followed by a 2 step reductive/oxidative process as shown in Scheme 43.[93] The synthesis of triphenylphosphine ethyl methylene carboxylate **108** was reported by Ross.[94] Reacting the phosphoranylidene with heptadienal in refluxing methylene chloride gave the triene ester **109** in 88% yield. Reduction of **109** with DIBAL-H in toluene produced silvery flakes of the trienol, **110**, in 90% yield. Allylic oxidation with manganese dioxide in hexanes provided the nonatrienal, **111**, in 95% yield.



Scheme 43.

The synthesis of undecatetraenal (Scheme 44) follows a procedure reported by Wollenberg.[95] Crude 1-methoxy-2-buten-3-yne is available as a methanolic solution from Fluka.

Pure **112** is required for the first reaction and can be obtained by performing fractional vacuum distillation of the Fluka material. Azobisisobutyronitrile catalyzed free radical addition of tributyltin hydride across the triple bond of **112** gave (1-methoxy-1,3-butadienyl)tributyltin **113** in 55% yield after fractional vacuum distillation. Generation of the vinyl anion of **113** with nBuLi in THF and *in situ* reaction of this anion with heptadienal gave the diallylic alcohol **114** in 81% yield. Compound **114** could be taken directly to the undecatetraenal **101** by adding a catalytic amount of p-toluenesulfonic acid and a few drops of water to the crude reaction mixture of **101**. This methodology resulted in a 36% yield of **101** from heptadienal.



Scheme 44.

6.3.3 Guanosine Adducts Study

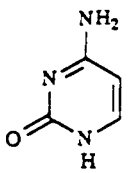
Acrolein formed an adduct with guanosine in conditions much milder than the reaction with crotonaldehyde and guanosine. It is not known what the difference in reactivity is due to, but the addition of a methyl group to acrolein, being the difference between the electrophiles, seems to alter the reactivity of the respective aldehydes drastically. In addition, Ames[96] used Salmonella tester strain, TA-104, to test the mutagenicity of various naturally occuring carbonyl compounds. He found that acrolein was approximately 15% more mutagenic than crotonaldehyde and that mutagenicity decreased with increasing carbon number within the enal series. It is of interest to

note that Ames found dicarbonyls to be the most mutagenic of the carbonyl compounds he tested.

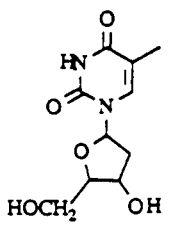
It was felt that an extended conjugated system of crotonaldehyde would lend greater reactivity toward nucleophilic attack by guanosine commensurate with reactivity observed in the acrolein reaction. To test this hypothesis heptadienal was subjected to treatment with various nucleoside bases under various conditions.

A typical procedure involved mixing heptadienal with the nucleoside in a solvent. If TLC indicated that a reaction had occurred then guanosine was removed by precipitation. In aqueous solutions, this was done by concentrating the mixture and cooling the concentrate. In DMSO solutions, 100 volumes of benzene were added and the mixture was allowed to stir for one day.

When guanosine was reacted with heptatrienal in DMSO, no reaction was observed, and when the reaction was monitored continuously by ^{1}H NMR no change in the spectrum was observed. This result indicated that no reaction took place, and no partial reaction was occurring under equilibrium conditions. Similarly, neither cytosine **115** nor thymidine **98** reacted with heptadienal.



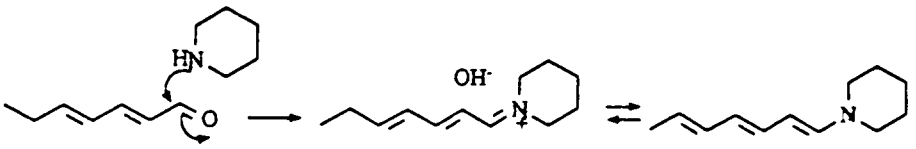
115



98

Significant reactions took place when slightly basic conditions were introduced. Basicity was imparted by using pH 9 buffer as a solvent or by adding a catalytic amount of piperidine to a DMSO solution. The use of buffers was introduced because it was felt that reactivity might be pH

dependent. Piperidine was added because reactivity of heptadienal would be enhanced by a secondary amine according to Scheme 45 since the iminium salt **116** would be far more



116 **117**

Scheme 45.

electrophilic than heptadienal. However, it should be pointed out that the enamine tautomer, **117** depicted in Scheme 45 would also be present in solution and that the use of piperidine might be precluded by structure **117**.

In the case of the pH 7 buffer and piperidine in DMSO, the mixtures were so complex that HPLC isolation was required on the filtrate from the guanosine precipitation performed as described previously. It was found, however, that all the compounds isolated by HPLC were aldol condensation products. These compounds were not further characterized because it was obvious, due to the absence of the sugar proton resonances in the ^{1}H NMR spectrum, that these compounds were not adducts of guanosine.

It was further demonstrated that even less basic conditions were adequate to decompose undecatetraenal. This was observed when undecatetraenal and guanosine were stirred in DMSO and TLC analysis of the reaction mixture revealed complete decomposition of undecatetraenal with no reaction of the guanosine.

The study conducted is summarized in Table 16 which demonstrates unequivocally that polyenals with allylic protons are more likely to undergo self condensation reactions than they are to form adducts with nucleoside bases. It is possible that the nucleoside base might catalyze the aldol condensation, but the likelihood of such a reaction could not be discovered from the data

Table 16. Summary of nucleoside base adduct formation with various enals.

Nucleoside Base	Aldehyde	Conditions	Result
Guanosine	heptadienal	pH 9	decomposition[a]
Guanosine	heptadienal	pH 7	"
Guanosine	heptadienal	DMSO[b]	"
Guanosine	heptadienal	DMSO	no reaction
Cytosine	heptadienal	DMSO	"
Cytosine	heptadienal	Ethanol	"
Thymidine	heptadienal	Ethanol	"
Guanosine	undecatetraenal	DMSO	decomposition
Guanosine	acrolein	DMSO	**105**
Guanosine	crotonaldehyde	pH 7	**107**

[a] Decomposition refers to heptadienal or undecatetraenal undergoing some sort of self condensation reactions.
[b] This particular reaction is the one where piperidine was added.

acquired. The data did seem to indicate, however, that the same reaction might occur in the nucleus of the cell, a scenario best mimicked by reactions run in pH 7 buffer. These data do not support the involvement of dodecatetraenal as a DNA-reactive intermediate, and thus tend to suggest that it is not a potential carcinogen.

6.4 Experimental

Preparation guanosine-acrolein adduct (**105**).

Guanosine (2 g, 6.85 mmol) was dissolved in 20 mL of DMSO and 4 mL (59.9 mmol) of acrolein was added. The solution was stirred for 2 days at room temperature. The solution was transferred to a beaker, 200 mL of benzene added and the mixture was stirred for 18 hours after which time a precipitate had formed. The solution was decanted off and the precipitate was triturated with 95% ethanol. The ethanol was filtered and the precipitate was recrystallized from water and ethanol to yield white crystals. Yield 1.6 g (67%). ^{1}H NMR (DMSO) δ 8.42 (bs, 1H, NH), 7.96 (s, 1H, C2), 5.94 (d, J=4.7 Hz, 1H, C1'), 5.71 (d, J=7.0 Hz, 1H, C8-OH), 5.40 (m, 1H, C2'-OH), 5.13 (d, J=5.3 Hz, 1H, C3'-OH), 5.02 (t, J=5.8 Hz, 1H, C6'-OH), 4.42 (m, 2H, C8 & C2'), 4.19 (m, 1H, C3'), 3.88 (m, 1H, C4'), 3.52 (m, 2H, C6'), 1.96 (m, 2H, C6), 1.76 (m, 2H, C7); IR (KBr) 3275, 1690, 1550, 1090, 1050 cm^{-1}; UV (EtOH) 253 nm (29,000).

Preparation of guanosine-crotonaldehyde adduct (**107**).

Guanosine (0.6 g, 2 mmol), 0.8 mL (10 mmol) of crotonaldehyde and 100 mL of pH 7 aqueous phosphate buffer were combined and the mixture was heated to 90°C for 16 hours. The mixture was concentrated *in vacuo* and set in the freezer overnight. The mixture was filtered, the precipitate was redissolved in 2 mL of DMSO, 200 mL of benzene was added and the mixture was stirred at room temperature for 18 hours. The liquid was decanted off and the precipitate was triturated with 95% ethanol. The ethanol mixture was filtered and the precipitate was recrystallized from ethanol and water to yield white crystals. Yield 148 mg (20%). ^{1}H NMR (DMSO) δ 7.96 (bs, 1H, NH), 7.92 (s, 1H, C2), 6.17 (d, J=1.3 Hz, 1H, C1'), 5.67 (d, J=5.3 Hz, 1H, C8-OH), 5.43 (m, 1H, C2'-OH), 5.18 (d, J=4.0 Hz, 1H, C3'-OH), 5.04 (t, J=4.6 Hz, 1H, C6'-OH), 4.36 (m, 2H, C8 &

C2'), 4.06 (m, 1H, C3'), 3.84 (m, 1H, C4'), 3.49 (m, 2H, C6'), 1.99 (m, 1H, C6), 1.39 (m, 2H, C7), 1.19 (d, J=7.3 Hz, 3H, CH_3).

Preparation of ethyl nona-2,4,6-trienoate (109).

Triphenylphosphine ethyl methylene carboxylate (108) (17.5 g, 0.05 mol) was dissolved in methylene chloride. Heptadienal (5.7 mL, 0.045 mol) was added and the solution was refluxed under argon for 13 hours. The solvent was removed *in vacuo*, and the crude oil was passed through a short column of silica gel 60 eluted with hexanes. The hexanes were removed *in vacuo* and the oil was purified on a flash column packed with silica gel 60 and eluted with 5% ether in hexanes to yield a yellow oil which appeared as a single spot on TLC. Yield 7.62 g (88%). ^1H NMR ($CDCl_3$) δ 6.54 (dd, J=11.5, 17.1 Hz, 1H, =CH), 6.20 (dd, J=17.8, 13.2 Hz, 1H, =CH), 6.14 (dd, J=13.2, 16.8 Hz, 1H, =CH), 6.11 (dd, J=11.5, 17.8 Hz, 1H, =CH), 5.97 (dt, J=6.9, 16.8 Hz, 1H, =CH), 5.83 (d, J=17.1 Hz, 1H, =CH), 4.29 (q, J=7.9 Hz, 2H, CH_2), 2.16 (dq, J=6.9, 8.6 Hz, 2H, allylic CH_2), 1.28 (t, J=7.9 Hz, 3H, CH_3), 1.04 (t, J=8.6 Hz, 3H, CH_3); IR (neat) 3020, 1730, 1630, 1445, 1195, 1175 cm^{-1}; UV (EtOH) 311 nm (31,318); MS (EI) *m/z* (relative intensity) 180 (M^+, 37), 107 (58), 79 (100).

Preparation of nona-2,4,6-trien-1-ol (110).

In a flame dried flask under argon, 7.62 g (0.04 mol) of ethyl nona-2,4,6-trienoate (109) was dissolved in 50 mL of toluene and the solution was cooled to -80°C. DIBALH (68 mL, 25% by weight in toluene, 0.10 mol) was added dropwise over 30 minutes. After 1 hour 50 mL of 50% aqueous methanol, 100 mL of ether and anhydrous magnesium sulfate was added and the mixture was stirred for 13 hours. The mixture was filtered, washed several times with ether and the solvent was removed from the filtrate *in vacuo* to yield silvery platelets which were recrystallized from ether

and hexanes.. Yield 5.0 g (90%). ^{1}H NMR ($CDCl_3$) δ 6.41 (dd, J=10.8, 14.0 Hz, 1H, =CH), 6.25 (m, 1H, =CH), 6.15 (m, 1H, =CH), 6.08 (dd, J=10.1, 11.1 Hz, 1H, =CH), 5.83 (m, 1H, =CH), 5.59 (m, 1H, =CH), 4.34 (m, 2H, CH_2O), 2.14 (m, 2H, allylic CH_2), 1.35 (m, 1H, OH), 1.01 (t, J=8.2 Hz, 3H, CH_3); IR (KBr) 3450, 3020, 2975, 2925, 1700, 1485, 1475 cm^{-1}; MS (EI) *m/z* (relative intensity) 138 (M^+, 10), 81 (98), 57 (100).

Preparation of nona-2,4,6-trien-1-al (111).

Nona-2,4,6-trienol (110) (100 mg, 0.72 mmol) was dissolved in methylene chloride and 2 g of manganese dioxide were added. The heterogenous mixture was stirred at room temperature for 4 hours and the mixture was filtered and the filtrate was evaporated to dryness *in vacuo* to yield a yellow oil, which appeared as a single spot on TLC. Yield 98 mg (99%). ^{1}H NMR ($CDCl_3$) δ 10.17 (d, 1H, CHO), 7.13 (dd, 1H, =CH), 6.96 (dd, 1H, =CH), 6.54 (dd, 1H, =CH), 6.54 (dd, 1H, =CH), 6.21 (dd, 1H, =CH), 6.08 (dt, 1H, =CH), 5.82 (dd, 1H, =CH), 2.19 (dq, 2H, allylic CH_2), 1.04 (t, 3H, CH_3); IR (neat) 2990, 2960, 1665, 1650, 1615, 1020 cm^{-1}; MS (EI) *m/z* (relative intensity) 136 (M^+, 93), 110 (48), 79 (100); UV (EtOH) 350 nm (60,293).

Preparation of 4-(1-methoxy-buta-1,3-dienyl)-tributyltin (113).

Freshly distilled 4-methoxy-but-2-en-1-yne (7.0 mL, 0.08 mol) and 26 mL (0.1 mol) of tributyltin hydride were combined, 94 mg (0.6 mmol) of AIBN was added and the solution heated to 80°C for 12 hours. Pure **113** was obtained by fractional vacuum distilling the crude mixture. Yield 15.7 g (55%). bp 113-115°C (0.1 mm Hg). ^{1}H NMR ($CDCl_3$) δ 6.84 (d, J=12.9 Hz, 1H, OCH=), 6.60 (dd, J=9.7, 17.7 Hz, 1H, =CH), 6.08 (m, 2H, =CH), 5.78 (dd, J=11.3, 21.5 Hz, 1H, =CH), 3.80 (s, 3H, CH_3O), 1.75 (m, 6H, CH_2Sn), 1.57 (m, 6H, CH_2), 1.13 (t, J=6.4 Hz, 9H, CH_3); IR (neat) 2925, 2875, 1635, 1600, 1460, 1215, 1115 cm^{-1}; UV (EtOH) 255 nm (20,632).

