TOWARD THE DESIGN, SYNTHESIS AND EVALUATION OF PROTEIN KINASE C INHIBITORS

by

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Dissertation Submitted to the Faculty of the
Virginia Polytechnic Institute and State University
In Partial Fulfilment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY
in
CHEMISTRY

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April, 1996

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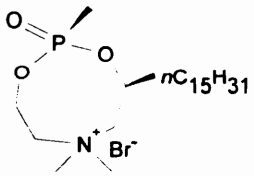
(Abstract)

Protein Kinase C (PKC) represents an important regulatory element in the signal transduction pathways of mammalian cells. Research interest has increased enormously since the discovery that PKC plays critical roles in cell differentiation, tumor promotion, oncogenesis and cell regulatory processes.

The primary driving force of this project was the study and development of enantioselective PKC inhibitors. To accomplish this objective the four stereoisomers, (2*S*/4*S*)-, (2*R*/4*S*)-, (2*R*/4*R*)-, and (2*S*/4*R*)-6-*N*,*N*-dimethyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphacyclooctane bromides (**1a-d**) were synthesized and evaluated.



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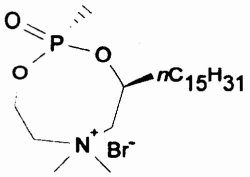

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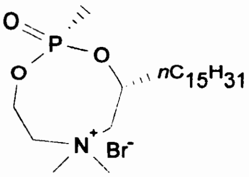

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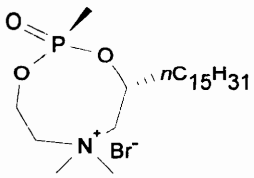

1d

Long-alkyl chain optically pure epoxides, the key intermediates for the synthesis, were prepared from relatively inexpensive glyceraldehyde surrogates. Several other intermediates exhibited other biological responses including spermicidal, anti-HIV, mycobactericidal, and anti-cancer activities.

ACKNOWLEDGMENTS

First, I would like to extend my gratitude toward the Faculty of the Chemistry Department of Louisiana State University (LSU) where I began my graduate studies. Dr. Steve Watkins and Dr. Daniel Fogel deserve special mention for their help and support during my initial adaptation to graduate school. My gratitude is also extended to my friends, Drs. Alberto Ugaz, Rosario Benites, Javier Nakamatsu and Ms. Liliana Unten. We all were part of the Peruvian crew who migrated north to continue advanced studies. Their friendship and companionship helped me get through the transition to a new country and made my stay at Baton Rouge more enjoyable.

Equally appreciated are the people from Virginia Tech. Many thanks to my graduate committee: Drs. Richard Gandour, Tomas Hudlicky, Neal Castagnoli, Karen Brewer and James Tanko, for their support and tolerance of my graduate efforts. Thanks are also due to Dr. Joseph Merola, Dr. Michael Calter, and Dr. Paul Deck; who helped me when necessary and for participating in my oral examinations.

My appreciation is extended to the Analytical Services, especially to Tom Glass who had the patience to teach me how to run critical NMR experiments and other spectral data handling. Thanks Tom for taking the time. I am similarly indebted to Kim Harich for recording the mass spectra.

Special thanks to Dr. Michael Natchus for his continuing educational and moral support. I feel indeed very fortunate to have the opportunity to learn about chemistry and life from an outstanding scientist and a wonderful person.

The author also wishes to express her gratitude to the Contraceptive Research and Development (CONRAD) for funding this project, and to Virginia Tech for providing me with an assistantship.

Among those whose contributions to the development of this work cannot go unmentioned are our collaborators: Dr. Curtis Ashendel at Purdue University, and Drs. Scott Franzblau and Anita Biswas from the Hanson Disease Center at LSU; and to the National Institute of Health for providing the biological data presented in this manuscript.

On a personal level, I am forever indebted to my parents for their financial and continuous emotional support. Their education and their perseverance contributed to my withstanding the rigor of formal education.

I also want to express my gratitude to all past and current members of Dr. Gandour's research group. Special acknowledgments go to Dr. G. Kumaravel for his stimulating discussions and helpful suggestions and to Dr. P. Savle for covering for me when necessary.

Finally, I offer my appreciation to my research director, Dr. Richard D. Gandour, for his support during the completion of this project.

To My Parents,

Manuel and Marina

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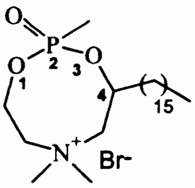
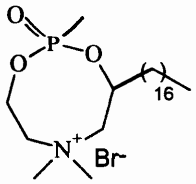
I. INTRODUCTION

The protein Kinase C (PKC) family of kinases, regulated by lipids, is a prime target for most potent tumor-promoting phorbol esters.[1] PKC is implicated in many malignant cell diseases and plays a crucial role in signal transduction.[2] These implications have stimulated immense interest in scientists worldwide.

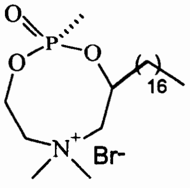
In the early 1990, our research group became involved in the area of PKC inhibition research. Previous work in our laboratories resulted in the synthesis of the lipids diasteromers **2a-b**, which were effective PKC inhibitors.[3] A two-fold inhibition decrease was observed on going from the *cis* (**2a,** $IC_{50} = 4.8\ \mu M$) to the *trans* (**2b,** $IC_{50} = 9.9\ \mu M$) diastereomer. This result attracted our attention and we chose to study inhibiton of PKC with selected enantiomers (**1a-d**), which are lower homologues of **2a-b**. We were also interested in investigating the possible side effects of a particular enantiomer and therefore the therapeutic applications of these agents.



1a (2*S*/4*S*)
1b (2*R*/4*S*)
1c (2*R*/4*R*)
1d (2*S*/4*R*)



2a

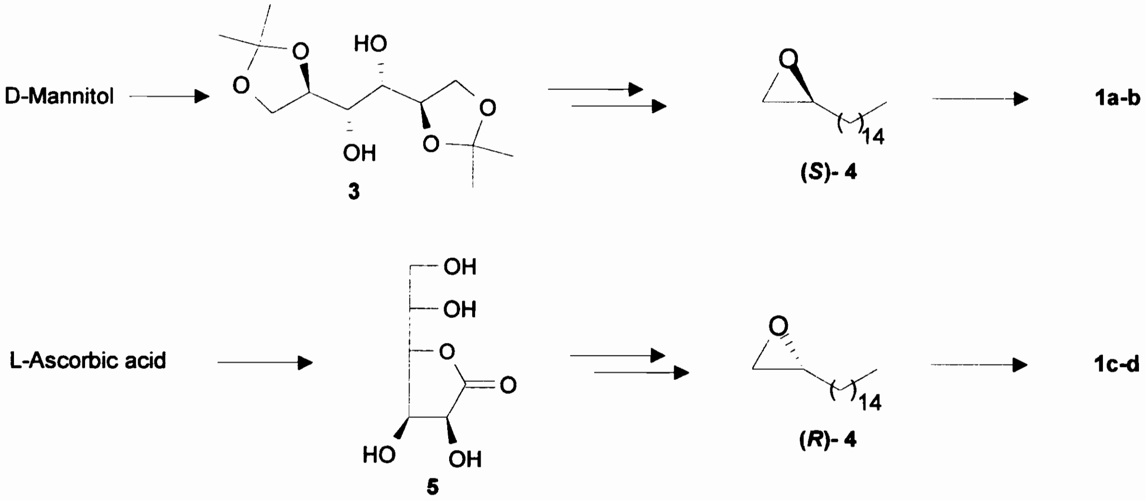


2b

Our lipids (**1a-d** and **2a-b**) represent the cyclic combination of phosphatidylcholine and the synthetic analog hexadecylphosphocholine , two known inhibitors of PKC.[4, 5] Conformationally cyclic "rigid" analogues were selected because of their greater usefulness as molecular probes of the topographies of the binding sites in enzymes.[6,7]

In order to accomplish the synthesis of the four stereoisomers (**1a-d**), the carbon stereocenter need to be set with absolute stereochemistry and high enantiomeric excess (≥ 98 %) to ensure correct assignment of enantioslective inhibition. After several initial approaches, the synthesis was successfully accomplished using optically pure glyceraldehyde surrogates as the starting materials, as shown in Scheme 1. The key intermediates for the synthesis were the long-alkyl chain optically pure epoxides. Several intermediates and other derivatives are expected to exhibit other therapeutic applications, based on previous results obtained from their corresponding racemates or related compounds.

A review covering several aspects of PKC research is given for a better understanding of this work.



Scheme 1

II. HISTORICAL

II.1 Introduction to Protein Kinase C Research

Protein kinase C (PKC), a threonine/serine kinase, was first isolated in 1977 by Nishizuka and co-workers from brain tissues as a proteolytically activated form.[8,9] PKC plays a pivotal role in signal transduction. Once activated, PKC phosphorylates other proteins, including other protein kinases, leading to a cascade of abnormal cellular information that can modulate gene expression at both the transcriptional and translational levels. Malignant transformations can be explained as a dysfunction of signal transduction, and result in cells that either generate their own growth-promoting stimuli (cell proliferation) or cells that do not respond to growth-inhibitory signals (cell differentiation). The discovery that PKC is the receptor of various tumor-promoting agents such as phorbol esters, telocidin and others has made PKC a very popular target for rational anti-cancer drug development. Inhibitors of PKC are not only very useful in cancer treatment, but also they help probe the modes by which the enzyme is activated and inhibited.

PKC is ubiquitously distributed throughout different tissues and organs, with the brain providing a site for the highest activity.[10,11] Although the enzyme was initially thought to be a single entity, studies of the genes encoding PKC show that it comprises a complex family of isoenzymes.[12,13] So far, twelve isoforms of PKC have been identified in mammalian tissues.[14] Following the nomenclature of Nishizuka,[15] these isoforms can be divided into three groups based upon the biochemical properties and sequence homologies of individual enzymes: classical or conventional PKC's (cPKC) are Ca^{+2}-dependent and phorbol ester-activated; novel or new PKC's (nPKC) are Ca^{+2}-independent and phorbol ester activated; and atypical PKC's (aPKC) are phorbol ester unresponsive. Further subdivision based on the primary structure of the individual isoforms denotes them with Greek letters (Table 1).

Table 1. PKC Isoenzymes in Mammalian Tissues.[16]

	Subspecies	Amino acid residues	Tissue expression
cPKC	α	672	universal
	βI	671	some tissues
	βII	671	many tissues
	γ	697	brain only
nPKC	δ	673	universal
	ϵ	737	brain and others
	η(L)	683	skin, lung, heart
	θ	707	muscle, T cell, etc
	μ	912	NRK cells*
aPKC	ξ	592	universal
	λ	586	many tissues
	ι	587	many tissues

* NRK cells = normal rat kidney cells

To date, the biological importance of this heterogeneity has not been fully established. In mammalian species, there is a high degree of conservation; e.g. the human PKCα is more closely related to bovine PKCα than it is to human PKCβ suggesting that each isoenzyme could have a particular relevance for the organism. Indeed, there are marked differences in the cellular distribution and some *in vitro* substrate selectivity by different PKC isotypes have been reported.[17] These isozymes may have different *in vitro* activation modes and different responses to extracellular signals.[14,18] However, the possibility that these isoforms may play the same role in some cases, cannot be ruled out.

II.2 Biological Significance

The most relevant biological significance of PKC is its role in tumor promotion.[15] In addition, the family of PKC isoforms has been further attributed to several other biological responses including: neuronal disorders, platelet activation, muscle contraction, cell growth and cell differentiation as well as others. Throughout the years the number of biological activities owing to PKC has increased enormously and it is not unreasonable to think that the biological implications of PKC is far from being exhausted. Table 2 summarizes the possible roles of PKC in cellular responses.[15]

Table 2. Possible Roles of PKC in Cellular Responses.

Tissues and cells	Responses
Endocrine systems	
Adrenal medulla	Catecholamine secretion
Adrenal cortex	Aldosterone secretion
	Steroidogenesis
Pancreatic islets	Insulin release
Insulinoma cells	Insulin release
Pituitary cells	Pituitary hormone release
	Growth hormone release
	Luteinizing hormone release
	Prolactin release
	Thyrotropin release
Parathyroid cells	Parathyroid hormone release
Thyroid C cells	Calcitonin release
Leydig cells	Steroidogenesis
Exocrine systems	
Pancreas	Amylase secretion
Parotid gland	Amylase and mucin secretion
Submandibular gland	Mucin secretion
Gastric gland	Pepsinogen secretion
	Gastric acid secretion
Alveolar cells	Surfactant secretion
Nervous systems	
Ileal nerve endings	Acetylcholine release
Neuromuscular junction	Transmitter release
Caudate nucleus	Acetylcholine release
PC 12 cells	Dopamine release
Neurons	Dopamine release

cont.

Table 2 cont.

Muscular systems	
Vascular smooth muscle	Muscle contraction
	Muscle relaxation
Inflammation and immune systems	
Platelets	Serotonin release
	Lysosomal enzyme release
	Arachidonate release
	Thromboxane synthesis
Neutrophils	Superoxide generation
	Lysosomal enzyme release
	Hexose transport
Basophils	Histamine release
Mast cells	Histamine release
Lymphocytes	T-lymphocyte activation
Metabolic and other cell systems	
Adipocytes	Lipogenesis
	Glucose transport
Hepatocytes	Glycogenolysis
	Inhibition of gap junction
Epidermal cells	Inhibition of gap junction
Fibroblasts	Inhibition of gap junction

II.3 Molecular Structure

The primary structures of mammalian isoenzymes of PKC show conserved structural motifs. The cPKC group comprises the isoforms α-, βI- βII- and γ which have four conserved sections (C_1-C_4) and five variable ones (V_1-V_5).[19,] The carboxy-terminal half (C_3, C_4, and V_4) forms the catalytic domain, which contains the ATP- and the substrate-binding sites. The amino-terminal half (V_1, C_1, V_2, C_2, and part of V_3) is the regulatory domain, which accounts for the Ca^{2+}, zinc, phospholipid, diacylglycerol (DAG), and phorbol ester binding sites. The nPKC group comprises the isoforms δ, ε,η, θ, and μ and differs from the cPKC group by lacking the C_2 region in the regulatory domain. The aPKC group includes the ξ, λ, and ι isoenzymes. This group lacks the C_2 region and one cysteine rich sequence motif included in the C_1 region of both cPKC and nPKC (Figure1).