Preparation of 1-methoxyundeca-1,3,6,8-tetraene-5-ol (114).

In a flame dried flask under argon, 2.9 g (7.9 mmol) 4-(1-methoxybuta-1,3-dienyl)-tributyltin (113) was dissolved in THF. The solution was cooled to -78°C at which time 3.2 mL of 2.6 M (8.3 mmol) of nBuLi in hexanes was added over 10 minutes and stirred for 1 hour. Heptadienal (1.03 mL, 8.3 mmol) was added. After 15 minutes, 10 mL of saturated aqueous sodium bicarbonate was added and the mixture was warmed up to room temperature. The mixture was extracted with 4X100 mL of ether and the ether layers were combined, washed with 3X20 mL of brine, dried over magnesium sulfate, filtered, and the ether removed *in vacuo* to yield a yellow liquid which was loaded on flash column packed with triethylamine pretreated silica gel 60 and eluted with 1:1 ether:hexanes to yield a yellow liquid which appeared as a single spot on TLC. Yield 931 mg (84%). ^{1}H NMR ($CDCl_3$) δ 6.30-7.20 (m, 2H, 2=CH), 5.85-6.30 (m, 3H, 3=CH), 5.40-5.85 (m, 3H, 3=CH), 5.01 (m, 0.5H, CHO), 4.62 (m, 0.5H, CHO), 3.55-3.65 (2s, 3H, CH_3O), 2.07 (m, 2H, allylic CH_2), 0.98 (t, J=7.9Hz, 3H, CH_3); IR (neat) 3420, 3050, 3000, 2960, 2910, 1670, 1635, 1480, 1280, 1225 cm^{-1}; UV (EtOH) 230 nm (21,524).

Preparation of undeca-2,4,6,8-tetraen-1-al (101).

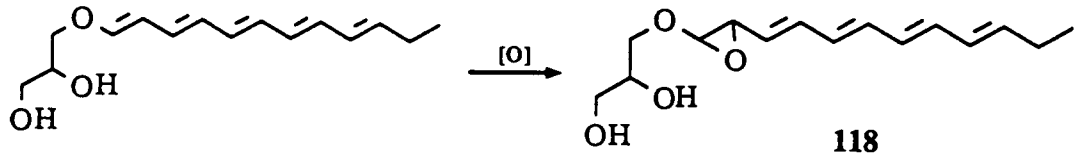
In a flame dried flask under argon, 10 g (0.03 mol) of 4-(1-methoxybuta-1,3-dienyl)tributyl tin (113) was dissolved in THF. The solution was cooled to -78°C at which time 11.0 mL of 2.6 M (0.03 mol) of nBuLi in hexanes was added and the solution was stirred for 1 hour. Heptadienal (2.75 mL, 0.02 mol) was added and the solution was stirred for 3 hours at -78°C. The workup was the same as described for 114. The crude liquid was not purified on a flash column but immediately dissolved in THF and cooled to 0°C. p-Toluenesulfonic acid (210 mg, 1.1 mmol) and 2 drops of water was added and the solution was stirred for 1 hour. The solvent was removed *in vacuo* and the crude residue was loaded on a flash column packed with triethylamine pretreated

silica gel 60 and eluted with 1:1 hexanes:ether to yield yellow crystals. Yield 1.29 g (36%). ^{1}H NMR ($CDCl_3$) δ 9.53 (d, J=7.2 Hz, 1H, CHO), 7.11 (dd, J=10.7, 13.9 Hz, 1H, =CH), 6.67 (dd, J=10.0, 13.5 Hz, 1H, =CH), 6.43 (dd, J=9.6, 9.8 Hz, 1H, =CH), 6.39 (dd, J=9.6, 10.0, 1H, =CH), 6.22 (dd, 13.5, 10.7 Hz, 1H, =CH), 6.11 (m, 1H, =CH), 5.94 (m, 1H, =CH), 2.15 (m, 2H, allylic CH_2), 1.03 (t, J=7.2 Hz, 3H, CH_3); IR (KBr) 3025, 1695, 1610, 1130, 1010 cm^{-1}; UV (EtOH) 350 nm (72).

VII Epoxy Ether as a Fecapentaene-12 Metabolite.

7.1 Introduction

Another possible reaction fecapentaene-12 may undergo *in vivo* prior to a reaction with DNA is an oxidative process. This possibility was alluded to in chapter 6 and is restated in Scheme 46. This discussion maintained that the epoxy ether **118** was the actual reactive species in the nucleus.



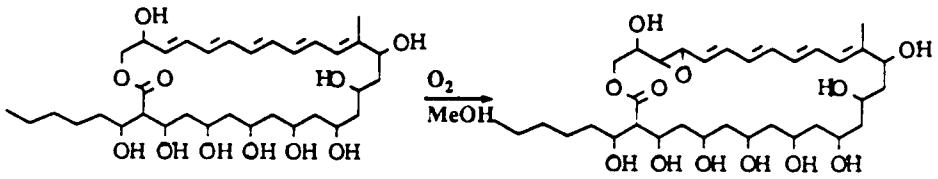
118

Scheme 46.

Although the colonic environment is anaerobic, aerobic regions exist at the cell surface. The oxygenated blood is the vehicle for carrying the oxygen to the cell surface. Oxidation of FP-12 in aerobic epithelial cells therefore becomes a reasonable metabolic pathway. It should be remembered that incubation of 6-[^{3}H]-FP-12 with DNA displayed enhanced binding under aerobic conditions, albeit only slightly. In addition, the Ames test used to quantify the mutagenicity of FP-12, was done in the presence of air.

Examples of autooxidation of pentaenes are not abundant in the literature, however, Golding, Rickards and Smith[97] reported that autooxidation of filipin, **119**, occurs on exposing a methanolic solution of the macrolide antibiotic to air, forming oxidofillipin, **120**, in 50 % yield (Scheme 47).

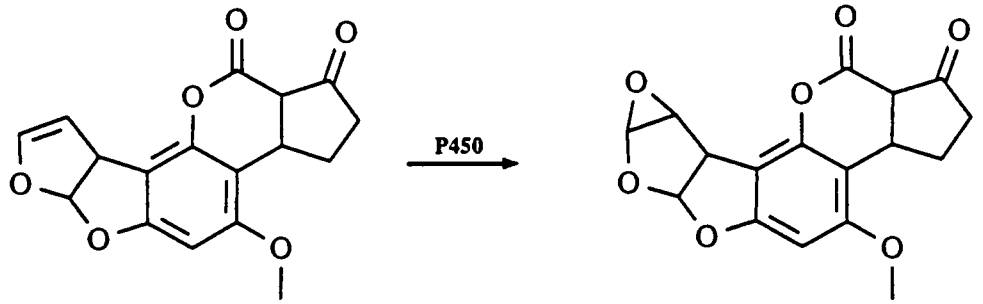
Likewise, examples of epoxy ethers as biologically important intermediates have not attracted a great amount of attention. The most important study dealt with the mode of action of



119 120

Scheme 47.

the carcinogen aflatoxin, **121**. There has been extensive evidence that the epoxy ether, **122**, is implicated as the key intermediate in the aflatoxin carcinogenesis pathway (Scheme 48), apparently through cytochrome P-450 monooxyenase mediated oxidation.[96] This intermediate immediately reacts with nucleophiles such as the guanine moiety of DNA. It is for this reason that the



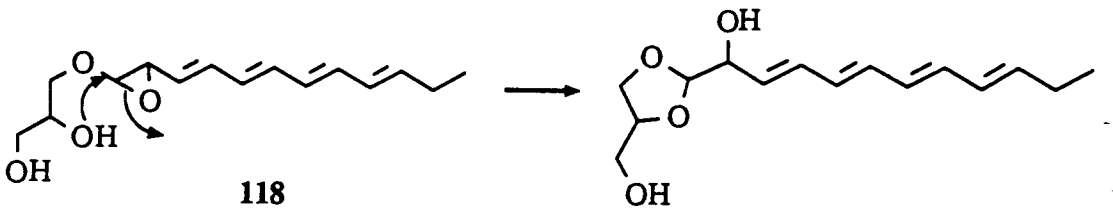
121 **122**

Scheme 48.

oxidoaflatoxin B has never been isolated. Until very recently attempts to synthesize oxidoaflatoxin have failed, making it very difficult to prove conclusively that oxidoaflatoxin B (**122**) is the key intermediate in this pathway.

We first decided that if autooxidation of FP-12 could occur, then the most likely product would be 1,2-oxidofecapentaene-12, **118**, since the first double bond is reasoned to be the most reactive. We wanted to see if autooxidation of FP-12 could occur and therefore, following the procedure described by Golding and coworkers,[97] FP-12 was dissolved in d_4-methanol and the

solution set in the dark at 10°C for 1 day. ^{1}H NMR of the crude mixture indicated almost complete decomposition of FP-12 with no evidence of any epoxide formation. Since, it was already known that FP-12 is unstable in air, no new data were revealed by this experiment. None of the data thus far discussed excludes the possibility of an epoxy ether as an intermediate in mutagenesis by FP-12. Perhaps oxidofecapentaene-12 (**118**) is not an isolable system due to the presence of the free hydroxyls of FP-12; thus Scheme 49, shows how an intramolecular nucleophilic attack on an epoxy ether by the 2-hydroxy function would cause the system to be extremely short-lived.

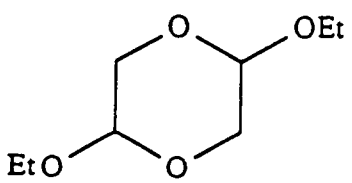


118

Scheme 49.

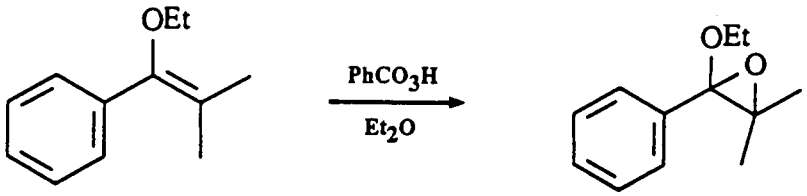
7.2 Synthesis of Epoxy Ethers - Literature Review.

The synthesis of 1,2-epoxy ethers has been known since 1921. In that year, Bergmann[99] reported that the reaction of ethyl vinyl ether with perbenzoic acid gave an epoxy ether, which he later described as being the dimer,[100] 1,2-diethoxy-1,3-dioxane (**123**). In 1949, Hurd[101] reported the



123

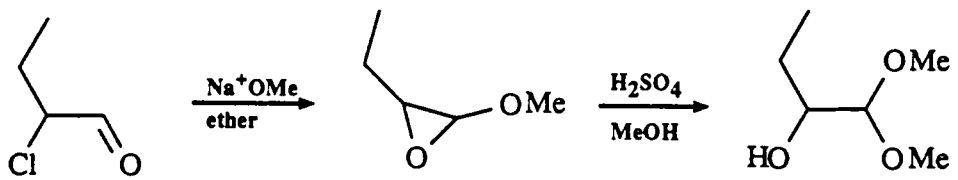
isolation of a crude epoxy ether from the reaction of dihydropyran with perbenzoic acid. A year later, Mousseron[102] used the same conditions to achieve the corresponding epoxy ether of 1-cyclohexenyl ethyl ether in 75% yield. In 1953 Stevens and Tazuma[103] epoxidized 2-methyl-1-ethoxy-1-phenylpropene, **124**, with perbenzoic acid to give the epoxy ether, 1,2-epoxy-2-methyl-1-ethoxy-1-phenylpropene (**125**) in 90% yield (Scheme 50).



124 **125**

Scheme 50.

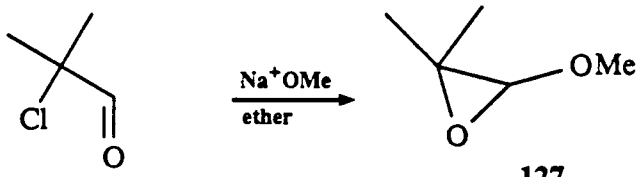
In 1953 the beginning of new methods of epoxy ether synthesis appeared when Stevens[104] reported the synthesis of 1-methoxy-2-ethyloxirane (**126**) by reacting 2-chloro butanal with sodium methoxide in ether. They claimed that the oxirane was stable in methanol but decomposed to 2-hydroxybutanal dimethyl acetal upon addition of a few drops of sulfuric acid (Scheme 51).



126

Scheme 51.

Kirrmann[105] repeated the work of Stevens to make **126**, except he used dioxane as the solvent instead of ether. In 1957 Meerwein[106] and coworkers reported the synthesis of 2-methoxy oxirane by reacting methyl formate with diazomethane. In that same year Stevens and Gillis[107] reported a synthesis of 3,3-dimethyl-2-methoxyoxirane (**127**) from 2-chloro-2-methylpropanal and sodium methoxide in ether (Scheme 52).



127

Scheme 52.

In 1973, Kraus and Sturtz[108] extended the methodology reported by Stevens and coworkers by utilizing α-phosphate aldehydes as well as α-halo aldehydes as the electrophiles. Their work was done on the corresponding straight chain aliphatic aldehydes of carbon number ranging from four to seven.

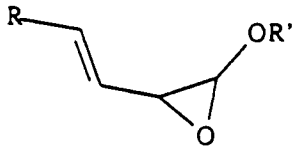
Finally, in 1976, Havel and Chan[109] irradiated nitrous oxide decomposition products with a mercury lamp to generate molecular oxygen. They performed gas phase reactions of ground state (^3P) oxygen atoms with vinyl ethers to get the corresponding epoxy ethers of ethyl vinyl ether, 2-methoxy-1-propene and 1-methoxy-2-methyl-1-propene in 50%, 21% and 46% yields, respectively.

7.3 Attempted Synthesis of Epoxy Ethers

Our laboratory was faced with the task of generating an oxidized derivative of fecapentaene-12 and reacting this epoxy ether with a nucleoside base in hopes of gaining information as to the possible genotoxic effects oxidofecapentaene-12 might have. This study, if

successful, would lend credence to the theory that fecapentaene-12 is a biological precursor that undergoes oxidation *in vivo* to yield the highly reactive oxidofecapentaene-12 (**118**). Upon inspection of the epoxy ethers that have been synthesized it became necessary to simplify the system drastically because the known epoxy ethers are much simpler than the epoxy ether of FP-12. In addition, the simple fact that an epoxy ether of FP-12 would almost certainly be impossible to isolate (due to the previously mentioned intramolecular hydroxyl attack on the labile acetal carbon) warranted attempts at synthesizing simpler epoxy ethers.

The system chosen was one in which at least partial integrity of the fecapentaene-12 molecule was maintained. Our goal was to make an epoxy ether with at least one double bond present as shown by the generic structure **128**. Incorporating what was known about epoxy ether



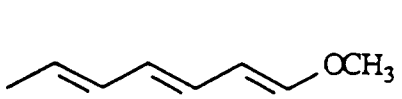
128

synthesis with what was required for our study, we set out to synthesize such an epoxy ether before any nucleoside adduct study could be pursued. The reason for this approach is manifested by the fact that we were trying to make a compound whose unique array of functional groups had never been reported.

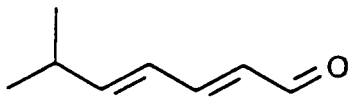
7.3.1 Attempted Epoxy Ether Synthesis Via Epoxidation of Enol Ethers with MCPBA.

One approach to form an epoxy ether with double bonds was to epoxidize an enol ether with MCPBA. This reaction was reported for vinyl ethers in the literature[100] except perbenzoic acid was used instead of MCPBA. Heptadienal had proved to be a valuable starting material for many reactions discussed in this report thus far and was therefore, an obvious starting material for us

here. Initially, making a methyl enol ether would be simpler than an analogous derivative of heptadienal, however, it was soon discovered that methyl heptatrienyl ether (**129**) could not be synthesized from heptadienal and methyl iodide. What was consistently made from this procedure

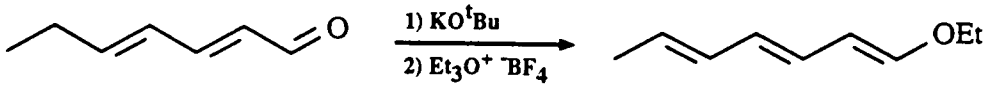


129



130

was 6-methylheptadienal (**130**). Utilizing the premises of hard-soft symmetry in chemical reactivity,[110] the potassium salt of the heptatrienolate anion was quenched with triethyl oxonium tetrafluoroborate[111] to afford the required O-alkylated product, heptatrienyl ethyl ether (**99**) in 74% yield (Scheme 53).



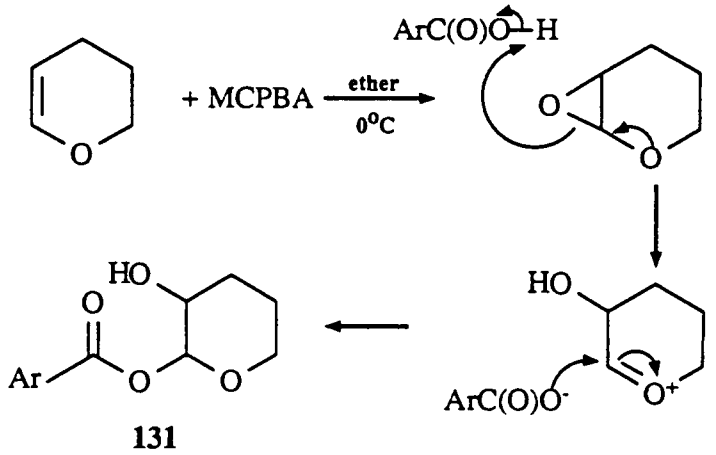
99

Scheme 53.

Epoxidation of **99** with MCPBA in ether did not yield the desired epoxy ether; even when conditions were used exactly as described by Stevens, a complex mixture of products were formed, none of which were easily interpreted by standard spectroscopic techniques.

We felt it necessary to acquire an epoxy ether of any kind and perform *ab initio* guanosine adduct studies to at least show that such reactivity was reasonable. We repeated the work of Hurd[101] and epoxidized dihydropyran with MCPBA in ether at 0°C. What we found was a very good yield of the ester **131**. We next relied upon the work of Stevens whose work up of the epoxy ether in question seemed more reasonable. Stevens[103] loaded the reaction mixture onto a basic alumina

column within thirty minutes of perbenzoic acid exposure to his enol ether, ethoxy cyclohexene, in contrast to Hurd's simple removal of solvents and carrying the crude epoxy ether to the next synthetic step. However, when we stirred dihydropyran with MCPBA and within four seconds loaded the crude on a basic alumina column we discovered a 68% yield of **131**. The presumed mechanism for the formation of **131** is shown in Scheme 54. As can be seen, the need for immediate loading onto a basic medium becomes obvious since acid hydrolysis of the epoxy ether is a very facile process. To counteract this rapid hydrolysis, we tried adding basic alumina to the



131

Ar= m-Cl benzene

Scheme 54.

reaction medium before MCPBA introduction and found that no change in the reactivity of the system had occurred.

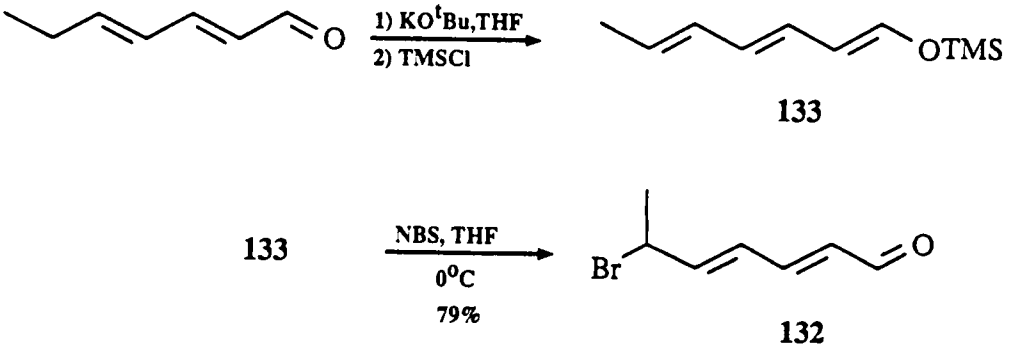
7.3.2 Epoxy Ether Synthesis Via Alkoxide Attack on Bromoenals.

Again borrowing from the methodology reported by Stevens,[104,107] we tried to make an epoxy ether by reacting a homolog of an α-chloro aldehyde with the salt of an alcohol. The homolog of interest in this case was 6-bromoheptadienal (**132**). The presence of the conjugated

system in heptadienal required that ε bromination proceed through the mildest conditions that could be used, since bromination under conditions successful with aliphatic aldehydes is precluded by the presence of the double bonds which can exhibit comparable reactivity with that of enols.

7.3.2.1 Synthesis of 6-Bromoheptadienal (**132**).

The method of bromination we selected was the reaction of the trimethylsilyl enol ether (**133**) with NBS.[112] Preparation of heptatrienyl trimethylsilyl ether was performed by treating heptadienal with potassium tert-butoxide in dry THF and trapping the trienolate with trimethylsilylchloride.[113] Purification of **133** was not carried out for three reasons. The first reason was that purification was extremely difficult without rapid decomposition of the requisite product. Purification by column chromatography using a variety of stationary phases resulted in a polymerization process yielding a viscous colorless gel. Distillation of the crude mixture at temperatures slightly above room temperature yielded no distillate but rather a black charred polymer. Secondly, the ^{1}H NMR of the crude showed the existence of various isomers along the triene system where the presence of E and Z isomers were present in indeterminable amounts. Third, the reaction of the crude silyl enol ether with NBS in THF proved not only to be a clean reaction but provided all trans 6-bromoheptadienal (**132**) in good yield (79%). The synthesis of **132** is shown in Scheme 55.



Scheme 55.

We observed rapid decomposition of 6-bromoheptadienal when trace amounts of the succinimide by-product were present. Base catalyzed aldol condensation of 6-bromoheptadienal was believed to be occuring; this reaction was more facile due to the presence of an acidic proton α to the bromine atom and conjugated to the carbonyl group. The pKa of this proton is not known but is probably close to 13. Reasoning for this estimated pKa arises from known pKa's reported in the literature. The pKa of acetone has been estimated to be 19[114] and the presence of a halogen α to the carbonyl group can increase the acidity of the proton by as much as 4 pKa units.[115] In addition, the presence of the double bonds also increases resonance stabilization of the resultant anion[116] formed by deprotonation. Although the magnitude of the increased acidity of the proton has not been carefully studied we estimated the increased acidity due to the presence of the double bonds to be 2 pKa units. We therefore believe that the proton ε to the carbonyl is acidic enough to react with succinimide.

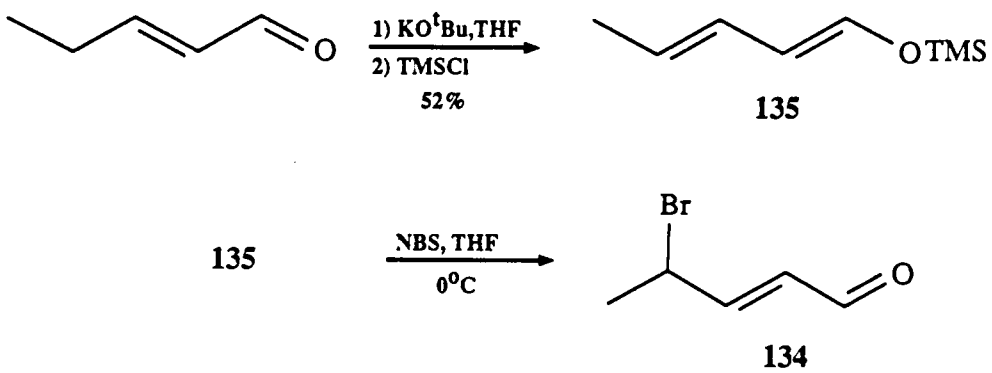
The most efficient way of removing succinimide was to wash the organic layer with aqueous sodium bicarbonate and sodium chloride and to add pentane to the crude residue (after solvent removal) and filter off the precipitant. Distillation of the product proved to be fruitless since the compound immediately decomposed upon slight heating and therefore, a compound of only 95% purity could be obtained.

6-Bromoheptadienal decomposed rapidly under argon at -10°C and was therefore reacted immediately with sodium solketal to yield what appeared to be aldol condensation products of widely varying constitution. It was not known whether the impurities carried over with the 6-bromoheptadienal compound were responsible for this result, so we decided to synthesize 4-bromopentenal. It was our hope to obtain a more stable homolog and still maintain the one double bond we needed for our study.

7.3.2.2 Synthesis of 4-Bromopent-2-enal.

The synthesis of 4-bromopentenal (**134**), was analogous to its homolog. Deprotonation of pentenal with potassium tert-butoxide and trapping the enolate with trimethylsilyl chloride provided the silyl enol ether (**135**) as a mixture of E and Z isomers which could be distilled in 52% yield.

During the synthesis of 4-bromopentenal it was discovered that the trimethyl silyl ether was more easily purified than the bromo enal and that reacting pure trimethylsilyl pentadienyl ether with NBS provided 99% pure 4-bromopentenal (**134**) as shown in Scheme 56.

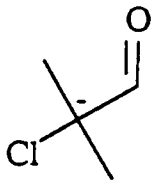


Scheme 56.

As with all of these enal derivatives spectroscopic evidence was difficult to obtain due to the rapid decomposition of the compounds. For this reason it was felt to be more important to pursue epoxy ether synthesis from 4-bromopentenal than to fully characterize the compound. If epoxy ether can be achieved by this methodology then complete characterization of all the compounds could be obtained at a later date.

Stevens alluded to a side reaction of his α-chloro aldehyde with sodium methoxide, namely deprotonation forming the enolate **136**.[107] We discovered that this "side" reaction was the major pathway undertaken in our system when 4-bromopentenal was reacted with sodium benzyloxide or

159



136

sodium phenoxide in THF. The only product that was characterized was the starting alcohols from the respective reaction; all other components in the mixture appeared to be aldol condensation products.

7.3.3 Epoxy Ether Via Sulfur Ylides.

Formation of epoxides by reacting sulfur ylides with carbonyl compounds is a synthetic methodology known since the early 1960's:[117] Figure 21 shows the basic mechanism involved. The reactivity of sulfur ylides towards electron-deficient functional groups lies in the fact that the sulfur ylides are nucleophilic alkylidene transfer agents.