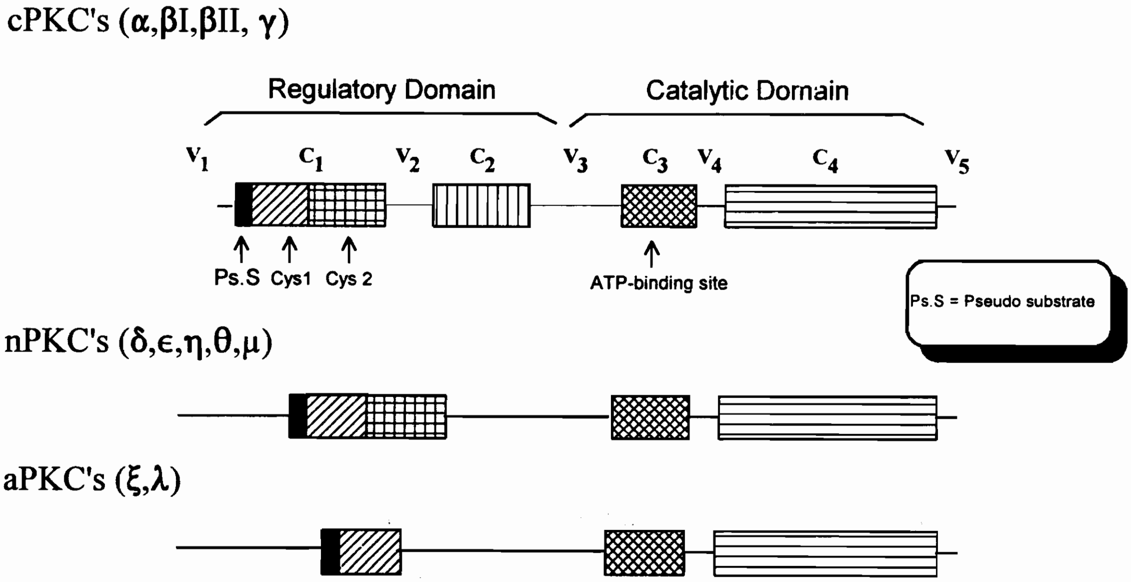

cPKC's (α,βI,βII, γ)
Regulatory Domain
Catalytic Domain
V1
C1
V2
C2
V3
C3
V4
C4
V5
Ps.S
Cys1
Cys 2
ATP-binding site
Ps.S = Pseudo substrate
nPKC's (δ,ε,η,θ,μ)
aPKC's (ξ,λ)

Figure 1. Molecular Structures of PKC.

II.3.1 The Regulatory Domain

The C_1 Region. The C_1 region in both cPKC and nPKC groups contains a repeat of a cysteine-rich sequence (Cys1, Cys2),[12] whereas the aPKC group contains only one.[21] The function of the cysteine-rich region is to bind Zn^{2+} ions. Although these regions are sometimes referred to as zinc fingers, they are structurally unrelated to any of the nucleic acid-binding zinc finger proteins. Extended X-ray absorption fine-structure of a purified sample of rat PKCβ I reveals four Zn^{2+} ions tightly bound to the cysteine-rich region of the enzyme. The average coordination is one histidine nitrogen and three cysteine sulfur atoms.[22,23] This interaction may stabilize a particular enzymic conformation that enhances the affinity of PKC for phorbol esters and other pharmacologically active natural products including the PKC-directed anticancer agent bryostatin.[24] For example, deletion and site-directed mutagenesis in the zinc finger sequence of the rat brain PKCγ results in the loss of the phorbol ester binding capability.[25,26] Furthermore, only one phorbol ester molecule binds to the enzyme, and probably this binding sterically blocks a second phorbol ester binding site on the second cysteine rich finger. The possibility of having two nonequivalent second messenger binding sites in cPKC's and nPKC's has been hypothesized to explain the different effects of several PKC activating tumor promoters.[27] In contrast, PKCζ, an aPKC type isoform that contains only one cysteine-rich motif, shows no affinity for phorbol ester. This result suggests that although only one cysteine-rich region is being used for coordination to Zn^{2+}, two are required for affinity to phorbol ester for PKCγ. Removal of Zn^{2+} inhibits phorbol ester binding[28] and binding of an additional Zn^{2+} ion in the presence of phorbol ester, enhances the attachment of PKC to the phospholipid membrane.[29] Zinc finger motifs have been found in more than 200 proteins, among those in particular *n*-chimaerin,[27] and the *unc*-13 gene product[29] of *Caenorhabditis elegans* bind phorbol ester; however, these proteins have no kinase activity.

The C_1 region of all PKC's contains a pseudo-substrate sequence, that autoinhibits the enzyme.[30] The sequence resembles that of a substrate but lacks the key residues serine or threonine or both for phosphorylation. The enzyme recognizes this pseudo-substrate and binds to itself without exerting kinase activity. Mutations in the pseudo-substrate sequence decrease the affinity for the active site and increase PKC activity as expected. Complete deletion of the pseudo-substrate sequence also enhances PKC activity.[31,32]

The C_2 Region. The C_2 region may be the Ca^{2+} binding site because the nPKC's and aPKC's that lack this region are insensitive to Ca^{2+}.[33] Unexpectedly, this region does not show characteristic Ca^{2+}-binding-site sequences as seen for other proteins.[12,34] *In vitro* assays show that the presence of Ca^{2+} is almost nearly absolute. Only Sr^{2+}, which is about 10% as active as Ca^{2+}, will poorly substitute for Ca^{2+}.[35] Deletion of the C_2 region makes the kinase activity independent of Ca^{2+}.[25]

The V_1 Region. The V_1 region may be associated with substrate selectivity.[36,37] For example, histone IIIs is a good substrate for PKCα, PKCβ, and PKCγ, but not for PKCε. However, proteolytic cleavage of PKCε generates a constitute kinase that is effective for histone IIIs,[36] suggesting the involvement of the regulatory domain in substrate specificity. A recombinant kinase with the catalytic domain of PKCγ and the regulatory domain of PKCε has a similar effect toward histone IIIs as that of native PKCε.[38] The V_1 region of cPKC is relatively short compared with that of compared with that of nPKC and aPKC groups. This lack of conservation suggests a possible role in substrate specificity and that each isoform may have specific substrates that cannot be phosphorylated by all members of the PKC family.

The V_3 Region. The V_3 region, or hinge region, contains a protease-sensitive site that upon cleavage by Ca^{2+}- activated neutral proteases (calpain) or by trypsin unfolds the enzyme into a kinase and a phorbol ester binding site (regulatory domain).[39] This region appears to be very flexible to allow the pseudo substrate region to be dislodged from the catalytic domain on binding to the activators.

II.3.2 The Catalytic Domain

The catalytic domain is active without cofactors after proteolytic removal of the regulatory domain by cleavage in the V_3 region. The catalytic domain possesses an amino acid consensus that shows sequence homologies with other protein kinases.[12] The C_3 region, which contains an ATP binding site, is the phosphate group donor region of the enzyme. The C_4 region has an additional ATP binding site, but the significance of having two ATP bindings sites still remains obscure. Deletion of the C_3 region inactivates the kinase.[25] Site-directed mutagenesis yields an inactive kinase[40] and a mutant that is resistant to phorbol ester-mediated down regulation suggesting a link between proteolytic regulation and kinase activity.

II.4 Model of Activation

Despite extensive studies, the mechanism of activation of PKC is not yet fully elucidated. The following is a concise description of the mode of activation of Ca^{2+}-dependent PKC's, as it is currently understood.

PKC plays a crucial role in signal transduction and tumor promotion. External cellular stimuli such as hormones, neurotransmitters, growth factors, and many other biologically active substances activate cellular functions and proliferation.[15,41] To coordinate external and internal cellular functions, such information must cross membranes. Membranes contain phospholipid bilayers that provide a permeable barrier to maintain the physical and chemical gradient between the cell plasma and the exterior. Membrane proteins function as cell receptors facilitating the transmission of signals into the cell, whereby information flows from one component to the next until the final effector system is activated. This flow of information is known as *signal transduction*. Figure 2 schematically summarizes the pathway of signal transduction.[18]

In general, during the earliest phase of PKC activation, inositol phospholipids are hydrolyzed by phospholipase C (PLC). The lipid precursor phosphatidylinositol-4,5-biphosphate (PIP_2), stored in the plasma membrane, is hydrolyzed to give diacylglycerol (DAG) and phosphatidylinositol-1,4,5-triphosphate (PI_3).[42]

DAG activates PKC, whereas PI_3 binds to its receptor and mobilizes calcium contained within intracellular compartments in the cytosol.[43] The concentration of Ca^{2+} in the cytosol is now elevated above its basal level and sensed by the C1 region in the regulatory domain of PKC (*vide infra*). Upon this signal, the enzyme translocates to the membrane. At this stage, the enzyme is still inactive but a conformational change has occurred that exposes the hinge region.[43]

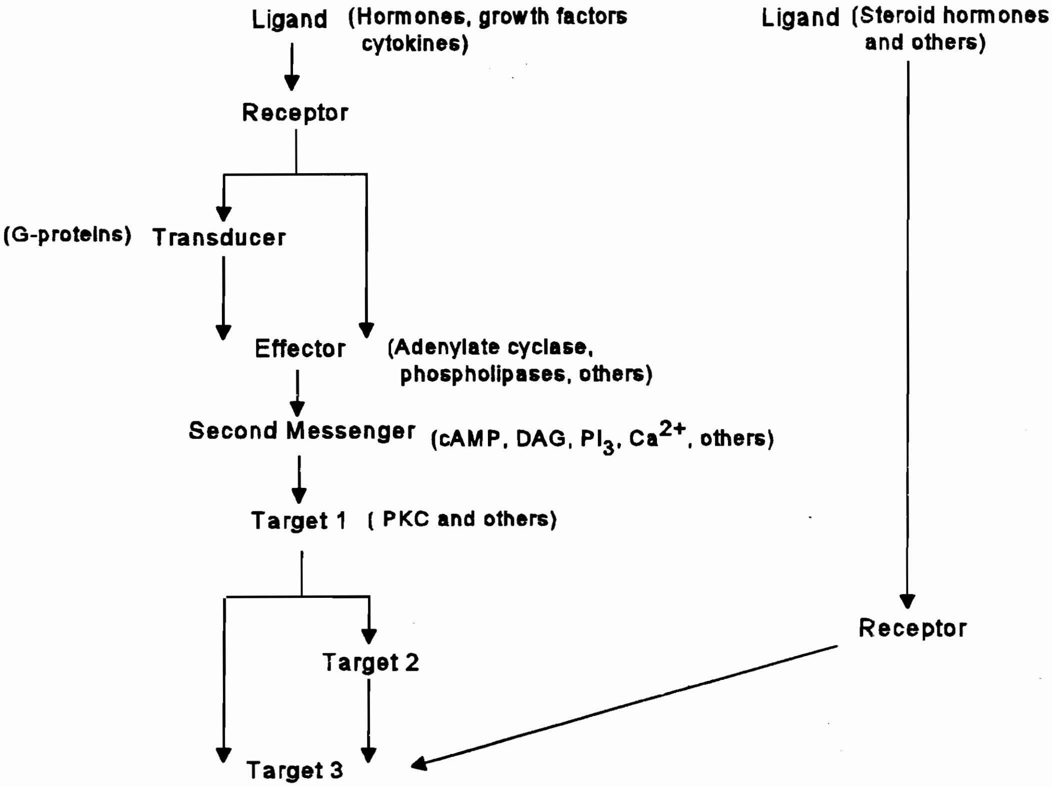

Ligand (Hormones, growth factors cytokines)
Receptor
(G-proteins) Transducer
Effector (Adenylate cyclase, phospholipases, others)
Second Messenger (cAMP, DAG, PI3, Ca2+, others)
Target 1 (PKC and others)
Target 2
Target 3
Ligand (Steroid hormones and others)
Receptor

Figure 2. The Signal Transduction Pathway: a Schematic Overview.

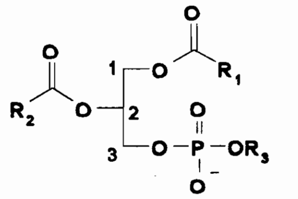
Extracellular signals (Ligands) bind to their cellular receptors. The receptors as such can directly reach the target(s) or interact with a transducer that stimulates an effector to produce second messengers. These second messengers activate a target cascade that can modulate gene expression at both the trancriptional and translational levels.

Membrane Translocation. In an inactive state, PKC resides within the cytosol, probably in a particular conformation in which the catalytic domain is not exposed but locked with the regulatory domain through binding to the pseudo-substrate region.[44] On elevation of Ca^{2+}, PKC translocates to the membrane. The membrane phospholipid composition plays an important role in the PKC activating network. Lipid head groups modulate the activity of transmembrane proteins by altering the bilayer fluidity, bilayer thickness, lipid backbone structure, acidity and other factors.[45,46] The most common phospholipids found in membranes are the glycerol derivatives containing a diacylglycerol-3-phosphate backbone.

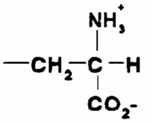
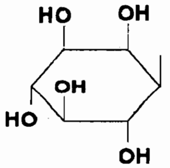
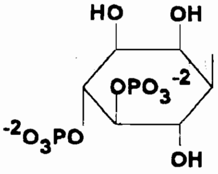
The glyceryl carbons are numbered 1, 2, and 3 from top to bottom. The prefix *sn-* preceding "glycerol" is used when the oxygen of the secondary hydroxyl group is drawn to the left of the central carbon (C2) in the Fischer projection. The hydroxyl groups on carbons 1 and 2 of natural phospholipids are acylated with fatty acids. In most cases, the fatty acids at carbons 1 and 2 are saturated and unsaturated, respectively. The phospholipids are generally named according to the alcohol (R_3OH) of their head group (Table 3).