The most common method of sulfur ylide formation is deprotonation of the corresponding sulfonium salt,[118] and the utility of sulfur ylides is thus dependent upon the availability of the sulfonium salt. In the vast majority of cases sulfonium salts are made by reacting the dialkyl sulfide with either trialkyl oxonium tetrafluoroborate[119] or alkyl iodide.[120]

It was our belief that by introducing an alkoxy substituent on the nucleophilic carbon, a viable method of epoxy ether synthesis could be realized. We therefore started our work by attempting to alkylate methoxymethyl phenyl sulfide (**137**) with methyl iodide, only to find the system to be completely stable even in refluxing methyl iodide. A review of the literature revealed to us why this might be so. Franzen[121] proposed that phenyl sulfides have reduced nucleophilicity and cannot be alkylated without assistance from silver salts. We proceeded to repeat the same reaction with methoxymethyl phenyl sulfide and one equivalent of methyl iodide in the presence of silver

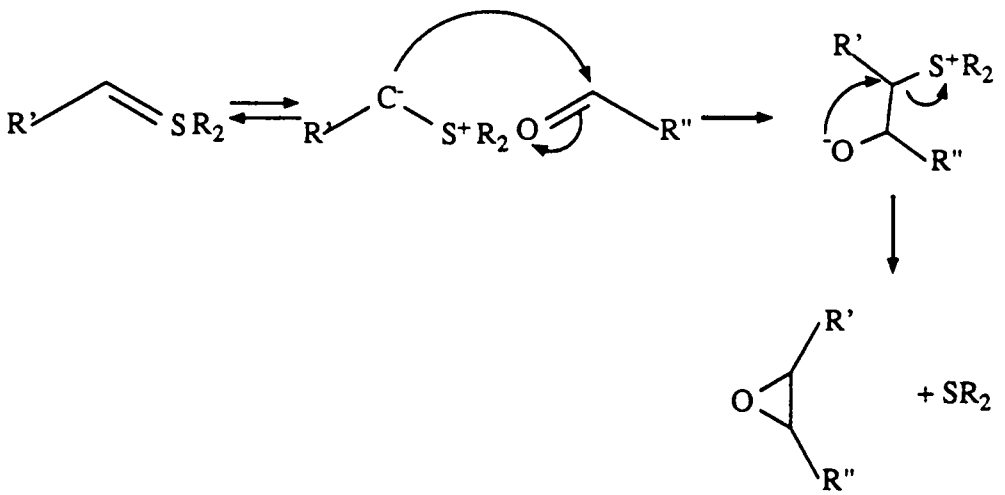


Figure 21. Mechanism of epoxide formation via sulfur ylides.

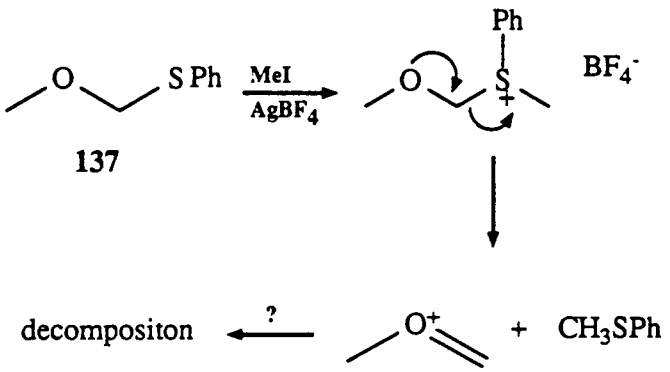
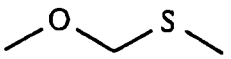


Figure 22. Reaction of 137 with MeI and $AgBF_4$.

tetrafluoroborate. Although alkylation occurred, the requisite sulfonium cation was not isolated. The reason for this was presumably that the highly reactive cation decomposed rapidly due the lone pair on the α-oxygen eliminating methyl phenyl sulfide, as shown in Figure 22. It was not known what path the rest of the reaction took.

In order to gain a better understanding of what may have occurred, a more nucleophilic dialkyl sulfide was needed where alkylation by methyl iodide could occur under milder conditions. The simplest sulfide needed was methoxymethyl methyl sulfide; the absence of the phenyl group should have allowed alkylation to occur without the need for silver salts. Unfortunately, this compound was not reported in the literature. Gokel[122] synthesized a series of alkoxy methyl sulfur derivatives in which he found it necessary to oxidize any sulfides to the sulfoxide or the sulfone if the alkoxy group present was an ethoxy (**138**) or a methoxy (**139**) group. We were able to get a

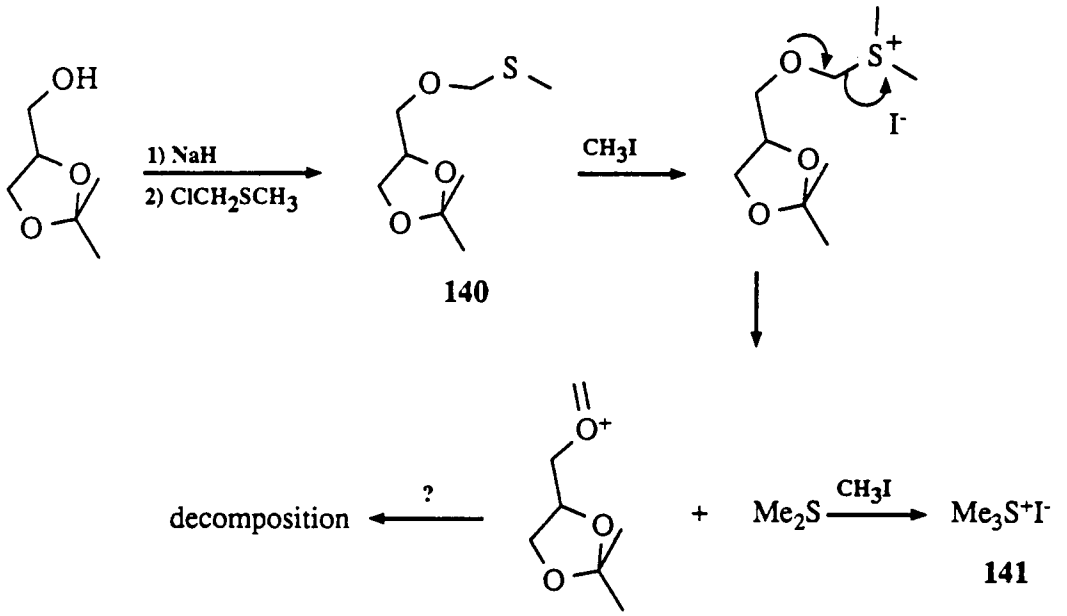
138



139

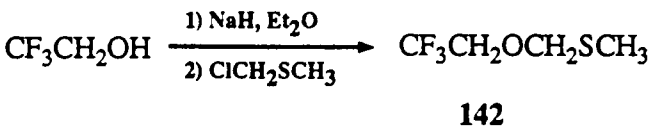
21% yield of 2,3-O-isopropylideneglyceroxymethyl methyl sulfide (**140**) by reacting the sodium salt of solketal with chloromethyl methyl sulfide in THF. Methylation of **140** with methyl iodide gave a 17% yield of trimethyl sulfenium iodide (**141**). This result tended to confirm the mechanism shown in Scheme 57, where the dimethyl sulfide formed reacted with another molecule of methyl iodide to form the sulfenium cation **141**.

If our proposed mechanism for the decomposition of alkoxymethyl dimethyl sulfenium iodide was correct, than perhaps introduction of an electron withdrawing functionality on the alkoxy substituent would prevent the lone pair of electrons on oxygen from eliminating dimethyl sulfide. For this reason we synthesized 2,2,2-trifluoroethoxymethyl methyl sulfide (**142**), in 22% yield by



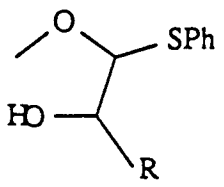
Scheme 57.

adding chloromethyl methyl sulfide to the sodium salt of 2,2,2-trifluoroethanol (Scheme 58). When **142** was reacted with methyl iodide we again observed the formation of trimethyl sulfenium iodide.



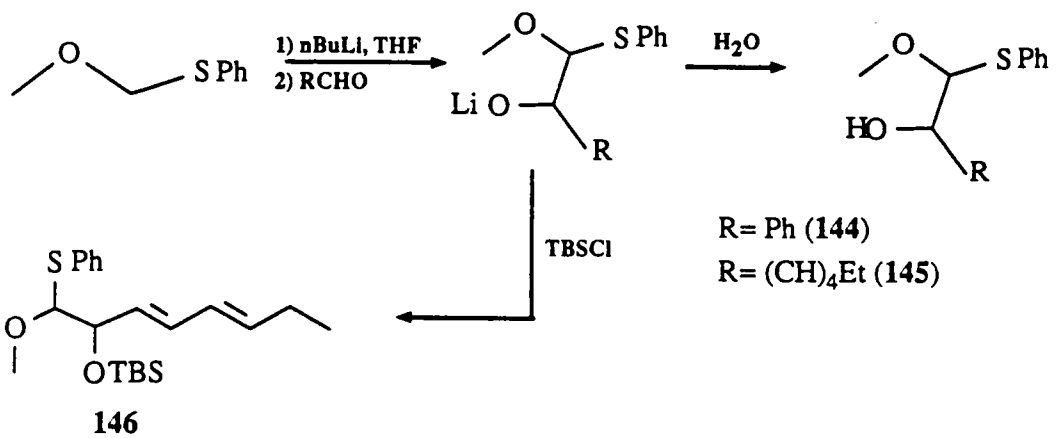
Scheme 58.

It became apparent that the formation of a sulfur ylide with an alkoxy group present was not feasible. We decided at this point that formation of an epoxy ether might be favorable over the formation of the alkyl oxonium cation formed in the above experiments. In order to pursue this we needed to synthesize molecules like structure **143**. It was our hope that selective S-alkylation of **143** would afford the desired epoxy ether instead of decomposition. We synthesized three



143

derivatives of **143** (Scheme 59) and the synthesis of all three included deprotonation of methoxy-methyl phenyl sulfide with nBuLi in THF and reaction of the resultant anion with an aldehyde.[123] To make 1-methoxy-1-phenylthio-2-hydroxy-2-phenylethane (**144**) the aldehyde of choice was benzaldehyde; to make 1-phenylthio-2-hydroxy-octa-3,5-dien-1-yl methyl ether **145**, the aldehyde used was heptadienal.[124] The synthesis of 1-phenylthio-2-('butyldimethyl)silyloxy-octa-3,5-dien-1-yl-methyl ether (**146**) was performed by simply trapping the alkoxy anion formed *in situ* with heptadienal with 'butyldimethylsilyl chloride.

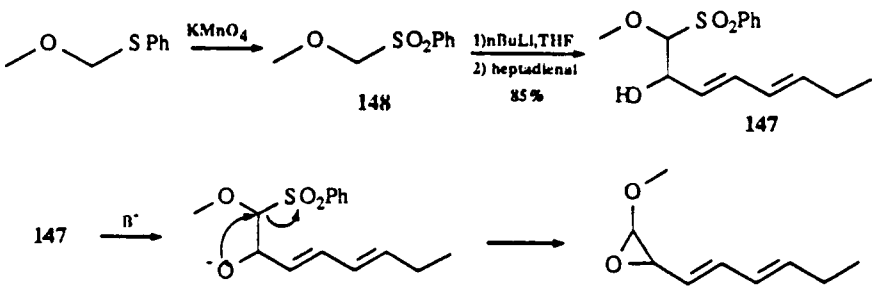


146

Scheme 59.

Reaction of 1-methoxy-1-phenylthio-2-hydroxy-2-phenylethane (**144**) with methyl iodide yielded starting materials; adding silver tetrafluoroborate to the medium resulted in decomposition of the mixed thio acetal. It is well known that selective hydrolysis of thio ketals in the presence of dioxane ketals or alcohols can be done with mercuric salts[125] and therefore, we decided that this methodology could be applied to our system. We wanted to include a diene in this reaction and therefore, we mixed 1-phenylthio-2-hydroxy-octa-3,5-dien-1-yl methyl ether (**145**) with mercuric chloride. We observed complete decomposition of the starting material, **145**, into at least 10 different compounds, none of which contained an epoxide or vinyl functionality. We repeated the same reaction with 1-phenylthio-2-(ᵗbutyldimethyl)silyloxy-octa-3,5-dien-1-yl methyl ether (**146**) as the substrate only to find the same myriad of products resulted.

Since it became apparent that sulfur ylide chemistry was precluded by the instability of α-alkoxy sulfenium cations, we aborted the sulfur ylide idea to afford epoxy ether. However, there was one other approach to be considered that utilized the presence of a leaving group α to a hydroxy group that could give epoxy ether. Replacing the sulfenium cation with a sulfone and imparting intramolecular attack by an alpha alkoxide might give the desired epoxy ether as shown in Scheme 60. Our compound used to test this theory was 1-phenylsulfonyl-2-hydroxy-octa-3,5-dien-1-yl methyl ether (**147**). The synthesis of **147** was performed by reacting the lithium salt of methoxymethyl phenyl sulfone (**148**), made by potassium permanganate oxidation of methoxy-



Scheme 60.

methyl phenyl sulfide, with heptadienal in THF to yield **147** in 85% yield. Deprotonation of the hydroxyl proton with sodium hydride in THF with a catalytic amount of 18-crown-6 did not yield any sulfone displaced product; no reaction occurred at all.

In order to get an epoxy ether it was decided that we needed to start with a much simpler system and that another methodology should be employed. The only evidence of an epoxy ether being made by the reactions thus far tried was when dihydropyran was epoxidized with MCPBA, which of course immediately hydrolyzed to the hydroxy arylate **131**. This decomposition of the epoxy ether formed was facilitated by the presence of acid, therefore, if epoxidation could be performed under neutral conditions then the epoxy ether might be isolable. Dimethyldioxirane has been shown to be a powerful epoxidation reagent of double bonds and the by-product of reduced dioxirane is the relatively unreactive acetone molecule. This was our next attempt at synthesizing epoxy an ether.

7.3.4 Epoxy Ether Synthesis Via Epoxidation of Enol Ethers with Dimethyldioxirane.

Our last attempt at synthesizing an epoxy ether was to use dimethyl dioxirane (**149**) as an epoxidizing reagent of an enol ether. Our enol ether of choice was one that was readily available and resembled precursors of epoxy ethers reported in the literature. Recalling Stevens' work on epoxidation of α-ethoxystyrene, we decided to employ epoxidation on β-methoxystyrene (**150**), a compound which is available from Aldrich. It should be pointed out that **150** does have a conjugated system to an enol ether, which is the type of system we were originally looking for as a reasonable model for **118**.

The synthesis of dimethyldioxirane, which was reported by Curci in 1980[126] and later by Murray[127] in 1985, entailed reacting an aqueous mixture of acetone and sodium bicarbonate with potassium monopersulfate (oxone) and entraining the resulting dimethyldioxirane with helium gas

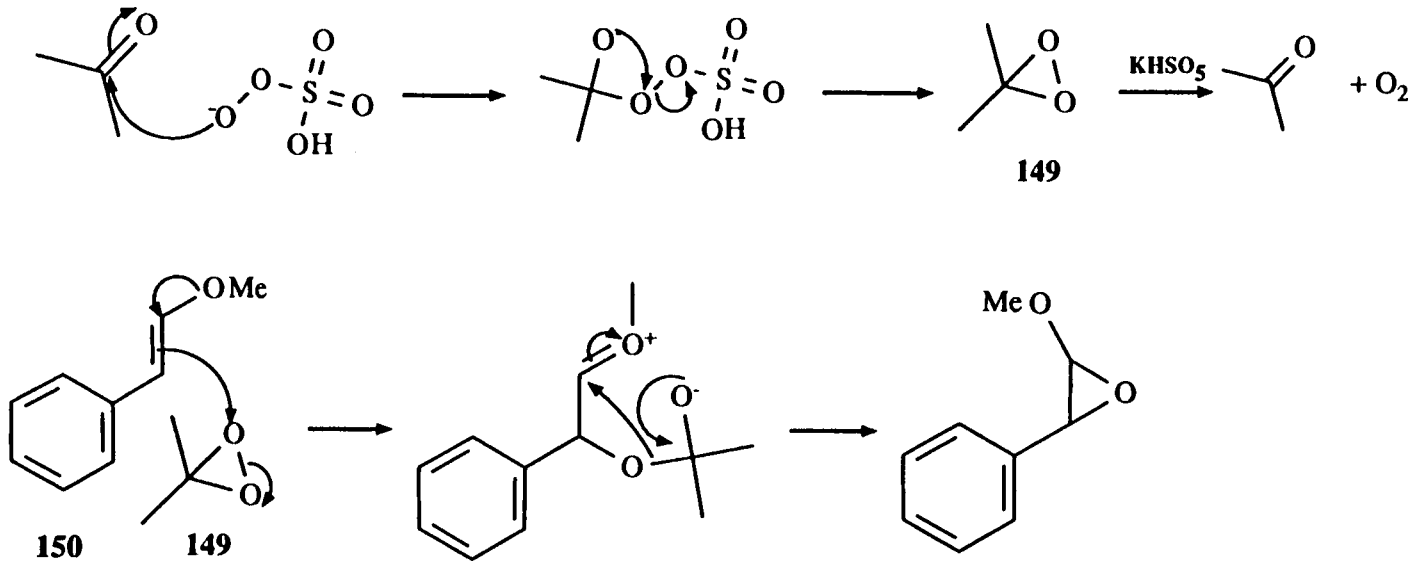
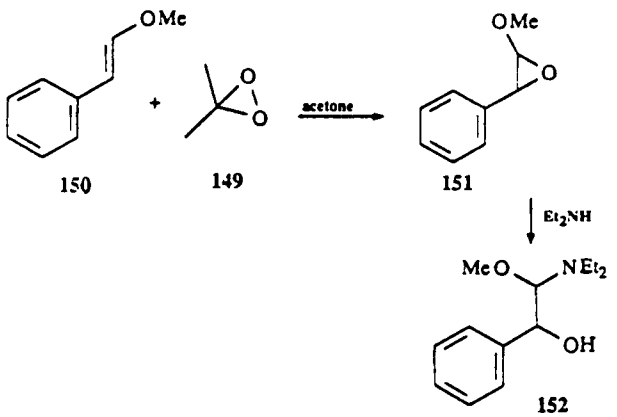


Figure 23. Epoxy ether via dimethyldioxirane.

and trapping in a liquid nitrogen trap. The difficulty in this procedure is to remove the highly reactive dimethyldioxirane from the oxone medium before continuous oxidation to oxygen occurred. The proposed mechanism for dioxirane formation and oxidation is shown in Figure 23.

The dimethyldioxirane trapped existed as a solution in wet acetone which could be dried over magnesium sulfate providing a solution of dimethyldioxirane. Determination of the concentration of dimethyl dioxirane was performed by adding a known amount of triphenylphosphine and quantitating the relative peak areas of triphenylphosphine and triphenylphosphine oxide as observed on a GC column, since triphenylphosphine oxide is the product formed by dimethyldioxirane oxidation of triphenylphosphine.

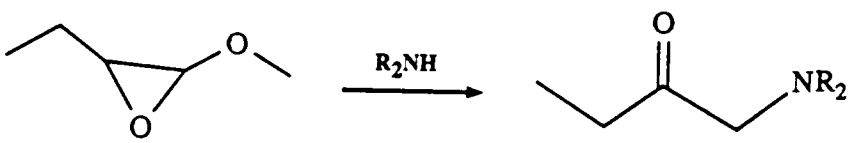
β-methoxystyrene was added to a freshly prepared solution of dimethyldioxirane in acetone[128] and the reaction was monitored by TLC to look for the disappearance of the aromatic starting material. β-methoxystyrene was consumed within seconds. The acetone was immediately removed *in vacuo* at -78°C to yield a yellow liquid which solidified into a white powder within five minutes. ^{1}H NMR of the solid did not indicate the presence of an epoxy ether and it was believed that the epoxy ether formed, and then polymerized when the acetone was removed. The observation that the yellow liquid solidified supported the theory that a polymerization process had occurred.



150 149 151 152

Scheme 61.

It was decided to trap the epoxy ether *in situ* with a nucleophile to generate a stable compound that could be characterized and therefore help prove that an epoxy ether did exist in solution. β-methoxystyrene (**150**) was added to 0.4 M acetone solution of freshly prepared dimethyldioxirane (**149**) and 400 equivalents of diethylamine was added after a few seconds (Scheme 61). After workup and purification of the major component in the crude mixture a 12% yield of 1-phenyl-2-methoxy-2-diethylaminoethanol (**152**) was isolated. This is in contrast to the compound (**153**) Kirrmann[105] isolated in a similar experiment (Scheme 62) and provided us with very good evidence that the epoxy ether had indeed been made.



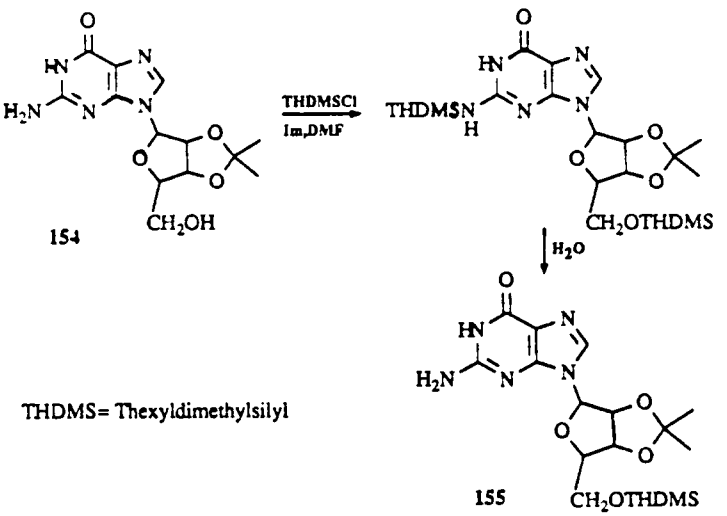
153

Scheme 62.

7.4 Reaction of Epoxy Ether with a Guanosine Derivative.

Our final task was to react the epoxy ether with guanosine. We decided to use a guanosine derivative, since guanosine is not soluble in acetone and we feared that the epoxy ether would decompose in solution before guanosine could react. Isopropylideneguanosine (**154**), which is available from Sigma, is only sparingly soluble in acetone so it was required to derivativize isopropylideneguanosine further. Protection of the primary hydroxyl of isopropylideneguanosine with chlorodimethylthexylsilane and imidazole in DMF worked best when two equivalents of the silane and four equivalents of imidazole were added. The reason for this was that selective protection of a primary hydroxyl in the presence of a primary amine is unlikely due to the fact that the amine functionality would consume the silyl chloride before the alcohol functionality could react. Selective

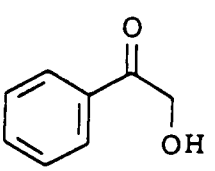
hydrolysis of a nitrogen-silicon bond in the presence of an oxygen-silicon bond is very feasible due to the fact that the silicon-oxygen bond is stronger than the silicon-nitrogen bond. The desired monoprotected derivative was thus obtained by the protection-deprotection sequence shown in



Scheme 63.

Reacting two equivalents of thexyldimethylchlorosilane and four equivalents of imidazole in DMF and using aqueous workup provided thexyldimethylsilylisopropylideneguanosine (**155**) in 74% yield. This compound proved to be soluble in acetone.

We repeated the epoxy ether trapping experiment except diethylamine was replaced with thexyldimethylsilylisopropylideneguanosine (**155**). After usual workup and isolation of all the components in the reaction mixture, a nearly quantitative recovery of the starting guanosine derivative was isolated along with a 98% yield of hydroxymethyl phenyl ketone (**156**).



156

The presence of **156** indicated that most likely one of two reaction pathways occurred. The first conclusion drawn was that hydrolysis of the epoxy ether by water provided the observed product as shown in Figure 24. However, this product was never isolated in previous reactions, where 2-methoxy-3-phenyloxirane (**151**) was the key intermediate, and it thus seemed probable that a guanosine adduct (such as that shown by structure **157**) was formed. This adduct then proceeded to be hydrolyzed during workup to the α-hydroxy aldehyde, **159**. This notion was not unreasonable since we were working on a twenty milligram scale and aqueous contamination in the solvents used in the workup could have provided the needed amount of water for quantitative hydrolysis to occur. In addition, hydrolysis of the imine (**158**) was facilitated by the purification step which included loading the crude product on a silica gel 60 flash column (Figure 25). The α-hydroxy aldehyde (**159**) then rearranged to the highly favored α-hydroxy ketone (**156**) in the presence of base (i.e **155**). This reasoning was supported by the work of Riehl, et. al.,[129] in which they reacted 1-chloro-1-phenyl-acetaldehyde (**160**) with sodium acetate to give β-acetoxy-acetophenone (**161**) where they proposed a transposition mechanism as shown in Figure 26.

7.5 Conclusions

From the results discussed in chapter 6, we felt that oxidofecapentaene-12 (**118**), was the actual mutagen. Although we were only able to get an epoxy ether in a transient form, our attempt to isolate a guanosine adduct indicated the type of system one would expect with other epoxy ethers. In other words, we believed that a result obtained in our reaction of 2-phenyl-3-methoxy-oxirane (**151**) with guanosine would yield the same α-hydroxy ketone (**156**) with any epoxy ether unless the reaction could be greatly scaled up. The significance of the formation of hydroxymethyl phenyl ketone (**156**) as it related to the genotoxic effects of epoxy ethers (or oxidofecapentaene-12) could not be determined without more data. What seemed to be apparent was that the guanosine-

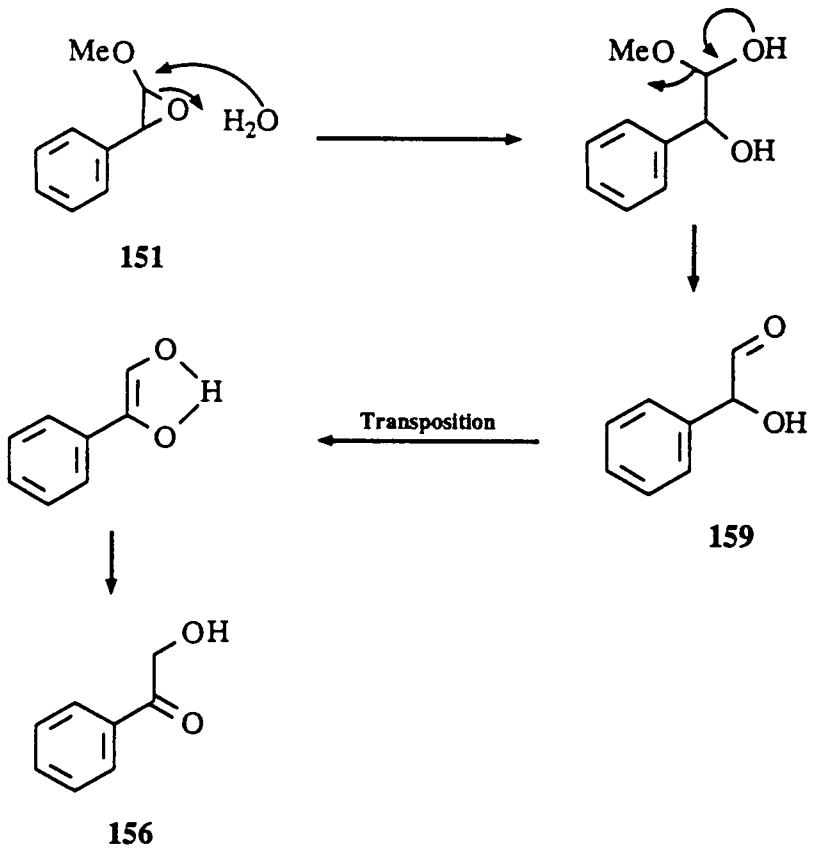


Figure 24. Hydrolysis of epoxy ether with water.

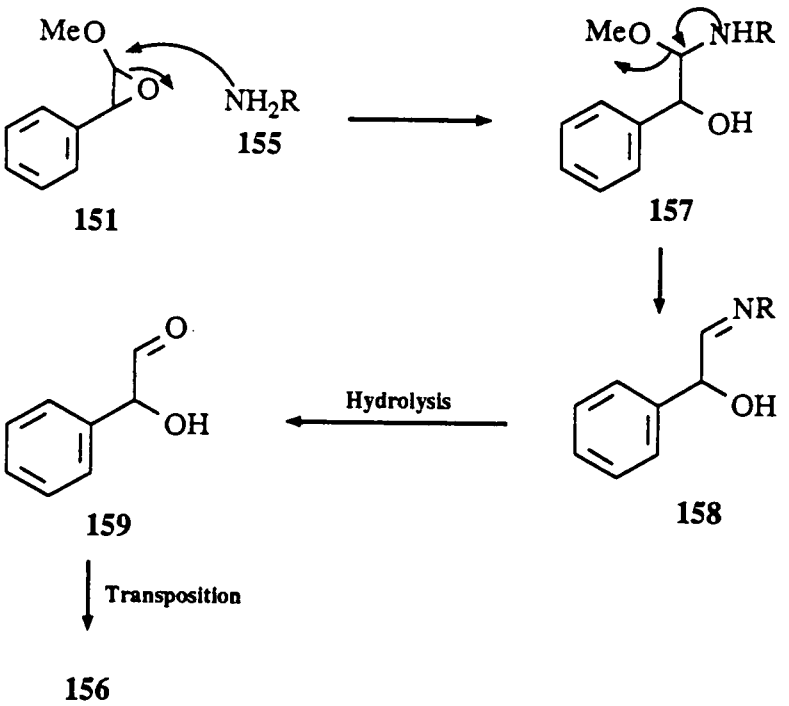


Figure 25. Hydrolysis of epoxy ether with **155** and water.