Several negatively charged phospholipid cofactors may partially activate PKC, but phosphatidyl serine (PS) is the most effective.[47] The major driving force for the association of PKC to acidic phospholipids, in general, seems to be unspecific electrostatic forces. Orr and Newton have shown that PKC binding to PS in lipid-detergent mixed micelles is relatively insensitive to increasing ionic strength compared to that of other phospholipids.[48] This result suggests that for PS there are more specific interactions in addition to the electrostatic interactions. There is a high degree of specificity in the PKC/PS interaction. For example, lyso-PS and 1-oleyl-2-acetyl PS, which are PS analogs, do not activate PKC,[49] suggesting that there is a requirement for an appropriate lipid interfacial conformation. Kinetic studies have revealed that the activation of PKC by PS is highly cooperative. A minimum of four PS molecules is required for activation; when ten-twelve molecules are involved, the activation becomes highly cooperative in the presence of Ca^{2+} and DAG's.[50]

Table 3. Different Types of Phospholipids Associated with the Function of PKC.



sn - diacylglycerol-3-phosphate

Name of R_3OH	Formula of $-R_3$	Phospholipid	Abbreviation
choline	$-CH_2CH_2N^+(CH_3)_3$	Phosphatidyl-choline	PC
ethanolamine	$-CH_2CH_2N^+H_3$	Phosphatidyl-ethanolamine	PE
serine	 $-CH_2-\overset{NH_3^+}{\underset{CO_2^-}{C}}-H$	Phosphatidyl-serine	PS
myo - inositol		Phosphatidyl-inositol	PI
myo - inositol- 4,5-biphosphate		Phosphatidyl-inositol- 4,5-biphosphate	PIP_2

R_1, R_2 are fatty acyl chains

Other phospholipids have shown variable cooperativity toward PKC activation. For example, phosphatidylethanol amine (PE), lysophospholipids, phosphatidylcholine (PC) and sphingomyelin are all inert, but when PS is added, PE further increases the affinity of the kinase for Ca^{2+}, whereas both PC and sphingomyelin show inhibitory effects instead. Some differences in the degree of activity supported by various phospholipids are seen in different assays systems.[51,52]

Recently, PKC activity has been measured with PC of different compositions (varying the number of *cis*-unsaturations) and by addition of PE and cholesterol.[47] In the absence of PE, the activity of PKC increases as the number of unsaturations (confined to the *sn*-2-chain) of PC increases from one to six carbons. Addition of cholesterol decreases lipid fluidity but increases PKC activity. This trend is proportional to the number of unsaturations of PC. Additional kinetic assays were used to investigate whether the head-group spacing or the lipid order excerts dominant effect in the regulation of PKC activity. These involved three approaches: *i*) fluorescence measurements (a reduced intensity indicates increased hydration or increased head-group spacing), *ii*) X-ray diffraction intrinsic bilayer curvature, *iii*) variation of the vesicle diameter. In the presence or absence of PE, the first two approaches give bell-shaped curves when PKC activity was plotted against fluorescence intensity and curvature index respectively. These results reveal an optimum value of the head-group spacing for maximal PKC activity. When PKC activity was measured against the vesicle diameter, an optimal activity was observed for the smallest diameter (~ 25 nm) in comparison with larger vesicles (~ 100 nm). This effect can only be explained on the basis of the head-group spacing because there were no compositional differences among the vesicles. Smaller vesicles, due to geometric constrains, have their outer monolayer head-groups more widely spaced than those of larger vesicles in which their packing arrangements are closer to a planar bilayer. Therefore, lipid membrane regulation of PKC activity is primarily sensitive to changes in the head-group spacing rather than in the lipid order. A wider head-group spacing might facilitate a greater degree of insertion of the hydrophobic regulatory domain of PKC into the membrane to a certain extent. Thus, the phospholipid membrane plays an important role in maintaining a particular enzyme conformation. If the membrane is too "tight" or too "loose", reflected in the head-group spacing, this conformation may not be fully maintained thus producing a sub-optimal activity.

Interestingly, PIP_2 a precursor of DAG, also modulates PKC activity.[53] In the absence of divalent metal ions such as Ca^{2+} or Mg^{2+}, PIP_2 inactivates PKC (*in vitro*).[54] However, in the presence of those metals, PIP_2 becomes an activator and causes a reduction of [^{3}H]phorbol-12,13-dibutyrate ([^{3}H]PDBu) binding by lowering the affinity of PKC for this ligand. The inactivation of PKC by PIP_2 is not likely to occur *in vivo* as the intracellular concentration of Mg^{2+} is usually in the millimolar range. Since the interaction of PKC with PS is stimulated by Ca^{2+} rather than by Mg^{2+}. Under physiological conditions at low concentrations of Ca^{2+} and millimolar concentrations of Mg^{2+}, PKC may bind to PIP_2 to form a PKC-PIP_2 complex that can further interact with PS upon elevation of $[Ca^{2+}]$.[55] This interaction can in turn sustain a moderate stimulation of PKC in the absence of DAG preceeding the breakdown of PIP_2 to generate PI_3 and DAG.

In summary, it is clear that PKC binding to the membrane surface occurs in presence of acidic phospholipids and is a prerequisite for activation. Many other lipid metabolites may potentially activate or inhibit PKC and extensive studies in this area by many research groups are currently in progress. Figure 3 shows a putative model for the activation of cPKC.

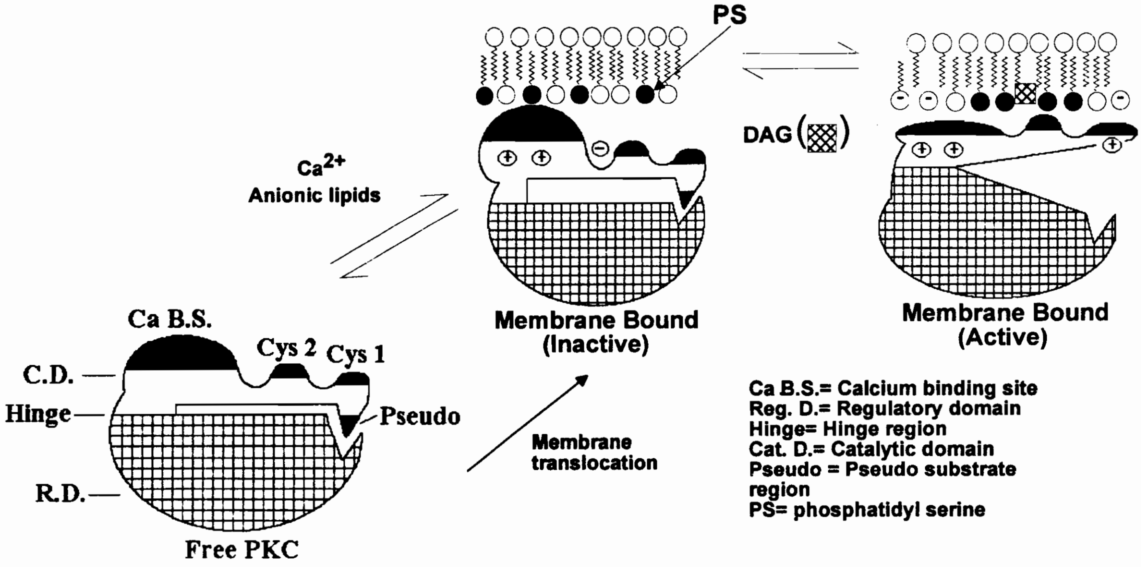

PS
Ca^{2+}
Anionic lipids
DAG (▩)
Ca B.S.
C.D.
Hinge
R.D.
Cys 2
Cys 1
Pseudo
Free PKC
Membrane Bound (Inactive)
Membrane translocation
Membrane Bound (Active)
Ca B.S.= Calcium binding site
Reg. D.= Regulatory domain
Hinge= Hinge region
Cat. D.= Catalytic domain
Pseudo = Pseudo substrate region
PS= phosphatidyl serine

Figure 3. Putative Model of Activation of cPKC.

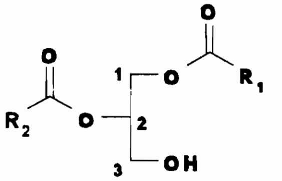
In its inactive state, free PKC resides in the cytosol. The pseudosubstrate region is autoinhibiting the kinase activity. Upon elevation of Ca^{2+}, PKC translocates and bind to the lipid membrane. This binding is mainly promoted by electrostatic forces between acidic phospholipids, especially PS (black circles) and the cysteine rich regions of the regulatory domain. At this stage, PKC is still inactive but a conformational change occurs exposing the hinge region. A subsequent interaction with DAG provides further insertion of PKC into the hydrophobic core of the membrane. Proteolysis of the hinge region releases the pseudosubstrate motif from the catalytic domain and activates the kinase.

Diacylglycerol Activation. Once PKC has translocated to the membrane surface, a conformational change occurs that brings the C_1 region closer to the membrane to allow further interactions with DAG. DAG, a second messenger, traverses the membrane phospholipid until it finds and binds the cysteine-rich region

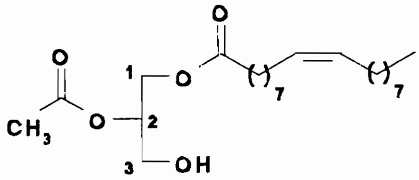
(C_1 region) already embedded in the membrane. This interaction promotes cooperative binding to PS but not to other anionic phospholipids. PKC can also be activated in absence of DAG, but in the presence of PS and high concentrations (millimolar) of Ca^{2+}. The specific role of DAG is to increase the affinity of the kinase for Ca^{2+}, enabling activity even under normal physiological concentrations of Ca^{2+}. The affinity for PS further promotes the insertion of the membrane into the hydrophobic core of the membrane. At this stage, the autoinhibitory pseudo-substrate region is released from the catalytic domain, and activation of the kinase occurs.[55]

Recently, Newton and Orr[56] have reported that conventional and non-conventional activators of PKC βII expose Arg19 of the autoinhibitory pseudo-substrate region to proteolysis. Molecular modeling of PKC's catalytic core suggests that this residue is pocketed by a cluster of acidic residues when the pseudo-substrate region is locked with the substrate binding site; therefore it is resistant to proteolysis when PKC is inactive. DAG's and phorbol esters activate all known PKC isoforms with one exception: PKC ξ an aPKC isoenzyme.[57,58]

The activation of PKC by DAG is stereospecific; only *sn*-1,2-diacylglycerol, but not 1,3- or 2,3-diacylglycerol is effective.[59] Both of the oxygen esters and the primary hydroxyl group are essential for DAG function.[60] The major requirement for the acyl chains appears to be in their length. The fatty acid chains might be an anchoring domain, immersed in the bilayer which may position DAG and phorbol ester for the binding to the protein. *In vitro*, six carbons are required for both acyl chains to induce maximal kinase activity. However for biological *in vitro* studies 1-oleyl-2-acetyl-*sn*-glycerol (OAG), or DAG with two short-chains (six to ten carbons) are most widely used. Because of their greater solubility in water, these molecules are more efficiently delivered to the cells.



Diacylglycerol (DAG)

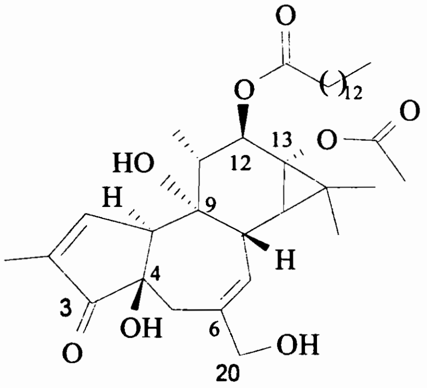


1-oleoyl-2-acetylglycerol (OAG)

The DAG obtained by hydrolysis of PIP_2 exists transiently and rapidly disappears. A second wave of DAG is provided by hydrolysis of PC. The fatty acid composition of the DAG derived from PC differs from that derived from PIP_2, but both DAG species are capable of activating PKC *in vitro*.[60] This second wave of DAG elevation can sustain PKC for several hours, which is essential for long-term responses such as cell growth and differentiation.[61] On the other hand, it is not yet clear whether continuous PKC activation is required for an elapsed time or whether multiple short time PKC activations during the cell cycle are required.

Phorbol Esters and Other Tumor Promoters. The active constituents from the oil of *Croton tiglium L* promote irritation and inflammatory effects when applied to the skin of mice.[62] These active principles were structurally elucidated and identified as phorbol esters.[63] Phorbol esters, which have DAG-like structures, when intercalated into the cell membrane, may substitute for DAG at an extremely low concentration and permanently activate PKC both *in vivo* and *in vitro*.

The high affinity of PKC toward phorbol esters exemplifies the important role of PKC in proliferation, differentiation, tumor promotion and carcinogenesis in animal cells and tissues.[1,64]



12-O-tetradecanoyl-phorbol-13-acetate

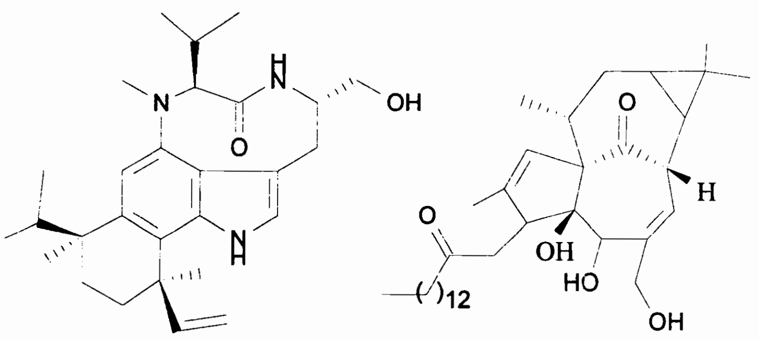
TPA

Kinetic studies have shown that 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), the most potent tumor promoter, like DAG dramatically increases the affinity of PKC for Ca^{2+}.[65] TPA alone has no activating effect, rather it requires the presence of Ca^{2+} and phospholipid for binding.