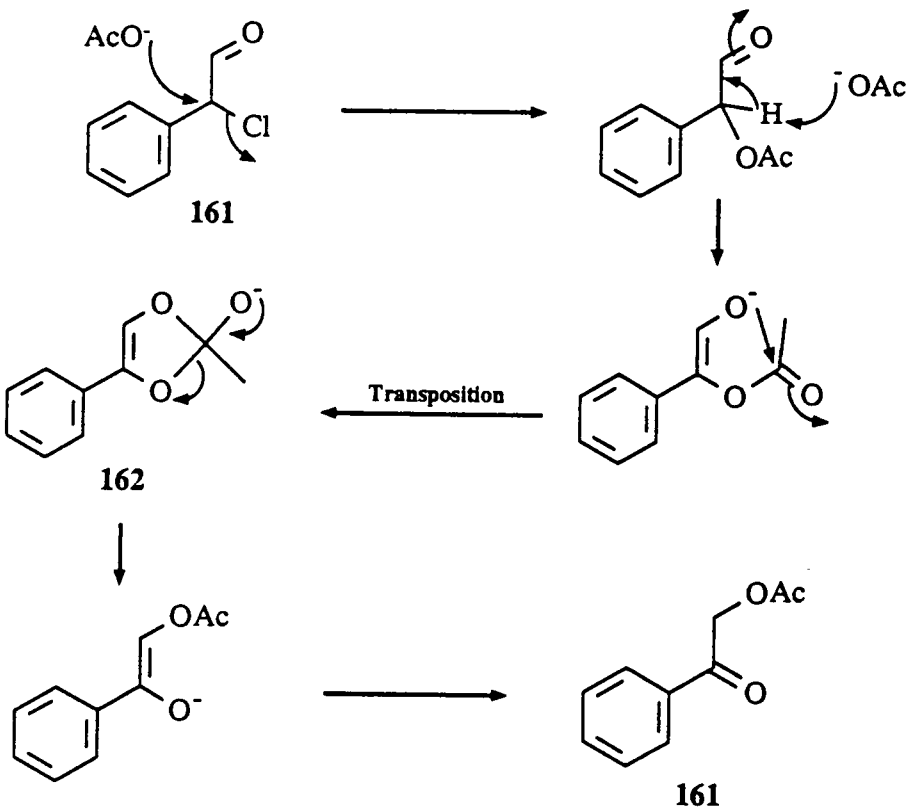


Figure 26. Transposition of α-acetoxy aldehyde.

epoxy ether adduct was easily hydrolyzed. This hydrolysis might not occur if another nucleoside base were in close proximity to the adduct where another reaction could took place leading to a DNA complex or intercalation. Perhaps a useful experiment would be to incubate an epoxy ether with DNA.

7.6 Experimental

Preparation of 1-methoxy-1-phenylthio-2-hydroxy-2-phenylethane (144).

In a flame dried flask under argon, 1 mL (6.8 mmol) of methoxymethyl phenyl sulfide (139) was dissolved in THF and the solution was cooled to -30°C at which time 3.0 mL of 2.5 M (7.5 mmol) of nBuLi in hexanes was added over 10 minutes and the solution stirred for 40 minutes. Benzaldehyde (0.76 mL, 7.5 mmol) in 5 mL of THF was added over 5 minutes. After 2 hours, water and ether were added, the aqueous layer was extracted with 5X50 mL of ether, the ether layers were combined, washed with 3X20 mL of brine, dried over magnesium sulfate, filtered and the ether removed *in vacuo*. The crude residue was loaded on a flash column packed with silica gel 60 and eluted with 20% ether in hexanes to yield a yellow oil which appeared as a single spot on TLC. Yield 330 mg (19%). ^{1}H NMR ($CDCl_3$) δ 7.42 (m, 4H, Ph), 7.37 (m, 2H, Ph), 7.29 (m, 4H, Ph), 4.61 (d, J=6.4 Hz, 1H, OCHS), 4.54 (dd, J=1.1, 7.8 Hz, 1H, OCH), 3.48 (s, 3H, OCH_3), 3.01 (d, J=1.6 Hz, 1H, OH); ^{13}C ($CDCl_3$) δ 134.0 (Ph), 128.9 (Ph), 128.5 (Ph), 128.1 (Ph), 127.2 (Ph), 126.4 (Ph), 97.8 (OCS), 74.0 (OCAr), 57.1 (MeO); IR (neat) 3510, 3110, 2980, 2940, 1600, 1490, 1470, 1455, 1195, 1110 cm^{-1}; MS (EI) *m/z* (relative intensity) 260 (M^+, 100), 240 (69), 228 (36); UV (EtOH) 214 nm.

Preparation of 1-methoxy-1-phenylthio-2-hydroxy-octa-3,5-diene (145).

In a flame dried flask under argon, 1.44 mL (9.8 mmol) of methoxymethyl phenyl sulfide (139) was dissolved in THF. The solution was to cooled to -40°C at which time 5.5 mL of 1.6 M (8.9 mmol) of nBuLi in hexanes was added over 5 minutes. After 2 hours, the solution was cooled to -78°C and 1.1 mL (8.9 mmol) of heptadienal in 10 mL of THF was added dropwise. After 1 hour, 20 mL of water was added and the solution was warmed up to room temperature. The

solution was extracted with 3X100 mL of ether, the ether extracts were combined, washed with 20 mL of water, 2X20 mL of brine, dried over magnesium sulfate, filtered and the ether removed *in vacuo*. The crude residue was loaded on a flash column packed with silica gel 60 and eluted with 40% ether in hexanes to yield a yellow liquid as a mixture as diastereomers as evidenced by 2 spots on TLC. Yield 1.67 g (72%). ^{1}H NMR ($CDCl_3$) δ 7.47 (m, 2H, Ph), 7.28 (m, 3H, Ph), 6.39 (dd, J=15.1, 10.8 Hz, 1H, =CH), 6.06 (dd, J=19.1, 10.8 Hz, 1H, =CH), 5.75 (dt, J=5.7, 19.1 Hz, 1H, =CH), 5.68 (dd, J=15.1, 6.7 Hz, 1H, =CH), 4.46 (d, J=7.1 Hz, 1H, OCHS), 4.20 (dd, J=6.7, 7.1 Hz, 1H, OCHAr), 3.51 (s, 3H, OCH_3), 2.51 (bm, 1H, OH), 2.10 (m, 2H, allylic CH_2), 1.00 (t, J=6.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 137.5, 133.7, 133.5, 133.0, 128.9, 128.5, 127.8, 127.6, 95.0 (OCS), 73.3 (OCAr), 56.6 (MeO), 25.6 (allylic C), 13.3; IR (neat) 3500, 3000, 2600, 1680, 1605, 1500, 1460, 1005, 760 cm^{-1}; MS (EI) *m/z* (relative intensity) 264 (M^+, 11), 248 (28), 232 (58), 218 (98), 153 (71), 110 (60), 77 (70), 65 (86), 57 (100); UV (EtOH) 234 nm (23,915).

Preparation of 1-methoxy-1-phenylthio-2-(ᵗbutyldimethyl)silyloxy-octa-3,5-diene (146).

The reaction procedure was identical to that to make **145** except 1.5 g (10 mmol) of ᵗbutyldimethylsilyl chloride was added at -78°C and the solution was stirred for 1 hour before the water was added. The workup was the same also. Purification was done by loading the crude residue on a flash column packed with silica gel 60 and eluted with 20% ether in hexanes to yield a yellow oil which appeared as a single spot on TLC. Yield 1.65 g (50%). ^{1}H NMR ($CDCl_3$) δ 7.49 (m, 2H, Ph), 7.24 (m, 3H, Ph), 6.17 (m, 1H, =CH), 6.02 (m, 1H, =CH), 5.70 (m, 2H, =CH), 4.58 (d, J=4.5 Hz, 0.5H, OCHS), 4.51 (d, J=5.4 Hz, 0.5H, OCHS), 4.35 (dd, J=3.8, 7.0 Hz, 0.5H, OCHAr), 4.28 (dd, J=5.4, 11.2 Hz, 0.5H, OCHAr), 3.42 (s, 1.5H, OCH_3), 3.38 (s, 0.5H, OCH_3), 2.09 (m, 2H, allylic CH_2), 0.99 (t, J=7.4 Hz, 3H, CH_3), 0.85 (s, 9H, ᵗBu), -0.01 (s, 6H, CH_3Si); ^{13}C NMR ($CDCl_3$) δ 136.8, 132.9, 132.4, 129.7, 129.4, 128.6, 127.1, 126.8, 97.4 (OCS), 96.2 (OCS), 75.9 (OCAr), 75.6

(OCAr), 57.0 (MeO), 56.4 (MeO), 25.6 (allylic C), 15.2 (^{t}BuC), 13.3 (CH_3), -4.4 (CSi), -4.6 (CSi), -4.7 (CSi); IR (neat) 2975, 2925, 2900, 1680, 1600, 1490, 1480, 1450, 1000 cm^{-1}; MS (EI) *m/z* (relative intensity) 269 (M-109, 26), 225 (82), 167 (49), 153 (99), 109 (20), 73 (100); UV (EtOH) 220 nm (47,250).

Preparation of methoxymethyl phenyl sulfone (148).

Methoxymethyl phenyl sulfide (139) (1.82 g, 11.8 mmol) was dissolved in 150 mL methylene chloride. Potassium permanganate (5.6 g, 35.4 mmol) in 250 mL of water was added and the solution was stirred vigorously for 96 hours. The solution was filtered and the purple aqueous filtrate was extracted with 3X100 mL of methylene chloride. The organic extracts were combined and washed with 1 g of hydrazine hydrochloride in 100 mL, 20 mL of brine, filtered and the solvent removed *in vacuo* to yield a white amorphous solid. The solid was recrystallized from THF and hexanes to yield white crystals. Yield 1.08 g (50%). mp 66-67°C (Lit. mp 67.5-68.5°C); ^{1}H NMR ($CDCl_3$) δ 7.96 (m, 2H, Ph), 7.62 (m, 3H, Ph), 4.54 (s, 2H, OCH_2SO_2), 3.69 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 134.1 (Ph), 129.2 (Ph), 128.8 (Ph), 87.8 ($OCSO_2$), 61.2 (OCH_3); IR (CCl_4) 1460, 1340, 1310, 1210, 1160, 1130, 1090 cm^{-1}; UV (EtOH) 210 nm (10,695).

Preparation of 1-methoxy-1-phenylsulfonyl-2-hydroxy-octa-3,5-diene (147).

In a flame dried flask under argon, 200 mg (1.08 mmol) of methoxymethyl phenyl sulfone (148) was dissolved in THF. The solution was cooled to -40°C at which time 0.64 mL of 1.6 M (1.03 mmol) of nBuLi in hexanes was added over 2 minutes and the solution was stirred at this temperature for 1 hour. The solution was cooled to -78°C and 0.13 mL (1.03 mmol) of heptadienal in 5 mL of THF was added dropwise. After 1 hour, 5 mL of water was added and the solution was warmed to room temperature. The solution was extracted with 3X50 mL of ether, the ether extracts

were combined, washed with 20 mL of brine, dried over magnesium sulfate, filtered and the ether removed *in vacuo*. The residue was loaded on a flash column packed with silica gel 60 and eluted with 40% hexanes in ether to yield a light yellow oil which appeared as a single spot on TLC. Yield 259 mg (85%). ^{1}H NMR ($CDCl_3$) δ 8.00 (m, 2H, Ph), 7.71 (m, 1H, Ph), 7.60 (m, 2H, Ph), 6.30 (dd, J=10.2, 8.0 Hz, 1H, =CH), 5.99 (m, 1H, =CH), 5.78 (m, 2H, =CH), 5.55 (m, 0.5H, $OCHSO_2$), 5.47 (m, 0.5H, $OCHSO_2$), 4.16 (d, J=2.9 Hz, 0.5H, OCH), 4.08 (d, J=7.3 Hz, 0.5H, OCH), 3.60 (s, 1.5H, OCH_3), 3.56 (s, 1.5H, OCH_3), 2.09 (m, 2H, allylic CH_2), 1.02 (t, J=3.8 Hz, 1.5H, CH_3), 0.98 (t, J=4.5 Hz, 1.5H, CH_3); ^{13}C NMR ($CDCl_3$) δ 138.2, 138.0, 133.9, 133.8, 129.6, 129.0, 128.8, 128.5, 128.0, 127.9, 126.8, 126.0, 124.8, 99.4 ($OCHOSO_2$), 87.8 ($OCHSO_2$), 70.4 (OCH), 70.2 (OCH), 53.3 (OCH_3), 25.6 (allylic CH_2), 13.2 (CH_3); IR (neat) 3540, 3000, 1500, 1460, 1310, 1160, 1120, 1090, 1000 cm^{-1}; MS (EI) *m/z* (relative intensity) 155 (M-141, 10), 141 (14), 125 (28), 77 (100); UV (EtOH) 230 nm (4933).

Preparation of 1-O-methylthiomethyl-2,3-O-isopropylideneglycerol (140).