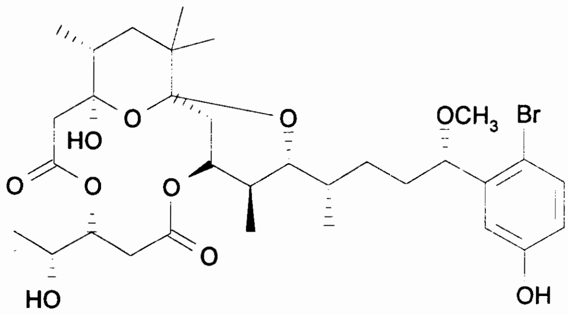
In terms of structural requirements, only 12,13- phorbol esters are PKC activators. The 12,13,20-triesters are de-esterified *in vivo* to the active 12-,13-diesters.[66] For many years the possible roles of other functional groups of phorbol esters such as the carbonyl, and the hydroxyl groups at C3, C4, C9, and C20 respectively have been debated. Computational studies by different groups worldwide have provided differing results.[67]

Recently, the first crystal structure for a member of the PKC family has been determined both alone and in a complex with phorbol-13-acetate.[68] This work provides evidence that the binding site of the enzyme contains a polar groove which *interacts with phorbol ester* through five hydrogen bonds formed with phorbol ester oxygens at C3 (acceptor), C4 (donor), and C20 (acceptor and donor (to two oxygens of the enzyme)). The hydroxyl group at C9 *intramolecularly* forms another hydrogen bond with the acetyl group at C13 rather than interacting with PKC. Thus, when phorbol ester binds to the enzyme filling up the polar groove, only a hydrophobic surface is exposed, probably promoting a further insertion of the enzyme into the plasma membrane. However, the fact that this study was performed on a solid-phase structure in the absence of phospholipid membrane should be carefully considered in the interpretation of the results and one should not overlook that PKC may assume different conformations *in vivo* where the environment is different. The choice of the substrate phorbol-13-acetate instead of the known activator tetradecylphorbol acetate (TPA) has been added to the debate. It is not clear whether phorbol-13-acetate is an agonist or an antagonist, and the interpretation could be misleading.

In addition to phorbol esters, an array of other tumor promoters with rather dissimilar structures bind to PKC with high affinity (nanomolar concentration), resembling phorbol esters in their biological activity. These compounds include the indole alkaloids such as telocidin B-1, polyacetates such as aplysiatoxin, and diterpenes such as ingenol-3-tetradecanoate among others (see Figure 4). All these compounds inhibit phorbol ester binding by overtaking the phorbol ester binding site. The precise structural requirements for the biological activities exhibited by these activators have not been fully established. However, because these unrelated structures interact at the same site, they would be expected to possess a common pharmacophore.[69]



Telocidin B-1 Ingenol 3-tetradecanoate



Aplysiatoxin

Figure 4. Chemical Structures of Various Tumor Promoters.

Activation of PKC by Fatty Acids. *Cis*-unsaturated fatty acids such as oleic, linoleic and arachidonic acids can potentiate the activity of PKC[70,71,72] to different degrees, depending on the isoenzyme. DAG enhances the effect of fatty acids in the presence of PS and at a physiological concentration of Ca^{2+}.[73] Saturated or *trans*-unsaturated fatty acids such as palmitic and stearic acid are inactive.[74] Recently, Kikkawa and Kasahara[75] have investigated the effects of saturated fatty acids with different chain lengths on PKC isoforms. Tridecanoic (C13) acid is as potent an activator as arachidonic (C20) acid. Palmitic (C16) and stearic (C18) acids have little effect. Lauric (C12), tridecanoic and myristic (C14) acids activate the α-, β-, γ- and ϵ- isoenzymes and these activations are further enhanced by addition of DAG. Fatty acids having chain lengths of fewer than 10 carbons are inactive. The ϵ- isoform is the most sensitive of all isoforms assayed. The δ- subspecies do not respond to any of these fatty acids.

The mechanism by which fatty acids modulate the activity of PKC is not yet clear and there have been conflicting results. Some studies reveal that activation is independent of Ca^{2+} while in others it is dependent.[76] The same conflicting results have been found for DAG's.[77] These controversial results are probably due to the different assay conditions and different PKC isoenzymes employed. What is known is that the mechanism of activation of PKC by fatty acids is different from that by DAG (*vide supra)*.[78] It is not known, however, whether fatty acids are recognized by the phospholipid site (C_1 and C_2 regions) or by a specific fatty acid recognizing site. As fatty acids do not take over the role of PS in the DAG/phorbol ester binding site of PKC, it has been suggested that only free non-membrane bound fatty acids are able to activate soluble PKC. Thus, *in vivo* fatty acids may sustain PKC activation in the cytosol, whereas DAG exerts its effect when the enzyme is bound to the membrane.[71]

Autophosphorylation. In addition to phosphorylating substrates, PKC also phosphorylates itself at both the regulatory and the catalytic domains.[79] Autophosphorylation alters the affinity of PKC for phorbol esters and increases the sensitivity for Ca^{2+}. The interaction of PKC to the phospholipid membrane seems to be dependent on autophosphorylation.[80] Autophosphorylation follows an intrapeptide mechanism and occurs in sections of the enzyme that are poorly conserved suggesting that each isoenzyme may undergo different autophosphorylating patterns.

II.5 Inhibitors of PKC

PKC, a key enzyme in signal transduction, is a suitable molecular target for anti-cancer drug development. Its ubiquitous nature, however, makes it difficult to effectively design selective inhibitors of tumor cell growth without inhibiting other cellular processes. Furthermore, PKC is not a single entity, rather it comprises a family of at least twelve isoforms, which may play particular roles in different cellular functions. Therefore, isoenzyme-specific inhibitors of PKC are necessary. Due to the important biomedical processes (See Table 2.) in which PKC is involved, a large number of inhibitors have been reported.[81] However attempts to devise specific inhibitors has not yet yielded a single "super drug".

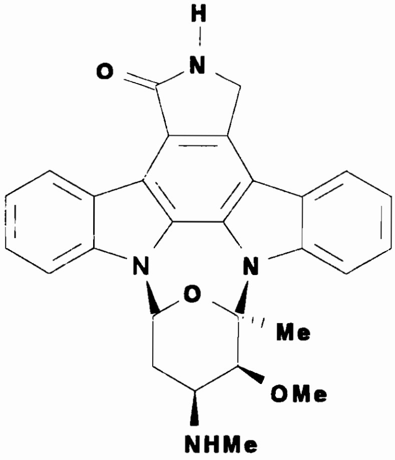
In general, PKC can be targeted at both the regulatory domain and the catalytic domain separately. A new approach is to use the signaling pathway that mediates the effect of growth factors and oncogenes on cell proliferation as the molecular targets. Recent advances in this field include, in particular, the development of growth factor antagonists, growth factor receptor-blockers, and targets in growth factor signal transduction at the post-receptor level.[82,83]

The structures of compounds that have been shown to inhibit PKC are quite diverse. PKC inhibitors have been found among ether phospholipids, alkyl phospholipids, dialkyl and alkyl-acyl-glycerols, lipoidal amines, isoquinoline sulphonamides, triphenylethylenes and an array of heterocyclic compounds.[84] To understand the scope of this work the inhibitors will be classified into four major groups (owing to the site at which they interact): Catalytic domain inhibitors, regulatory domain inhibitors, phospholipid metabolites and analogues, and peptide-substrate based inhibitors.

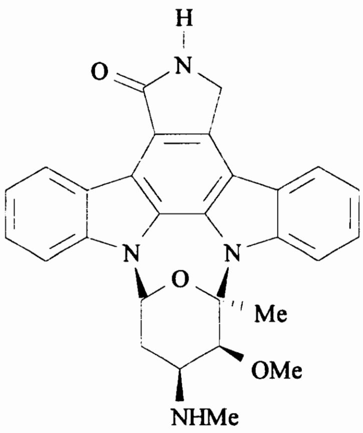
This is, however, by no means a rigid classification as many inhibitors possess multiple mechanisms of action. Only the most relevant inhibitors will be covered.

Catalytic Domain Inhibitors. These type of inhibitors have no effect on binding of phorbol ester to PKC. They interact instead with the ATP binding site at the catalytic domain. Most of these inhibitors lack specificity as other protein kinases have a similar ATP sequence. To date, the most potent inhibitor of protein kinases *in vitro* is staurosporine (**6**) (IC_{50}= 2.7 nM).[84] Staurosporine, a microbial alkaloid having an indolo[2,3,*a*]carbazole chromophore, is produced by *Streptomyces* species.[85] Staurosporine shows limited selectivity for different protein kinases *e.g.* Protein kinase A (PKA) and protein tyrosine kinase (PTK).

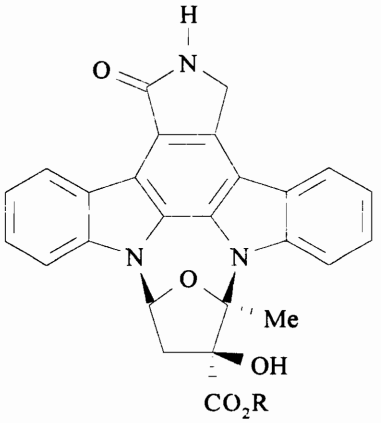
In contrast, UCN-01 (7), which possesses a staurosporine-like structure shows selectivity for PKC inhibition *in vitro* (IC_{50}= 4.1 nM).[86] K252a (**8**, IC_{50}= 28 nM) and K252b (**9**, IC_{50}= 20 nM),two naturally occurring alkaloids, are also potent inhibitors.[86]



Staurosporine 6

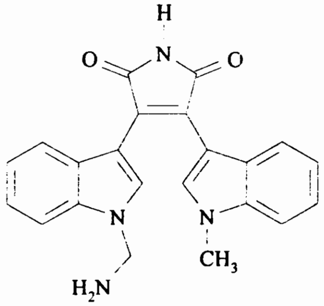

H
N
O
N
N
O
Me
OMe
NHMe

UCN -01 7

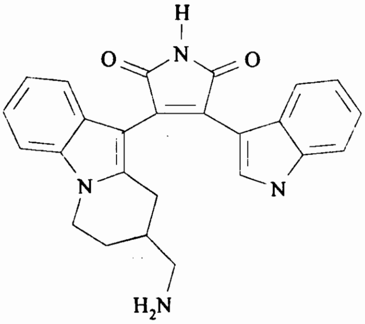

H
N
O
N
N
O
Me
OH
CO2R

R= Me **K-252a 8**
R= H **K-252b 9**

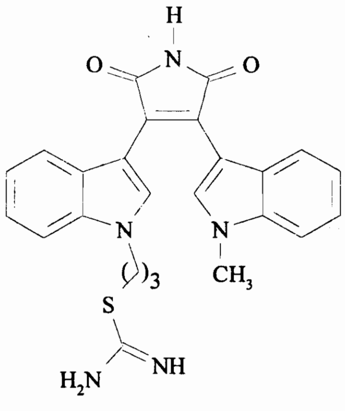
Most of the catalytic domain inhibitors to date are synthetic analogues of the naturally occurring staurosporine. A team from La Roche laboratories has developed PKC inhibitors containing a bis-indolyl maleimide structure such as Ro 31-7549 (**10**), Ro 31-8425 (**11**), Ro 31-8220 (**12**), and others (IC$_{50}$ = 10-500 nM).[87,88] These compounds strongly inhibit PKC but show little activity against many of the other kinases inhibited by staurosporine.[89]


H
N
O
O
N
N
H2N
CH3

Ro 31-7549 10

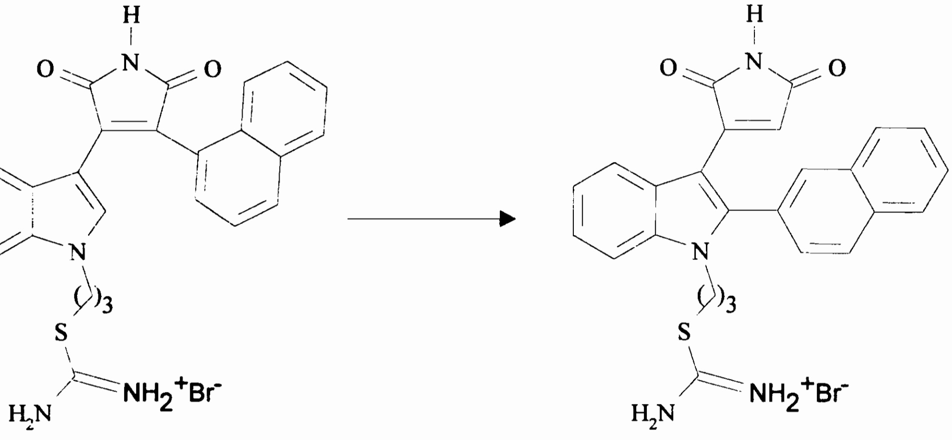


Ro 31-8425 11


H
N
O
O
N
N
( )3
CH3
S
H2N
NH

Ro 31-8220 12

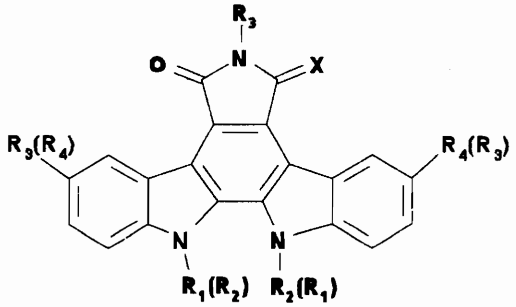
Recently, another group of researchers[91] investigated the effect of relocating one of the aryl substituents of an inhibitor such as **13** to the C2 position of the indole ring. The new inhibitor **14** is an excellent inhibitor of PKC-β without compromising the potency.



13 **14**

From a series of non-glycosidic/non-amino alkyl-substituted indolocarbazole lactams, Go 6976 (**15**) has emerged as a potent and selective inhibitor.[92] Compound **15** inhibits PKC-α and βI, but not δ, ϵ, and ζ isotypes.[93] However, the low solubility and bioavailability of Go 6976 represents an obstacle for therapeutic application.

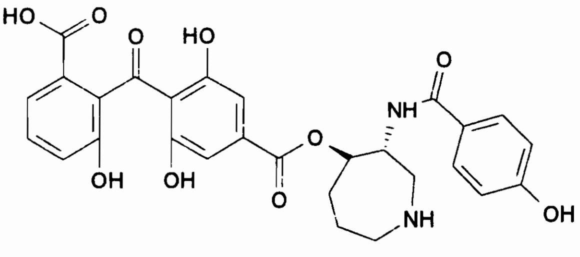
Several potent analogues of **15** with improved selectivity and solubility have been reported[94], not only in the lactam series Go 7852 (**16** , $IC_{50} = 0.03$ μM) but also in the imide series Go 7874 (**17**, $IC_{50} = 0.004$ μM).


R$_3$
O
N
X
R$_3$(R$_4$)
R$_4$(R$_3$)
N
N
R$_1$(R$_2$)
R$_2$(R$_1$)

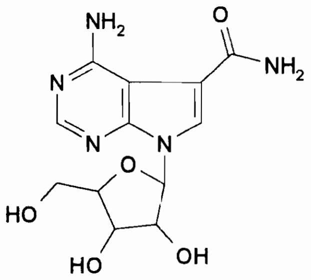
Go 6976 15	**Go 7852 16**	**Go 7874 17**
R_1=Me	$R_1(R_2)$=Me	$R_1(R_2)$=Me
$R_2=CH_2CH_2CN$	$R_2(R_2)=CH_2CH(OH)CH_2N(Me)_2$	$R_2(R_2)=CH_2CH(OH)CH_2N(Me)_2$
R_3=H	$R_3(R_4)$=H	$R_3(R_4)$=OMe
X=H_2	$R_4(R_3)$=H	$R_4(R_3)$=H
	X=H_2	X=O

*The residues R_1-R_4 given in parentheses denote the structure of the respective isomer in regioisomeric mixtures.

Among the staurosporine non-related structural compounds, (-)-balanol[95] (**18**) and its analogues[96] have been by far the most potent. Sangivamycin **19**, a nucleoside, is a competitive inhibitor with respect to ATP (K_i = 10 μM) and noncompetitive with respect to histone and lipid cofactors (PS and DAG) and it does not inhibit binding to [^{3}H]PDBu.[97]

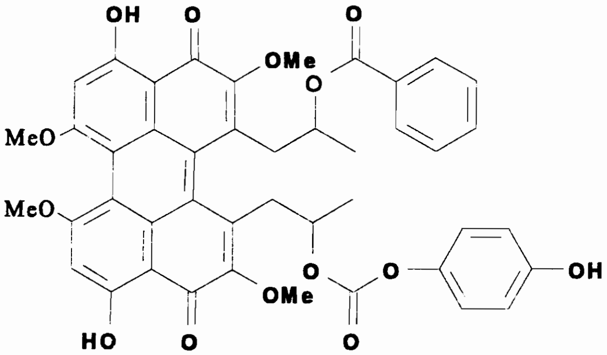

HO
O
O
HO
O
NH
O
OH
OH
O
NH
OH

(-) Balanol 18


NH$_2$
O
NH$_2$
N
N
N
O
HO
HO
OH

Sangivamycin 19

Regulatory Domain Inhibitors. There are now several PKC inhibitors acting at the regulatory domain, among them Calphostin C (**20**, UCN-1028C), a microbial compound isolated from *Cladosporium cladosporioides*, was the first potent (50-500 nM dose range) and selective inhibitor.[98] However, the inhibition proceeds by a complicated, light-dependent mechanism limiting its usefulness as a probe of PKC function.[99]

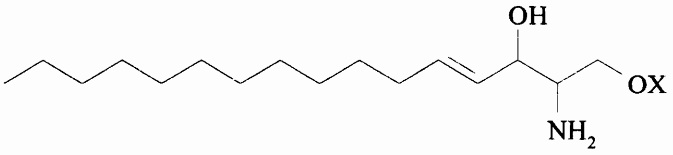


Calphostin C 20

Sphingolipid breakdown products such as sphingosine (**21**) and lysosphingolipids (**22-25**) are also potent and reversible inhibitors of PKC *in vitro* ($IC_{50} = 2.5\text{-}30\ \mu M$). However the biological response is not limited to alterations in PKC activity.[100]

Mechanistic studies reveal that sphingosine inhibits DAG and phorbol ester binding to PKC competitively, and Ca^{2+} non-competitively. The inhibition depends on the concentration of sphingosine in the membrane rather than by its molar concentration. The structural requirements for sphingosine to inhibit PKC are the hydrophobic character and the positively charged ammonium ion. *N*-acetyl and short-chain sphingosines with fewer than eleven carbons do not inhibit PKC.[96]

Lysosphingolipids

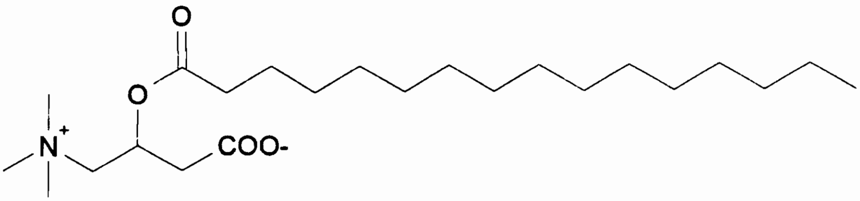


21	X= H	sphingosine
22	X=galactose	Psychosine (galactosphingosine)
23	X= Sulfogalactose	lysosulfatide (sulfogalactosylsphingosine)
24	X= phosphorylcholine	lysosphingomyelin
25	X= galNAc-gal(sia)glc	lyso GM_2

Phospholipid metabolites and analogues. Because a membrane bound PKC is needed prior to activation of PKC by DAG, phorbol esters, or both, it is not surprising that many lipid metabolites present in the membrane modulate the activity of PKC. The class of phospholipids that potentiate activation has been already described. Now in this section a class of lipid inhibitors is presented.

In vitro studies have shown that several phospholipids act synergistically with DAG to activate PKC at low concentrations and inhibit at high concentrations, among these phospholipids are PE, PC, and lyso-PC (A degradation product of PC).[101] The modulation of PKC by PC depends on the fatty acyl-chain length of PC. Short-chain length PC's activate, whereas long-chain length PC's inhibit. Although it is not totally clear how these phospholipids inactivate PKC, it seems that they act by changing the membrane composition.[97]

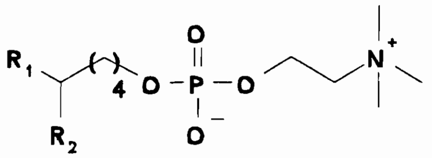
Palmitoylcarnitine **26**, a lipoidal amine intermediate in long-chain fatty acid metabolism, inhibits PKC[102,103] probably by altering the interaction between phospholipid cofactors at the regulatory domain of the enzyme.



Palmitoylcarnitine 26

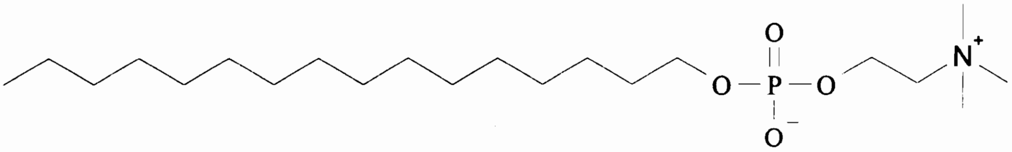
Other long-chain and medium-chain acylcarnitines, such as stearoyl, linoleylcarnitine, and octanoylcarnitine, respectively are less effective as inhibitors of PKC. It has been suggested that lipophilicity as well as other structural features are crucial for the ability of such compounds to regulate PKC activity.[98]

There are other related lipids, which are lyso-PC **29** analogues, that act as PKC inhibitors.[104] The ether lipid 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-OCH_3) **27** and the thioether lipid 1-*S*-hexadecyl-2-methoxymethyl-*rac*-glycero-3-phosphocholine (BM 41.440) **28** are active.



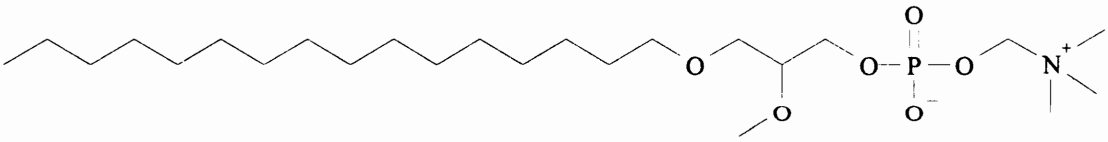
ET-18-OCH_3 27	**BM 41.440 28**	**Lyso-PC 29**
$R_1=O(CH_2)_{17}CH_3$	$R_1=S(CH_2)_{15}CH_3$	$R_1=O(CH_2)_{17}CH_3$
$R_2=OCH_3$	$R_2=CH_2OCH_3$	$R_2=OH$

Another important class of inhibitors is the alkyl phospholipids. Hexadecylphosphocholine[5] (miltefosine) **30**, is a prototype molecule in this class. Miltefosine has been approved by the German Health Organization for topical treatment of skin metastasis in breast cancer patients. However oral administration of this drug to cancer patients has undesirable side effects in the digestive tract.[105]

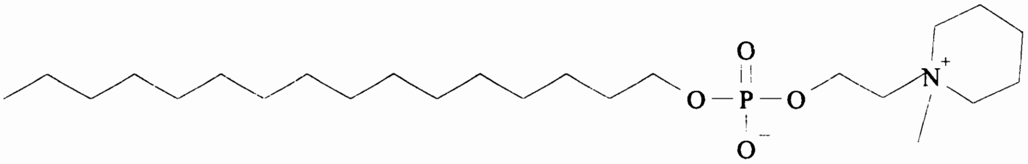


Miltefosine 30

Several new miltefosine analogues have been investigated. 2-*O*-Methyl-1-*O*-octadecyl-*rac*-glyceryl-3-phosphocholine[106] (**31**, edelfosine), and octadecyl-[2[(*N*-methylpiperidinio)ethyl]-phosphate (OMPEP, **32**)[107] are the most potent.

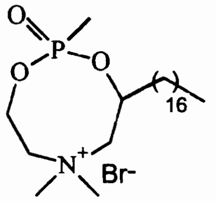


Edelfosine 31

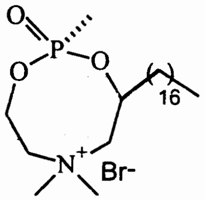


OMPEP 32

Recently, cyclic related compounds **2a** and **2b** have been synthesized in our laboratories.[3] These compounds contain a quaternary ammoniun salt and a fatty alkyl side-chain, as the structural features seen in other lipid inhibitors (*vide infra*). By using a phosphonate group instead of the phosphate group we were able to observe a diasteroselective PKC inhibition (IC_{50}= 4.8 μM, and 9.9 μM for the *trans* and the *cis* diastereoismers respectively). We have prepared conformationally rigid inhibitors because they should provide a better understanding of the topographical arrangement of the enzyme recognition sites.[6] Identifying the molecular interactions between our inhibitors and the enzyme is our long-term objective.



2a



2b

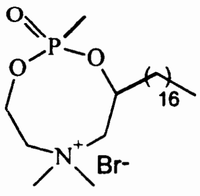
Pseudo Substrate-Based Peptide Inhibitors. Many protein kinases remain inactive due to the presence of an autoinhibitory region that masks the catalytic activity. The autoinhibitory region resembles the phosphorylating site of a protein substrate. The kinase becomes active upon proteolytic removal of the pseudo substrate region or by site-directed mutagenesis. Any structure that mimics that of the pseudo substrate region may fold back the protein into its inactive form and hence act as an inhibitor. A synthetic peptide corresponding to the pseudo substrate sequence of PKCα inhibits PKC catalyzed peptide phosphorylation (IC_{50} = 92 μM).[31] Unfortunately this synthetic peptide is too bulky and hydrophilic to enter cells. To overcome the permeability problems, *N*-myristoylated PKC peptide substrate analogues have been tested. In fact, these derivatives potently inhibit the histone kinase activity of purified PKC and antagonize the PKC-mediated events in intact cells.[108] However, it is not clear whether the *N*-acylated peptides actually enter the cells or merely associate with the plasma membrane. These compounds form a new class of cationic amphiphilic PKC inhibitors. In particular they could even provide the specific isoenzyme inhibitors, because fundamental differences have been observed among the substrate specificities of several PKC isozymes. However, a disadvantage is the limited bioavailability of such compounds.

Having covered the most significant aspects of PKC research, in the following sections we describe the synthesis of our first generation of PKC lipid inhibitors and a review of the synthetic methods revelant to the development of a second generation of optically pure PKC inhibitors. We will briefly discuss about the advantages/disadvantages of these methods.

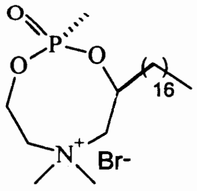
II.6 Rationale for the Design of PKC inhibitors

In the search for pharmacologically active compounds, hundreds, and often thousands of compounds are routinely evaluated. Naturally occurring compounds are a valuable starting point in the search for active leads which may provide insight into the structural features of molecules that can be optimized synthetically for greater potency and pharmacological profiles. Yet, sometimes results may deviate from what one may have expected. Nevertheless, this evidence becomes part of the data pool and is used by the scientific community to further its understanding to a given problem.

A great deal of work in our laboratories have been directed toward preparing the next generation of PKC inhibitors. To this end, we have investigated cyclic synthetic analogues of miltefosine **30**[5] and naturally occurring phosphatidylcholine.[4] Two of our promising leads are a diastereomeric pair of compounds **2a-b** containing an eight-membered phosphorus heterocycle with a quaternary ammonium ion and an alkyl side chain.[3]



2a



2b

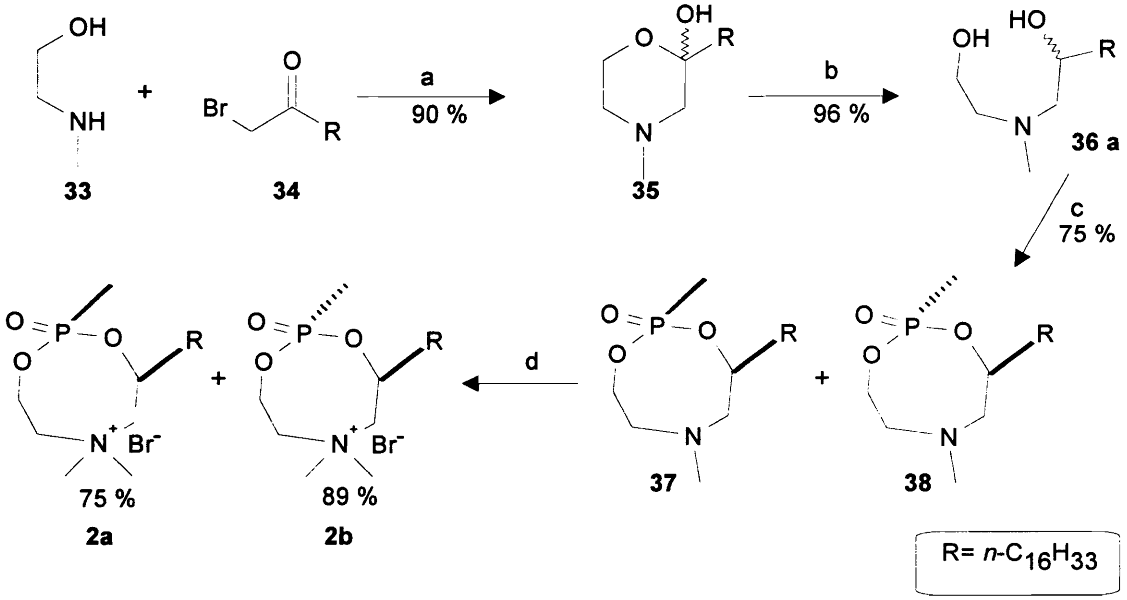
By using a phosphonate instead of a phosphate group, we demonstrated a diastereoselective inhibition of PKC by a factor of two (**2a**, $IC_{50} = 4.8\ \mu M$; **2b**, $IC_{50} = 9.9\ \mu M$).[3]

In view of these encouraging results, our attention recently turned to the study of enantioslective PKC inhibition. To accomplish this task, all four stereoisomers of **2** or analogues of these prototype molecules need to be synthesized selectively. In particular, we have chosen to prepare the stereoisomers of a lower homologue (alkyl chain-length one carbon shorter) that bears the palmitoyl chain length, which is present in nature and in several natural and synthetic PKC inhibitors such as palmitoylcarnitine and miltefosine. This added parameter may or may not produce a substantial change in the inhibition potency.