In a flame dried flask fitted with a dropping funnel and flushed with argon, 0.6 g (0.025 mol) of sodium hydride was stirred in THF and 3.1 mL (0.025 mol) of solketal in 20 mL of THF was added dropwise. The solution was stirred for 20 hours and 2.2 mL (0.025 mmol) of neat chloromethyl methyl sulfide was added dropwise over 5 minutes. The solution was stirred at room temperature for 2 hours where a precipitate formed. The mixture was filtered and washed with 3X100 mL of chloroform. The solvents were removed *in vacuo* to yield a yellow oil which appeared as a single spot on TLC. Yield 1.01 g (21%). ^{1}H NMR ($CDCl_3$) δ 4.70 (d, J=13.9 Hz, 1H, OCH_2S), 4.62 (d, J=13.9 Hz, 1H, OCH_2S), 4.27 (m, 1H, CH), 4.04 (dd, J=6.9, 8.2 Hz, 1H,), 3.70 (dd, J=6.5, 8.2 Hz, 1H), 3.59 (dd, J=4.8, 10.4 Hz, 1H), 3.52 (dd, J=5.8, 10.4 Hz, 1H), 2.10 (s, 3H, SCH_3), 1.39 (s, 3H, CH_3), 1.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 109.4 (OCH_2S), 75.7, 74.4, 74.3, 68.6, 66.8,

66.6, 26.7, 25.3, 15.8, 13.7.

Preparation of 2,2,2-trifluoroethanyl methylthiomethyl ether (142).

In a flame dried flask fitted with an addition funnel and an argon atmosphere, 660 mg (27.5 mmol) of sodium hydride was stirred with ether and the mixture cooled to 0°C. 2,2,2-Trifluoroethanol (2 mL, 27.5 mmol) was added dropwise over 2 minutes. The solution was warmed to room temperature and stirred for an additional 30 minutes. Chloromethyl methyl sulfide (2.3 mL, 27.5 mmol) was added dropwise and the solution was stirred at room temperature for 14 hours where a precipitate slowly formed. The mixture was drop filtered through 3 funnels. The ethereal solution was distilled at atmospheric pressure to yield a colorless oil. Yield 954 mg (22%). bp 107-110°C (1 atm.). ^{1}H NMR ($CDCl_3$) δ 4.71 (s, 2H, OCH_2S), 3.91 (q, J=7.9 Hz, 2H, CF_3CH_2O), 2.12 (s, 3H, SCH_3); ^{13}C NMR ($CDCl_3$) δ 144.8 (CF), 130.9 (CF), 117.1 (CF), 103.3 (CF), 75.9 (OCH_2S), 66.7 (CH_2O), 65.0 (CH_2O), 63.3 (CH_2O), 61.6 (CH_2O), 13.2 (SCH_3); IR (neat) 2970, 1440, 1295, 1170, 1110 cm^{-1}; MS (EI) *m/z* (relative intensity) 160 (M^+, 48), 113 (100), 83 (33), 61 (89).

Preparation of 2-m-chlorobenzoyl-3-hydroxy-2,3,5,6-tetrahydropyran (131).

In a flame dried flask under argon, 1 mL (10.96 mmol) of dihydropyran was dissolved in ether and the solution was cooled to -30°C. In a separate flame dried flask under argon, 2.36 g (ca. 10.96 mmol) of 80-85% MCPBA was dissolved in ether. The MCPBA solution was combined with the dihydropyran solution and the combined solutions were immediately poured onto a flash column packed with activated basic alumina and eluted with 100 mL of ether. The ether was removed *in vacuo* to yield white crystals. Yield 1.87 g (67%). ^{1}H NMR (DMSO) δ 7.99 (m, 2H, Ar), 7.74 (d, J=7.7 Hz, 1H, Ar), 7.58 (dd, J=7.7, 7.7 Hz, 1H, Ar), 5.70 (d, J=4.6 Hz, 1H, OCHOCOAr), 5.28 (d, J=4.6 Hz, 1H, OH), 3.94 (m, 1H, OCH), 3.57 (m, 2H, OCH_2), 1.97 (m, 1H),

1.80 (m, 1H), 1.62 (m, 1H), 1.44 (m, 1H); ^{13}C NMR (DMSO) δ 163.2 (OCOAr), 143.8 (Ar), 133.4 (Ar), 131.5 (Ar), 130.7 (Ar), 128.8 (Ar), 128.0 (Ar), 95.9 (OCOCOAr), 65.0 (OCH), 63.2 (OCH_2), 27.4, 21.0; IR (neat) 3500, 3000, 1760, 1480, 1400, 1260, 1080, 760 cm^{-1}; MS (EI) *m/z* (relative intensity) 256 (M^+, 4), 238 (12), 156 (100), 100 (100); UV (EtOH) 225 nm (9,371).

Preparation of 1-ethoxy-hepta-1,3,5-triene (99).

In a flame dried flask under argon, 2.2 g (19.6 mmol) of potassium tert-butoxide was dissolved in THF and the solution was cooled to -60°C. Heptadienal (1 mL, 8 mmol) in 5 mL of THF was added and the solution was warmed to room temperature. After 45 minutes, 19.6 of 1 M (19.6 mmol) of triethyloxonium tetrafluoroborate in methylene chloride was added and the solution was stirred for 30 minutes. The solution was filtered and the precipitate was washed with 5X50 mL of ether. The solvents were removed *in vacuo* and the crude product was loaded on a flash column packed with neutral alumina (activity I) and eluted with hexanes to yield a yellow liquid which proved to be ca. 95% pure by ^{1}H NMR. Yield 792 mg (72%). ^{1}H NMR ($CDCl_3$) δ 6.56 (d, J=12.2 Hz, 1H, =CH), 6.32 (dd, J=11.3, 14.7 Hz, 1H, =CH), 6.15 (m, 1H, =CH), 6.01 (m, 1H, =CH), 5.65 (dd, J=11.3, 12.4 Hz, 1H, =CH), 5.40 (m, 1H, =CH), 3.82 (q, J=6.6 Hz, 2H, OCH_2), 1.74 (d, J=6.6 Hz, 3H, allylic CH_3), 1.28 (t, J=6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 150.5 (=CHO), 129.9 (=CH), 129.0 (=CH), 123.3 (=CH), 122.9 (=CH), 107.3 (=CH), 65.5 (OCH_2), 14.6 (allylic CH_2), 13.1 (CH_3); IR (neat) 3010, 1645, 1605, 1500, 1460, 1425, 1410, 1190, 1120, 1000; MS (EI) *m/z* (relative intensity) 138 (M^+, 80), 95 (98), 85 (100), 82 (99); UV (EtOH) 275 nm (13,800).

Preparation of trimethylsilyl penta-1,3-dienyl ether (135).

In a flame dried flask under argon, 8.6 g (76.8 mmol) of potassium tert-butoxide was dissolved in THF and the solution was cooled to -40°C. Penta-2-en-1-al (3 mL, 30.7 mmol) was

added and the solution was stirred for 2 hours while warming up to room temperature. Trimethylsilyl chloride (9.8, 76.8 mmol) was added and the solution was stirred for 30 minutes. The solution was poured into a mixture of 200 mL of water and 200 mL of hexanes. The hexane extracts were combined and washed with 4X100 mL of water, dried over sodium sulfate, filtered and evaporated *in vacuo*. The crude residue was vacuum distilled to yield a yellow liquid which existed as a mixture of geometric isomers as evidenced by ^{1}H NMR. Yield 1.44 g (30%). bp 30-35°C (3.0 mm Hg). ^{1}H NMR ($CDCl_3$) δ 6.49 (d, J=11.6 Hz, 1H, =CHO), 5.95 (dd, J=11.6, 20.2 Hz, 1H, =CH), 5.85 (m, 1H, =CH), 5.40 (m, 0.5H, =CH), 5.24 (m, 0.5H, =CH), 1.68 (m, 3H, allylic CH_3), 0.20 (2s, 9H, CH_3Si).

Preparation of 4-bromo-pent-2-en-1-al (**134**).

In a flame dried flask under argon, 832 mg (5.33 mmol) of trimethylsilyl penta-1,3-dienyl ether (**135**) was dissolved in THF and cooled to 0°C. NBS (1.04 g, 5.86 mmol) was added and the solution was stirred at 0°C for 30 minutes. The solution was poured into a 100 mL hexane/100 mL aqueous bilayer where the water layer contained 1 g of sodium chloride and 1 g of sodium bicarbonate. The aqueous layer was extracted with 2X100mL of hexanes. The hexane extracts were dried over sodium sulfate, filtered and hexanes removed *in vacuo* to yield a light yellow liquid. Pentane was immediately added and the mixture was filtered and the pentane was removed *in vacuo* to yield a light yellow liquid which appeared as a single spot on TLC. Yield 593 mg (68%). ^{1}H NMR ($CDCl_3$) δ 9.56 (d, J=8.0 Hz, 1H, CHO), 6.88 (dd, J=17.2, 7.6 Hz, 1H, =CH), 6.12 (dd, J=8.0, 17.2 Hz, 1H, =CH), 4.77 (m, 1H, CHBr), 1.83 (d, J=3.1 Hz, 3H, CH_3).

Preparation of trimethylsilyl hepta-1,3,5-trienyl ether (**133**).

In a flame dried flask under argon, 6.75 g (60 mmol) of potassium tert-butoxide was

dissolved in THF and the solution was cooled to -40°C. Heptadienal (3 mL, 24 mmol) was added in one portion and the solution was stirred for 2 hours and allowed to warm up to room temperature. Trimethylsilyl chloride (7.8 mL, 60 mmol) was added in one portion. After 1 hour, the solution was poured into a 50 mL hexanes/50 mL water mixture. The hexanes were washed with 2X50 mL of water, 10 mL of brine, dried over magnesium sulfate, filtered and the hexanes evaporated *in vacuo* to yield a yellow liquid which was approximately 90% pure by ^{1}H NMR. Crude Yield 4.24 g (97%). ^{1}H NMR ($CDCl_3$) δ 6.55 (d, J=11.9 Hz, 0.5H, =CH), 6.42 (m, 0.5H, =CH), 6.30 (m, 0.5H, =CH), 6.14 (m, 0.5H, =CH), 6.02 (m, 1.5H, =CH), 5.76 (dd, J=11.6, 11.6 Hz, 0.5H, =CH), 5.60 (m, 0.5H, =CH), 5.48 (m, 1H, =CH), 5.28 (m, 1H, =CH), 1.73 (d, J=1.7 Hz, 1.5H, allylic CH_3), 1.70 (d, J=1.7 Hz, 1.5H, allylic CH_3), 0.19 (s, 4.5H, CH_3Si), 0.17 (s, 4.5H, CH_3Si).

Preparation of 6-bromo-hepta-2,4-dien-1-al (132).

In a flame dried flask under argon, 4.24 g (23.3 mmol) of trimethylsilyl hepta-1,3,5-trienyl ether (133) was dissolved in THF and the solution was cooled to 0°C. NBS (4.56 g, 25.6 mmol) was added and stirred at 0°C for 15 minutes. The solution was poured into 200 mL of an aqueous solution of 5 g of sodium chloride and 5 g sodium bicarbonate. The aqueous solution was extracted with 3X100 mL of hexanes and the hexanes extracts were washed with 20 mL of brine, dried over magnesium sulfate, filtered and the hexanes evaporated *in vacuo*. The crude product was dissolved in pentane and filtered and the pentane was removed *in vacuo*. The yellow liquid was dried under high vacuum for 8 hours to yield a yellow liquid which appeared as a single spot on TLC. Yield 3.57 g (81%). ^{1}H NMR ($CDCl_3$) δ 9.56 (d, 1H, J=7.9 Hz, CHO), 7.07 (dd, J=18.8, 9.8 Hz, 1H, =CH), 6.38 (m, 2H, =CH), 6.16 (dd, J=7.0, 18.8 Hz, 1H, =CH), 4.74 (m, 1H, CHBr), 1.82 (d, J=3.1 Hz, 3H, CH_3); IR (neat) 2990, 2960, 2900, 1700, 1655, 1480, 1000 cm^{-1}.

Preparation of 1-phenyl-2-methoxy-2-diethylamino-ethan-1-ol (152).

To 20 mL of 0.04 M (0.80 mmol) of freshly prepared dimethyldioxirane in acetone, 0.03 mL (0.24 mmol) of β-methoxystyrene was added and stirred at room temperature for 10 seconds. Diethylamine (10 mL, 100 mmol) was added and the solvents immediately removed *in vacuo*. The crude residue was loaded on a flash column packed with triethylamine pretreated silica gel 60 and eluted with 5% methanol in methylene chloride to yield a colorless liquid, which appeared as a single spot on TLC. Yield 6 mg (12%). ^{1}H NMR ($CDCl_3$) δ 7.39 (m, 2H, Ph), 7.30 (m, 3H, Ph), 5.72 (d, J=6.2 Hz, 1H, OCHN), 4.74 (m, 1H, PhCH), 3.39 (s, 3H, CH_3O), 2.95 (m, 4H, CH_2N), 2.70 (d, J=4.3 Hz, 1H, OH), 1.08 (t, J=6.5 Hz, 6H, CH_3); MS (EI) m/z (relative intensity) 223 (M^+, 1), 205 (2), 119 (17), 105 (100), 91 (50), 77 (98).

Preparation of 6-O-(thexyldimethyl)silyl-3,4-O-isopropylideneribofuranosylguanidine (155).

Isopropylideneguanosine (490 mg, 1.52 mmol) was dissolved in DMF. Imidazole (462 mg, 6.8 mmol) and 0.67 mL (3.4 mmol) of thexyldimethylsilyl chloride was added and the solution was stirred at room temperature for 2 hours. The solution was added to 100 mL of water. The aqueous mixture was extracted with 2X200mL of ethyl acetate. The ethyl acetate extracts were combined, washed with 4X100mL of water, 50 mL of brine, dried over magnesium sulfate, filtered and evaporated *in vacuo*. The residue was recrystallized from chloroform and hexanes to yield white crystals. Yield 526 mg (74%). mp 260-262°C. ^{1}H NMR ($CDCl_3$) δ 11.94, (bs, 1H, CONH), 7.69 (s, 1H, =CH), 6.45 (bs, 2H, NH_2), 5.95 (d, J=1.9 Hz, 1H, NCHO), 5.14 (dd, J=1.9, 5.3 Hz, 1H, OCH), 4.86 (dd, J=1.0, 5.3 Hz, 1H, OCH), 4.30 (m, 1H, OCH), 3.76 (m, 2H, OCH_2), 1.58 (s, 3H, CH_3), 1.55 (m, 1H, CH), 1.37 (s, 3H, CH_3), 0.84 (d, J=8.6 Hz, 6H, CH_3), 0.82 (s, 6H, CH_3), 0.07 (s, 6H, CH_3Si); ^{13}C NMR ($CDCl_3$) δ 160.2, 159.3, 156.1, 153.8, 151.8, 136.3, 114.2, 90.3, 87.0, 84.6, 81.4, 63.6, 34.2, 27.3, 25.5, 20.4, 18.5, -3.4, -3.5; IR (neat) 3350, 3175, 2960, 1700, 1630, 1610,

1600, 1540, 1375, 1090; MS (CI) *m/z* (relative intensity) 466 (M+1, 100), 381 (12), 234 (13).