Compounds **2a** and **2b**, on the other hand, contain an odd number of carbons in their chain-length, which is not found in nature. Many enzymes selectively discriminate different chain-lengths, and a factor of one or two carbons may be important. For example, other acylcarnitines of long-chain fatty acids such as stearoyl and linoleylcarnitine were less effective than palmitoylcarnitine as PKC inhibitors.[98] On the other hand, short-chain acylcarnitines such as octanoyl-, hexanoyl-, and acetylcarnitine were not effective. Another important factor to consider is the so-called "odd-even alternation effect". Homologous series of long-chain compounds often show alternating regularities in their melting points,[109] aggregation constants,[110] solubility,[111] and chromatographic retention.[112] Solubility and aggregation constants, in particular could affect the inhibition constant.

To understand the different synthetic demands between the racemic and the stereocontrolled synthesis of our inhibitors, we begin by presenting the racemic synthesis.[3] In general, in the homologous series of long-chain compounds, the odd-numbered ones are much more expensive than the even-numbered counterparts. For example, 1-octadecene 90% \$10.65/L; whereas 1-heptadecene 99% \$46.50/5 g.[113] Without taking into consideration the purity factor, in a rough approximation, 1-heptadecene is about 900 times more expensive than 1-octadecene, and it is not far from the truth to say that the chain-length outcome of the racemic synthesis was "mainly" driven by economic factors.[114]

As outlined in Scheme 2, the synthesis started with the coupling of commercially available *N*-methylaminoethanol (**33**) with 1-bromo-2-octadecanone (**34**) to yield racemic 2-heptadecanoyl-2-hydroxy-*N*-methylmorpholine (**35**).[3] 1-Bromo-2-octadecanone was obtained from 1-octadecene as previously described.[115] Compound **35** is then reductively opened with $NaBH_4$ in methanol to yield aminodiol **36 a**. The later compound is condensed with methyl phosphonic dichloride, using triethylamine (TEA) as the base to give diasteromers **37** and **38**, which are readily separated by column chromatography on neutral alumina. Quaternization of each diastereomer with bromomethane provides the ammonium salts **2a** and **2b** in 75% and 89% yield respectively. It is important to mention that the quaternary ammonium bromide derivatives of **36 a** also inhibit PKC.[112]

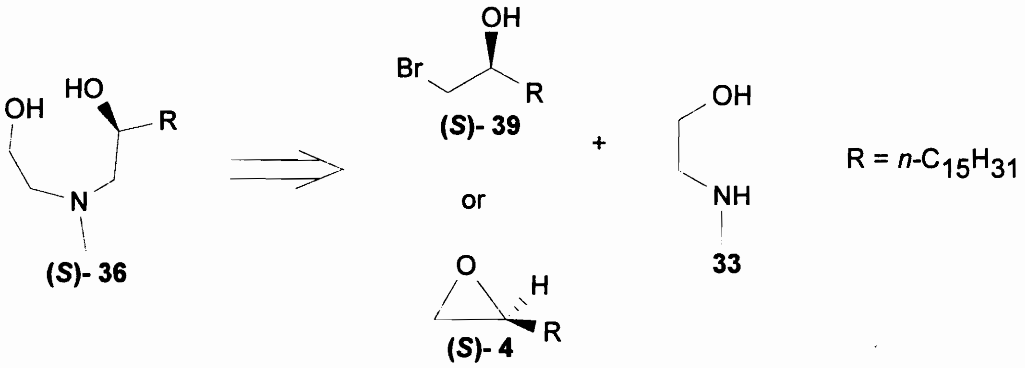


a) CH_3NO_2, rt. b) $NaBH_4$/MeOH, rt. c) $P(O)Cl_2CH_3$, NEt_3, CH_2Cl_2 d) CH_3Br, Et_2O, rt.

Scheme 2

For the stereocontrolled synthesis we require enantiopure aminodiols, from which the desired target molecules could be obtained . Therefore, the carbon stereocenter need to be set with absolute configuration. For our study we required the enantiomeric excess to be greater than 98% to ensure correct assignment of enantioselective inhibition. We envisioned the nonracemic long alkyl-chain epoxide ((*R*)- and (*S*)-pentadecyl oxyrane) or bromohydrin ((*R*)- and (*S*)-2-hydroxy-1bromoheptadecane) as the key intermediates to provide the optically pure aminodiols (Scheme 3).

With this in mind as our first goal, we initiated a literature search and found that both optically pure long alkyl-chain epoxides or bromohydrins have not been previuosly reported. However, as we found merit in some of the procedures reported for analogous compounds a review is given in the next section.



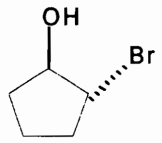
Scheme 3

II.7 Review of the Synthetic Methods Relevant to the Project

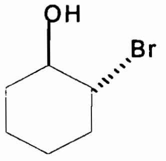
Synthesis of optically pure bromohydrins

Chemoenzymatic transformations have been widely used for the resolution of secondary alcohols.[116] These transformations include ketone reduction,[117,118,119] asymmetric hydrolysis,[120] and esterification.[121] For our purposes, kinetic resolution provided the advantage of ready access to both enantiomers.

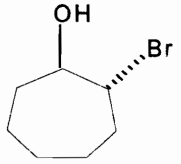
Most successful resolutions are usually obtained with molecules in which the stereocenter bears size distinguishable substituents, or when the stereocenter is part of a cyclic system.[114] There are some examples of bromohydrin resolution in the literature. For example, cyclic bromohydrins such as **40**, **41**, and **42** have been efficiently resolved by enzymatic hydrolysis of their respective butyrate esters.[122]



40

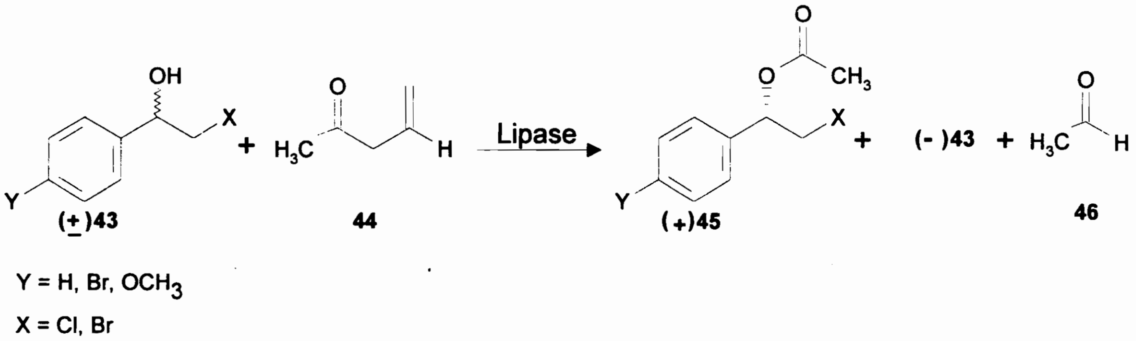


41



42

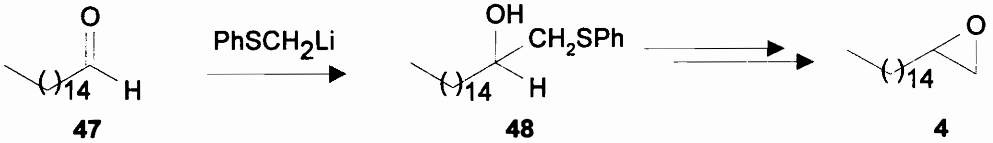
Enantiomerically pure 3-bromo-2-octanol has been prepared by microbial reduction of the corresponding α-bromo ketone.[114] Another example was provided by Hiratake and co-workers,[123] who reported highly enantioselective acylations of several 2-halo-1-arylethanols catalyzed by lipase from *Pseudomonas fluorescens* as shown in Scheme 4.



Scheme 4

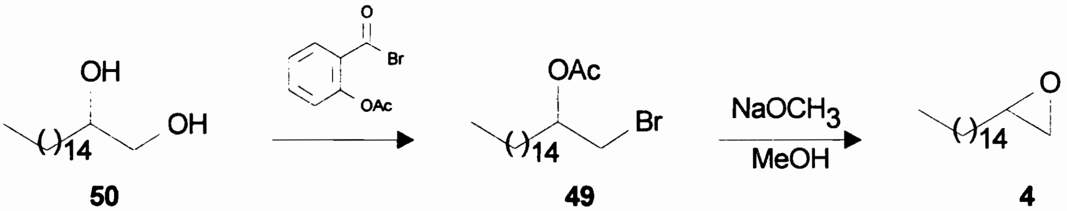
Synthesis of Optically pure Epoxides

Neither enantiomer of pentadecyloxirane **4** has been previously reported. However, there are precedents for the preparation of the racemic mixture. (±)-Pentadecyloxirane **4** has been made by nucleophilic alkylidene transfer from a sulfur ylide to hexadecanal **47** to yield a β-hydroxy sulfide intermediate **48**, which was converted to **4** by alkylation at sulfur followed by treatment with base.[124] An optically pure β-hydroxy sulfide can also be used for the preparation of nonracemic epoxides as we will discuss later.



Scheme 5

Rao *et al.*[125] reported the synthesis of (±)- **4** from 1,2-heptadecanediol *via* regiospecific formation of the bromo acetoxy intermediate **49**, which upon hydrolysis yielded the terminal oxirane.

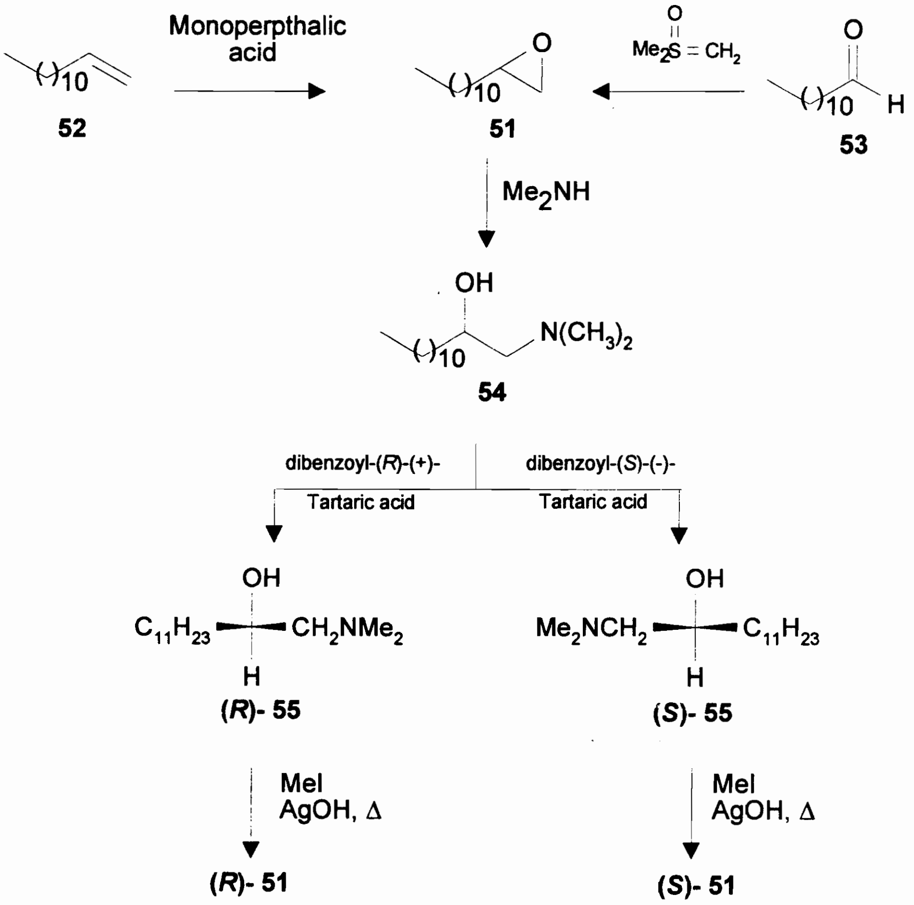


Scheme 6

These authors highlighted the convenience of this method for the synthesis of optically active terminal epoxides, however, from a series of diols employed as starting materials, only 1,2-propanediol was obtained optically pure. We also attempted the preparation of long-chain optically pure epoxides from nonracemic terminal diols. Our approaches will be addressed later in the next chapter.

Optically pure lower homologues of **4** such as (*S*)-tridecyloxirane and (*S*)-undecyloxirane appear in the literature. These compounds have been used as intermediates in the syntheses of natural products, namely oxoprenolol, a β-adrenergic blocking agent, and others.[126] Coincidently, nonracemic undecyloxirane was prepared as the key intermediate in all the following reviews for the synthesis of δ-*n*-hexadecalactone, a pherormone that regulates some behaviors of queens and workers of the Oriental hornet.[127]

In 1976, Coke and Richon[128] reported the first synthesis of optically pure long-chain oxiranes. Their approach began with racemic 1,2-epoxytridecane **51** as shown in Scheme 7. This epoxide was opened with dimethylamine and the resulting 1-dimethylamino-2-tridecanol was resolved as diastereomeric salts using the two enantiomers of dibenzoyltartaric acid to yield **(*R*)- 55** and **(*S*)- 55** after hydrolyses. The two aminoalcohols were then converted into the two optically pure epoxides**(*R*)-** and **(*S*)- 51** by quaternization followed by Hofmann elimination.

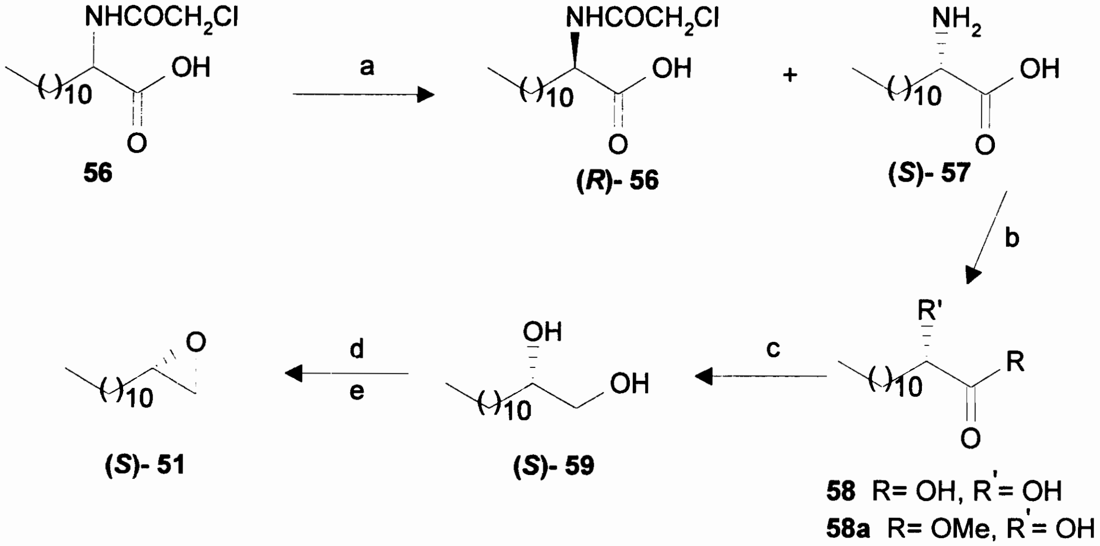


Monoperpthalic acid
52
51
53
$Me_2S=CH_2$
Me_2NH
54
$N(CH_3)_2$
dibenzoyl-(R)-(+)- Tartaric acid
dibenzoyl-(S)-(-)- Tartaric acid
$C_{11}H_{23}$
CH_2NMe_2
Me_2NCH_2
(R)- 55
(S)- 55
MeI
AgOH, Δ
(R)- 51
(S)- 51

Scheme 7

Almost 10 years later, in 1985; two groups independently published the synthesis of nonracemic **51**. Mori and Otsuka[129] resolved the unnatural long-chain **α**-aminoacid **57** employing aminocyclase of *Aspergillus* spp (Scheme 8), which provided for separation of both enantiomers. Deamination of **(*S*)- 57** with nitric acid yielded the **α**-hydroxy acid **58**, which was reduced to (*S*)-1,2-tridecanediol **(*S*)- 59** with lithium aluminum hydride (LAH). Diol **(*S*)-59** was then converted to the terminal epoxide **(*S*)-51** by hydrolysis of the acetoxy bromide intermediates in 86%

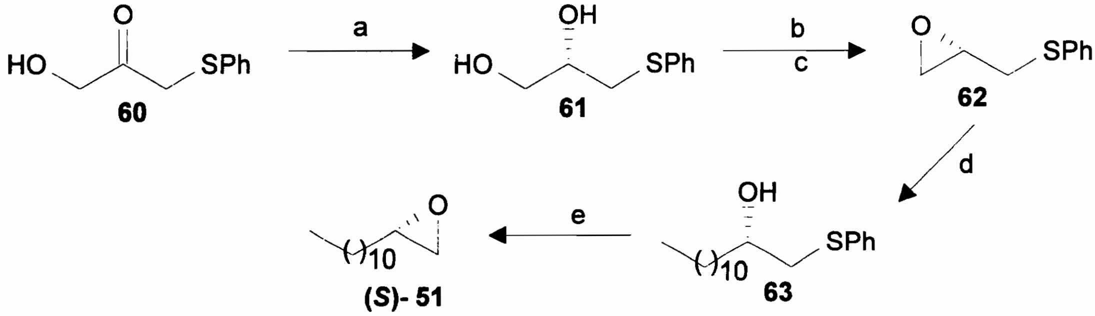
yield. The optical purities of **(*S*)-58a** and **(*R*)-58a** were calculated as 91.8% and 91.3%, respectively, by HPLC analysis of the corresponding α-methoxy-α-trifluoromethylphenylacetates (MTPA esters). These values indicate incomplete selectivity of the enzymic hydrolysis, and/or incomplete retention of configuration during the deamination step, or least probably, the partial racemization during the esterification of 598 to **58a.**[126]


NHCOCH2Cl
OH
10
O
56
a
NHCOCH2Cl
OH
10
O
(R)- 56
+
NH2
OH
10
O
(S)- 57
b
R'
R
10
O
58 R= OH, R'= OH
58a R= OMe, R'= OH
c
OH
OH
10
(S)- 59
d
e
O
10
(S)- 51

a) Amino acylase/ water. pH= 7.25 (NaOH/HCl), 37 ^oC, $CoCl_2$ cat.
b) H_2SO_4 2N, heat, $NaNO_2$. c) LAH/ THF
d) HBr-HOAc. d) NaOMe/ MeOH

Scheme 8

Fujisawa *et al.*[130] also employed an optically pure β-hydroxy sulfide intermediate obtained from the chemoenzymatic reduction of 1-hydroxy-3-phenylthio-2-propanone **60** as shown in Scheme 9.

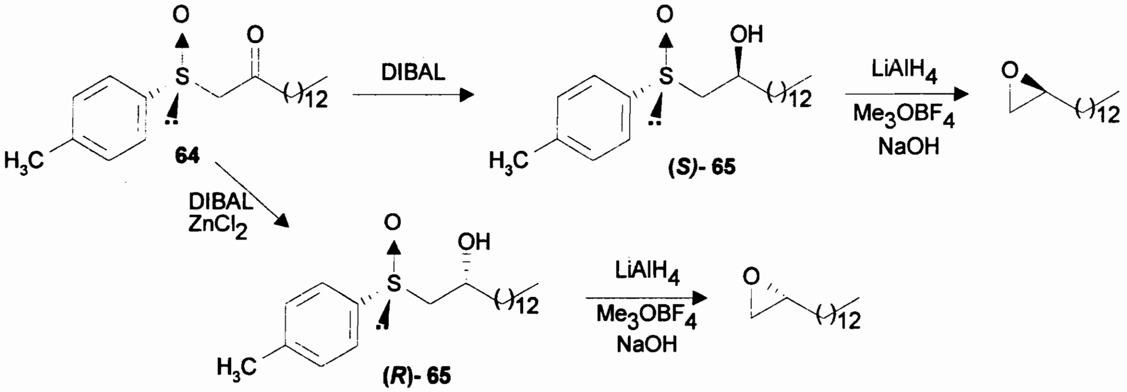


a) Baker's yeast. b) TosCl/Py. c) NaOH/MeOH, -10 °C, 4h. d) n-$C_{10}H_{21}MgBr$ CuI, THF-Me_2S(20:1), -30 °C. e) $Me_3O^+BF_4^-$ (1.8 equiv.) 2.5 M NaOH

Scheme 9

Selective tosylation of the primary hydroxyl group of **61** followed by an alkaline treatment afforded glycidyl sulfide **62**. A cooper (I)-catalyzed reaction with decylmagnesium bromide gave the secondary alcohol **63**, which then was treated with trimethyloxonium tetrafluoroborate to afford epoxide **(*S*)- 51** in 83% yield upon basification. The optical yield was determined to be 78 ee % by analysis of a derivative of **62** (1-phenylthio-2-butanol) and by comparison with the literature data.

Solladié *et al.*[131] prepared both enantiomers of tridecyloxirane from a β-hydroxysulfoxide intermediate (Scheme 10) obtained by reduction of optically active β-ketonesulfoxide with diisobutyl aluminum hydride (DIBAL) with high asymmetric induction (90-95%). The reduction of **64** with DIBAL afforded **(*S*)- 65** with 90 d.e.%. The same reduction in presence of one equivalent of anhydrous zinc chloride reversed the diastereomeric selectivity to give primarily **(*R*)- 65** with 90 d.e%. The diasteromeric ratio was determined by ^{1}H NMR (100 MHz) assignments of the crude product from the diastereoisotopic protons of the α-methylene group. Interestingly,

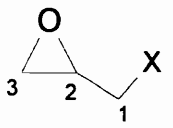


Scheme 10

this paper presented a discrepancy with the literature. All the assignments of the absolute configuration based upon the optical rotation data, are the opposite to what others have reported.[125,127,126]

Synthesis of Optically pure Epoxides from Glycerol Derivatives

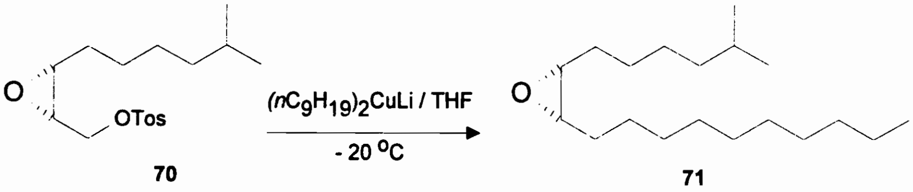
Both enantiomers of glycidol **66** are commercially available in about 88-91 ee %, and they could be readily enriched up to 99 ee % by recrystallization of their *p*-nitrobenzoyl derivatives in ethanol. These compounds are two of the most widely used 3-carbon chirons and their reactions are well documented.[132]



	X
66	OH
67	Cl
68	Br
69	OTos

Glycidol **66** has 3 potentially electrophilic centers and the reactivity order usually runs from $C_3 \approx C_2 > C_1$. There are several examples in which the regioselectivity (C_3 vs. C_2) can be controlled.[144,129,133] When X becomes an activating group (e.g. X= halides, sulfonate esters), the electrophilicity of C_1 is further increased and can be advantageously used when reactions at C_1 are desired.[129]

We investigated both nucleophilic epoxide opening (C_3) on glycidol, and nucleophilic displacement (C_1) on glycidol tosylate. In particular, we were interested in a long-alkyl organocuprate coupling reaction as seen in the final step of the synthesis of (+) disparlure **71** (Scheme 11).[134]



Scheme 11

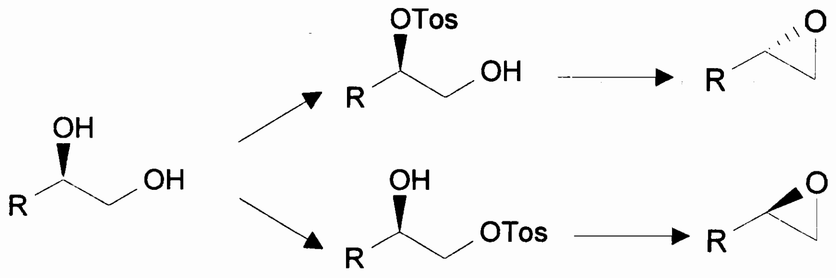
A further discussion of the methods presented in this section will be addressed in the next chapter.

III. DISCUSSION

Despite the structural simplicity of our key intermediates (*S*)-, (*R*)-pentadecyloxirane ((***S***)- and (***R***)- **4**) and (*S*)-, (*R*)-1-bromo-2-hydroxyheptadecane their syntheses are unknown in the literature. From our literature search we found no examples on the synthesis of long-chain acyclic bromohydrins and only a few examples on the synthesis of nonracemic lower homologues of **4**.

The first nonracemic synthesis of long-chain epoxides, such as (*R*)- and (*S*)-tridecyloxirane was accomplished in 1976 by Coke and Richon (Scheme 7).[125] However, we were discouraged by this synthesis due to the lack of information about the enantiomeric purity. The authors did not report the optical purity at any stage throughout the synthesis. Subsequent synthesis by others[126,127,135] showed that Coke and Richon's compound was of low enantiomeric purity (< 10 %) based on optical rotation data. Mori and Otsuka's approach[126] to the titled compound (Scheme 8) provided much higher enantiomeric purities (≈ 92 %). However, the overall result was still a lengthy process, taking into consideration the synthesis of the unnatural aminoacid employed.

The synthesis completed by Fujisawa *et al.*[127] presents an opportunity for partial racemization (Scheme 9). For the sequence diol → monosulfonate → epoxide to be successful, a high degree of regioselectivity in the initial sulfonylation step is required. As the cyclization of regioisomeric sulfonates leads to opposite enantiomers with a loss in the enantiomeric purity (Scheme 12).



Scheme 12

Sharpless and co-workers,[147] have more often found that, even employing sterically more encumbered areneresulfonyl chlorides such as 2,4,6-triisopropylbenzenesulfonyl chloride and 2,4,6-trimethylbenzenesulfonyl chloride, a mixture of regioisomeric monosulfonates and bis-sulfonates are formed.

The lack of an efficient synthesis with high optical purity was then evident. While the syntheses of chiral lipids may not be complicated, the purification and enantiomeric enrichment could represent challenging tasks. Long-chain compounds are particularly prone to polymorphism probably as a consequence of the different lipid-chain packing arrangements.[136] In other words, the packing of the hydrophobic chain could be the driving force for crystallization, rather than the polar head containing the chiral information, therefore the resistance of lipids to enantiomeric enrichment.

With this in mind, having explored what was investigated, we decided to embark on other alternative routes due to our high demand for high enantiomeric purity. The description of our initial approaches is presented below.