Preparation of phenyl hydroxymethyl ketone (156) .

To 20 mL of 0.02 M (0.4 mmol) of freshly prepared dimethyldioxirane in acetone was added 0.02 mL of β-methoxystyrene followed immediately by 186 mg (0.4 mmol) of thexyldimethylsilylisopropylideneguanosine (155). The solution was stirred for 15 minutes at which time the solvent was removed *in vacuo*. Unreacted methoxystyrene was removed under high vacuum for 12 hours. The residue was loaded on a flash column packed with silica gel 60 and eluted with 10% hexanes in ether to yield white crystals which were recrystallized from methanol. Yield 20 mg (98%). mp 85-86°C (Lit. mp 86-87°C); ^{1}H NMR ($CDCl_3$) δ 7.91 (dd, J=1.1, 6.8 Hz, 2H, Ph), 7.62 (dd, J=1.1, 8.1 Hz, 1H, Ph), 7.49 (dd, J=8.1, 6.8 Hz, 2H, Ph), 4.87 (d, J=4.1 Hz, 2H, OCH_2), 3.49 (t, J=4.1 Hz, 1H, OH); (Lit.[130] ^{1}H NMR δ 7.25-7.90, 4.86, 3.63); ^{13}C NMR ($CDCl_3$) δ 198.5 (C=O), 134.2 (Ph), 133.7 (Ph), 129.0 (Ph), 127.7 (Ph), 65.5 (CH_2O); (Lit. ^{13}C δ 198.6, 134.4, 129.1, 127.8, 65.6); MS (EI) *m/z* (relative intensity) 136 (M^+, 4), 105 (100), 77 (74).

VIII Summary

8.1 Review of Precursor Work.

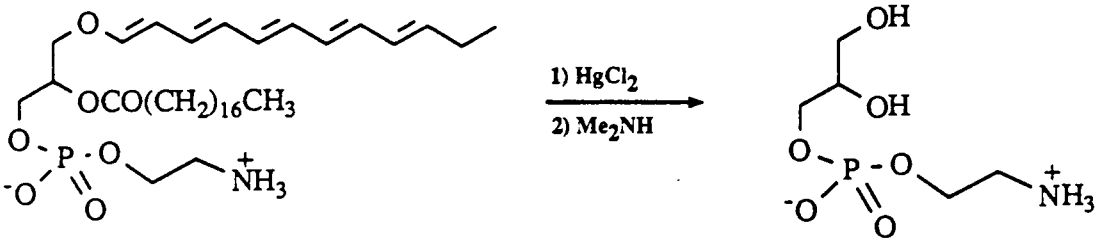
The existence of precursor has been well documented by the work by Wilkins, Kingston and coworkers.[48,49,52,53] The incubation of fecal samples with Bacteroides sp. to form FP-12 was the initial result indicating the presence of the precursor. This work was further pursued by Wilkins and coworkers and they were able to show that the precursor was a compound that was found most readily amongst excreters of FP-12.[52] The source of the precursor may come from colonic flora, the host or the diet. Although it is not known which of these sources the precursor originates from, recent work by Wilkins and Van Tassell has shown that fecal samples from germ free-pigs produced fecapentaene-12 when incubated with Bacteroides spp.[50] and this result indicates that the precursor does not originate from the colonic flora.

The structure of the precursor is not at present elucidated. The evidence obtained thus far indicates that the precursor is a phospholipid with the pentaenyl moiety present. The pentaenyl moiety is known to be present because the UV spectrum of the precursor is identical with that of FP-12. The fact that precursor is a phospholipid was proved by the following experimental facts:

1. Hydrolysis of the precursor and subsequent GC/MS analysis of the crude products indicated the presence of a series of fatty acid esters.
2. Comparative TLC analysis with the precursor and the synthetic perhydroprecursor (**22**) and the observance of similar amphipolar nature of both of the compounds indicated that a lipophilic fatty acid ester and a hydrophilic polar group existed in the precursor.
3. The positive result of precursor reacting with phosphoesterase indicated that the hydrophilic functionality was a phosphate ester. The precursor was shown to be

a plasmalogen with extended conjugation of the enol ether moiety. Since plasmalogens historically have proven to be of the phosphatidylethanolamine or the phosphatidylcholine variety, the focus of the attention of determining the nature of the phosphate ester was limited to these two.

It should be pointed out that very recently Kingston, Keyes, Wilkins, Van Tassell and coworkers have provided preliminary evidence as to the nature of the phosphate ester. By subjecting the precursor to a series of hydrolysis reactions, a phosphatidyl molecule could be isolated. The hydrolyses removed the pentaenyl and the fatty acid ester functionalities from the glycerol backbone of the precursor, retaining the phosphate ester moiety (Scheme 64). The phosphatidyl structure could then be determined by comparative chromatographic techniques with



Scheme 64.

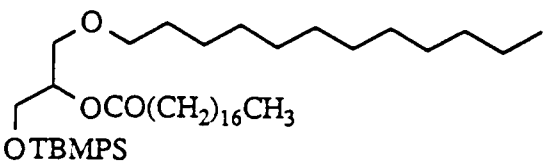
standard phosphatidyl molecules. The preliminary results obtained indicated that the phosphate ester of the precursor was phosphorylethanolamine. This recent result might indicate that a synthesis of a proposed precursor should include a phosphorylethanolamine as the hydrophilic portion.

8.1.1 Review of the Synthetic Work of Perhydroprecursor and Precursor

The synthesis of perhydroprecursor and precursor proved to be a challenging problem, the

constraints that we felt were necessary, (i.e. avoidance of acidic conditions, strongly basic conditions, hydrogenolysis or free radical reactions) limited the methodologies available for this work.

In the synthesis of perhydroprecursor we were able to circumvent all of these potential problems by applying a protecting group on the key primary hydroxyl group which could be deprotected under mildly basic conditions. We found that ester migration is best reduced by using a protecting group that can be removed under conditions that minimize ester migration. The protecting group that afforded this desired result was the silyl ketal (**40**). The work of Guindon,[71]



40

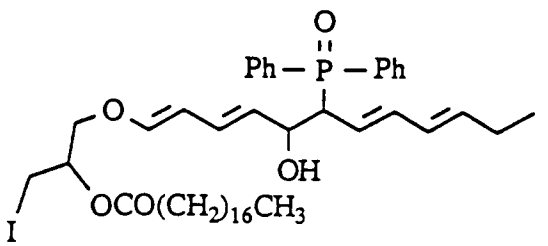
using silyl ketals as a selective protecting group of alcohols was extended as a potential protecting group of alcohols where unwanted side products could be prevented.

We discovered that the presence of the primary tosylate and the presence of the primary silyl ketal on a glycerol backbone prevented acylation of the secondary hydroxyl of the glycerol backbone. We developed a unique way to generate a 2-acyl glycerol compound. This methodology included the preparation of 2-stearoyl-1,3-O-benzylideneglycerol (**62**) and subsequent hydrolysis of the acetal with magnesium bromide etherate[74] to form 2-stearoylglycerol (**63**). The use of magnesium bromide etherate provided a method to hydrolyze the acetal center without hydrolysis of an ester or a silyl ether.

The presence of an acyl group alpha to a tosylate greatly reduced the reactivity of the tosylate toward nucleophilic substitution. It was for this reason that it was necessary to introduce

the glutaconate functionality early in the synthesis of the precursor. This approach obliged us to apply a series of innovative synthetic steps to get an intermediate which could later be taken to the precursor.

The first of such steps was to use the Wittig reagent as a method for protection of an aldehyde. It has been shown in the past that lithium salts of the hydroxyl of the Wittig adduct undergo retro-Wittig reactions and that the analogous potassium salt undergoes elimination typical of Wittig reactions.[31,32] The potassium salt of our Wittig adduct (**89**) underwent a retro-Wittig which



89

was a potential problem when the precursor synthesis was to be completed. It is our belief that conditions could be worked out to afford the desired pentaenyl ether.

We also found success in the opening of an epoxide by using sodium iodide and ammonium chloride. This technique was important because the acid concentration was maintained at a low level. Therefore, any acid that was introduced by the slow solvolysis of ammonium chloride was immediately neutralized by the alkoxide salts before hydrolysis of the enol ether could occur.

It is our belief that the major obstacle in our second generation precursor synthesis is the displacement of the iodide in the key intermediate (**89**). I firmly believe that the iodide can be substituted for a phosphate without affecting the other functionalities present on the substrate.

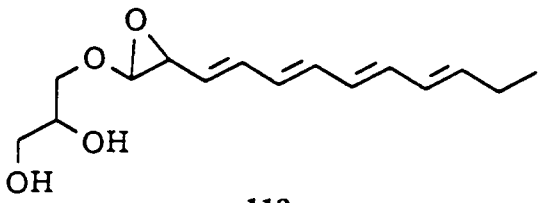
Since silver salts decompose the substrate in preference to iodide displacement, then perhaps a metal could be used which has comparable iodide reactivity to that of silver but does not complex with double bonds quite as readily.

8.2 DNA Binding Review.

The most significant result to come out of this work was the experiments which indicated that fecapentaene-12 does not bind to DNA. The lack of guanosine adduct formed with FP-12, and the low level of binding with DNA as seen from the UV studies and radiolabelled FP-12 binding studies were the key experiments that supported this theory.

The species which actually does bind to DNA was not definitely determined. We considered hydrolyzed FP-12 (i.e. dodecatetraenal) as the molecule which FP-12 was metabolized to *in vivo* which then reacted with DNA. It has been shown that undecatetraenal was not significantly mutagenic by the Ames test,[41] however, this information by itself can not preclude tetraenal as the FP-12 metabolite. Alternatively, FP-12 may serve as a protected tetraenal and hydrolysis of FP-12 occurs in close proximity to the nucleus where DNA binding then occurs. However, undecatetraenal did not form adducts with guanosine and since acrolein and crotonaldehyde did form guanosine adducts[91,92] we felt that the tetraenal was not a DNA-reactive intermediate.

The alternative FP-12 metabolite we considered was oxidofecapentaene-12 (**118**). It should be remembered that the Ames test was run in an aerobic environment and that radiolabelled FP-12 did not show any decreased binding when the incubation experiment was performed in the presence of air. These two experiments did not provide conclusive evidence that oxidofecapentaene-12 (**118**) was the metabolite which binds to DNA but they did not exclude this possibility either.



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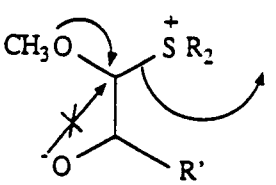
8.2.1 Review of the Work on Epoxy Ether Synthesis.

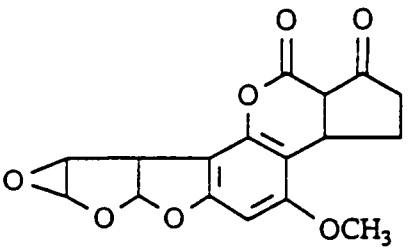
Oxidofecapentaene-12 is an epoxy ether, and it therefore became necessary to make an epoxy ether of similar constitution as that of oxidofecapentaene-12 (i.e. at least one double bond) to test the feasibility of oxidofecapentaene-12 as the metabolite that would bind to DNA.

The synthesis of an epoxy ether proved to be very difficult; it was much more difficult than the previous reports indicated. We initially tried to make an epoxy ether by epoxidizing the enol ether, dihydropyran, with MCPBA and found that rapid hydrolysis of the presumed epoxy ether to an α-hydroxy acetal ester (**131**) occurred. Reaction of 6-bromoheptadienal (**132**) or 4-bromopentenal (**134**) with alkoxide salts resulted in deprotonation of the acidic proton instead of alkoxide attack on the carbonyl carbon. This result did not agree with what Stevens[104,107] observed when he reacted sodium methoxide with α-chloro aldehydes. Apparently the extra double bond increased the acidity of the proton to a point which surpassed the electrophilicity of the carbonyl carbon. The sulfur ylide chemistry studied showed that decomposition of α-alkoxy sulfenium cations (**163**) occurred before epoxy ether could form.



163

Finally an epoxy ether was obtained through epoxidation of β-methoxystyrene by dimethyldioxirane.[126,127,128] It should be noted that oxidoaflatoxin B (**122**) has just recently been made by this method.[131] Although an epoxy ether from this method was never observed directly, the isolation of 1-phenyl-2-methoxy-2-diethylaminoethanol (**152**) by *in situ* trapping of the epoxy ether with diethylamine indicated that an epoxy ether had indeed been formed.



122

Reaction of diprotected guanosine (**155**) with the epoxy ether (**151**) was done in hopes of isolating an adduct. The isolation of a guanosine adduct would have provided preliminary evidence on the reasonableness of oxidofecapentaene-12 (**118**) in the mutagenic pathway of FP-12. The isolation of hydroxymethyl phenyl ketone (**156**) indicated that a transient guanosine adduct may have been formed but that the adduct proved to be easily hydrolyzed. There is a distinct difference between the reaction of the epoxy ether, **151**, with diethylamine and the same reaction with protected guanosine. Guanosine, being a primary nucleophile, would form an adduct with the epoxy ether which could very easily eliminate methanol forming the α-hydroxy imine **158** as shown in Figure 25; conversely the analogous adduct with diethylamine does not have the same ability to eliminate methanol. Rearrangement of **159** is the final mechanistic process that led to the formation of hydroxymethyl phenyl ketone (**156**). Isolation of **157** or **158** may or not be possible, but as mentioned earlier an experiment just as critical would be to incubate an epoxy ether with DNA.

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