III.1 Initial Synthetic Approaches

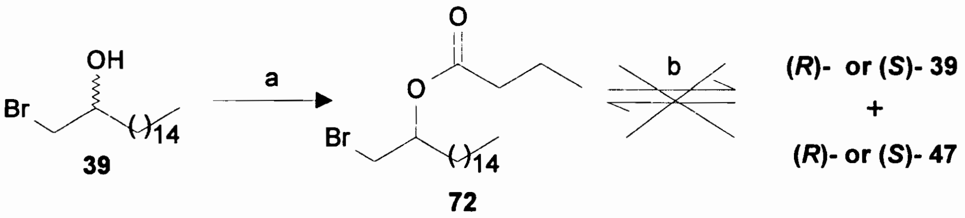
Approach Toward the Synthesis of Optically Pure Bromohydrins

One of our earliest approaches to obtain enantiopure long-chain bromohydrins, employed an enantioselective reduction of the ketone funtionality of 1-bromo-2-heptadecanone **34** by using (+) or (-)B-chlorodiisopinocamphenylborane ((+) or (-)DIP-chloride).[110] However, the reduction resulted in a mixture of low enantiomeric excess.[137]

We did not find examples of resolution of acyclic 1-bromo-2-hydroxyalkanes (long chain); however, we found merit in such a process and attempted a series of enzymatic hydrolysis conditions with (1-bromoethyl)hexadecylbutanoate **72**. Lipases are presently the most widely used enzymes for the kinetic resolution of esters and alcohols. Lipases are relatively stable in organic media which broadens the applicability of these enzymes to substrates which are not suited to aqueous media for reasons of solubility and stability.[138] Lipases catalyze both stereoselective esterification, transesterification and hydrolysis.[118,139,140]

In particular, we chose to carry out hydrolytic reactions because they offer some advantages over esterification and transesterification reactions. In general, these two latest reactions are considerably slower.[141] Lipases catalyze reactions in both directions causing a net decrease in optical purity as the reverse reaction begins to compete kinetically.[142] Therefore we prepared our substrate **72** from racemic **39** by esterification with butyric anhydride, employing triethylamine as the base and catalytic amounts of 4-dimethylaminopyridine (DMAP) in dichloromethane. The yield was 94 % after purification by column chromatography (Scheme 13).

The enzymes were supported on Celite and washed with a buffer solution (pH = 7.0) made of Na_2HPO_4 0.1M and NaH_2PO_4 0.1M (8 :5). The hydrolyses were run at room temperature in organic solvents such as dichloromethane and ether pre-washed with the buffer solutions. The resulting mixtures are commonly called "wet" solvents.



a) Butyric anhydride, TEA, DMAP, CH_2Cl_2, rt. b) Lipases, pH= 7. 0, CH_2Cl_2, rt, 7 d.

Scheme 13

Lipases:

a) Lipase EC 3.1.1.3 type VII from *Candida cylindracea* purchased from Sigma.

b) Lypozyme IM from *Mucor miehei* lipase immobilized on a ion-exchange purchased from Novo Nordisk Bioindustrial INC.

c) Lipase PS-30 from *Pseudomonas cepacia*

d) *Pseudomonas fluorescens* (SAM 2) purchased from Fluka.

e) Same as d.

* Lipases a-c were provided by Dr. T. Hudlicky.

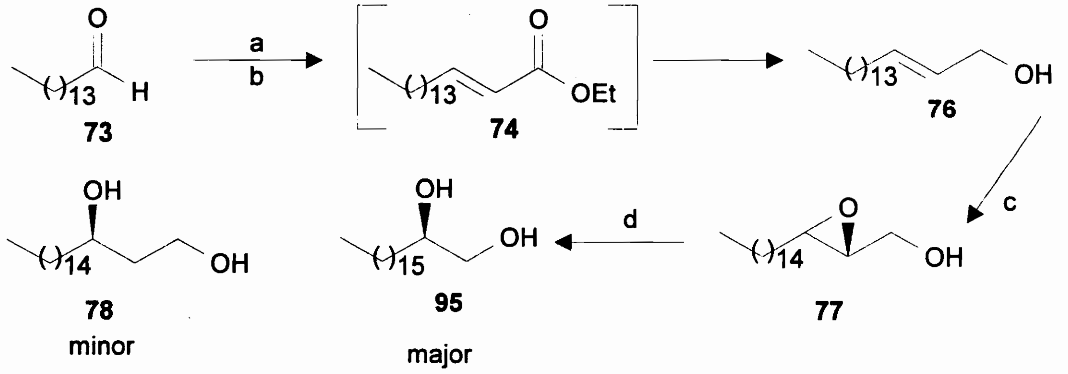
Compound **72** was screened with several different lipases following a modified procedure.[143] We monitored the reaction by thin layer chromatography, each day for seven days, but we did not observe formation of a product. Only starting material was detected. A major problem was the low solubility of **72** in these solvents, thus, the reactions were run under much more dilute conditions. This factor may have accounted for the failure of these reactions.

Approach Toward the Synthesis of Optically pure Epoxides from Aldehydes

Oxidation of pentadecanol with an excess of pyridinium chlorochromate (PCC) in dichloromethane gave pentadecanal **73** in 94% yield after purification by silica gel filtration. Tentatively, we prepared pentadecanal **73** by using pyridinium dichromate (PDC) and by employing Swern conditions.[144] PDC and Swern oxidations gave comparable chemical yields, although the Swern conditions were more demanding. Pentadecanal then was converted to the epoxy alcohol **77** as reported by Roush and Adam.[145] The known allylic alcohol[146] **76** was prepared in two steps (one pot) from **73** emplying diisopropyl(ethoxycarbonylmethyl)phosphonate followed by *in situ* diisobutylaluminum hydride (DIBAL) reduction of the intermediate α,β-unsaturated ester **74** (Scheme 14).

With the epoxy alcohol **77** in hand we attempted a Lewis acid mediated regioselective epoxide opening at C3 as we found reported in the literature for lower homologues.[147] The Lewis acid, titanium tetraisopropoxide, complexes with both the oxirane oxygen and the free hydroxyl group to yield the 1,2-diol **95** and the 1,3-diol **78** as the major and minor products, respectively. Indeed, we obtained this isomeric mixture, but our attempts to separate the diols failed. We required the terminal 1,2-

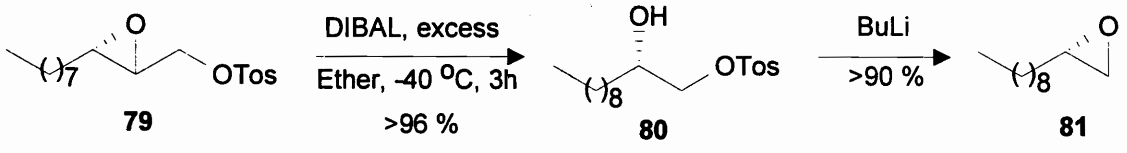
diol for subsequent conversion into the homoallylic terminal epoxide following a published procedure.[148]


a
b
73
74
OEt
76
OH
c
77
d
95
major
78
minor

a) $EtOOCCH_2P(O)(O\textit{i}\text{-}Pr)_2$, $K^{+}\textit{t}\,BuO^{-}$, 0 °C to rt. b) DIBAL, Et_2O, 0 °C to rt.
c) (+)DET, TBHP, Ti $(O\textit{i}\text{-}Pr)_4$, CH_2Cl_2, - 20 °C, 3 d. d) $LiBH_4$, $Ti(O\textit{i}\text{-}Pr)_4$, benzene, 10 °C

Scheme 14

As we ere developing another route, Chong and Johansen[149] reported a new direct reduction of 2,3-epoxy tosylate **79** to 2-hydroxytosylate **80** with DIBAL. The hydroxy tosylates prepared by this method provide ready access to simple or functionalized secondary alcohols and optically pure terminal epoxides such as **81** of high enantiomeric purity.


OTos
79
DIBAL, excess
Ether, -40 °C, 3h
>96 %
OH
OTos
80
BuLi
>90 %
81

Scheme 15

Syntheses of Nonracemic Long-chain 1,2 diols

Another promising route to optically pure epoxides is the asymmetric dihydroxylation (AD) reaction developed by Sharpless and co-workers.[150] Sharpless *et al.* have studied the AD reaction on a series of aliphatic terminal olefins. They demonstrated that the enantioselectivity strongly depends on the chain length of the olefins. The ee % initially increases with the number of carbons atoms (from propene to pentene), but then it reaches a plateau at *ca* 89% when the chain-length is greater than five. However, higher homologues have never been explored with the AD reaction conditions to reach a conclusion on the ee % vs. chain-length relationship.

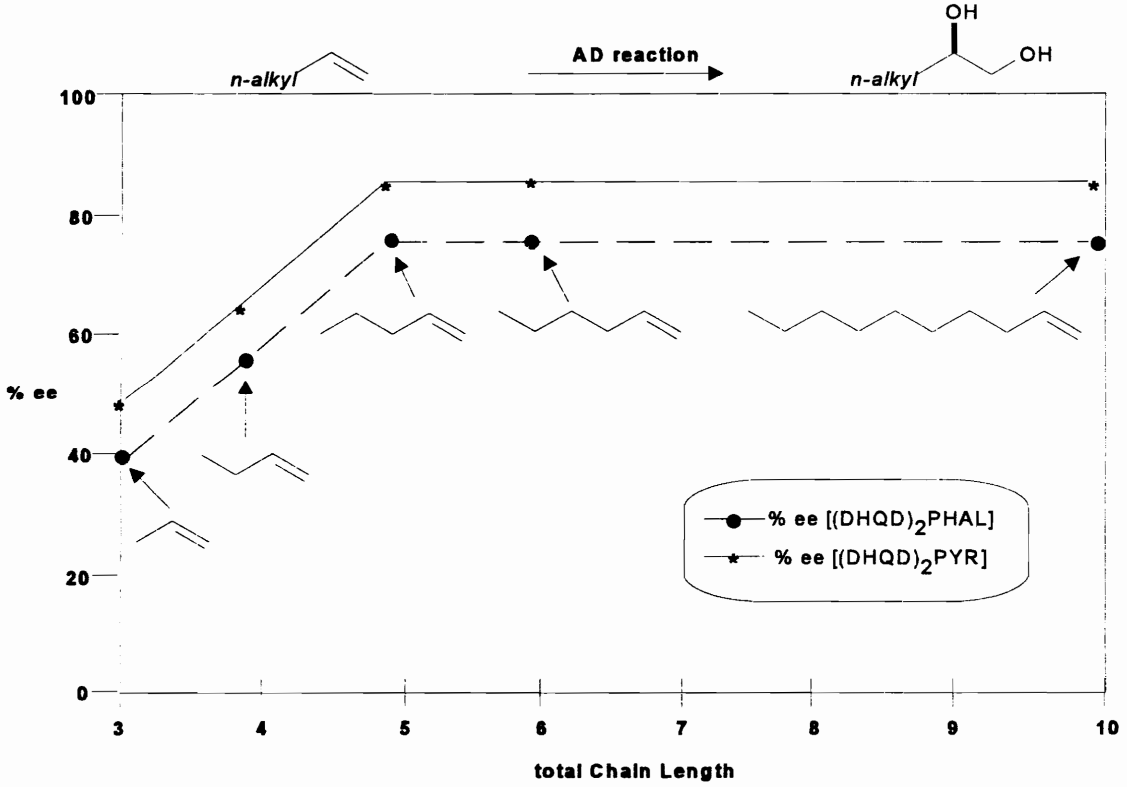


Figure 5. Dependende of the ee % on the Chain-length of Aliphatic *N*-Alkenes.

We evaluated the AD reaction on 1,2-heptadecene with the following series of ligands. The pyrimidine derivatives,[151,152] bis-dihydroquinine pyrimidine $(DHQ)_2PYR$ and bis-dihydroquinidine pyrimidine $(DHQD)_2PYR$; and the anthraquinone derivatives,[153] bis-dyhydroquinine anthraquinone $(DHQ)_2AQN$ and bis-dihydroquinidine anthraquinone $(DHQD)_2AQN$. In general the AD reaction for "standard" olefins is run under the following conditions:[147]

For 1 mmol of olefin:

$K_3Fe(CN)_6$,(3 equiv.)
K_2CO_3,(3 equiv.)
Ligand, (1% mol)
$K_2OsO_2(OH)_4$, 0.2% mol)
t-BuOH:water (1:1), 10 mL.
Concentration of the olefin 0.001M
Temperature, 0 °C
Reaction time, ≈ 6-24 h (depending on the olefin).

We had difficulties with the above-mentionedmethod. Again, we encountered problems due to the low solubility of 1,2-heptadecene in the amount of solvent employed by Sharpless. Thus, we carried out the AD reactions under two modified versions of the standard conditions.

Method A

For 1mmol of olefin:

$K_3Fe(CN)_6$, 1000 mg (3.03 equiv.)

K_2CO_3, 430 mg (3.12 equiv.)

Ligand, 14 mg (1.75 % mol)

$K_2OsO_2(OH)_4$, 10 mg 0.25% mol)

t-BuOH:water (1:1), 14 mL.

Concentration of olefin 0.007M

Temperature, 0 °C to rt

Reaction time, ≈ 3 days (1 day at 0 °C and 2 days at room temperature).

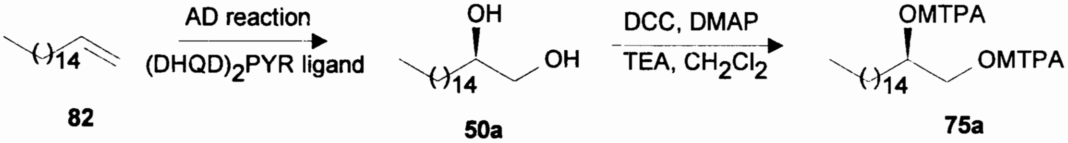
Method B

Same as method A

Temperature, room temperature

Reaction time, ≈ 30 hours.

We obtained high chemical yields of purified 1,2-heptadecanediols (≥ 95%). Similarly, we prepared 1,2-heptadecanediols **50d** and **50e** employing the chiral ligands $(DHQ)_2AQN$ and $(DHQD)_2AQN$ following method B. The enantiomeric excesses were evaluated by using ^1H NMR techniques on the bis-MTPA ester derivatives. We first tried to prepare these derivatives using standard esterification conditions.[154] We employed (*R*)-MTPA-chloride, triethylamine and a catalytic amount of DMAP, however, under these conditions the diol did not react , even with a prolonged reaction time. Subsequently, we tried the reaction using stoichiometric amounts of DMAP, but we did not obtain the product. We also tried generating the Mosher's acid chloride *in situ* by reacting the acid with thionylcloride and again did not observe any differences from the previous attempts. What seemed to be a trivial transformation turned out to be more difficult than we initially thought. Finally, we were able to obtain the bis-MTPA derivatives by using dicyclohexylcarbodiimide (DCC), (*R*)-MTPA acid and DMAP in equivalent amount.[155] Using this reaction we prepared bis-MTPA derivatives of racemic and nonracemic diols (Scheme 15). The ^1H NMR spectrum of racemic **75** was obtained for corroboration of the expected 1:1 diastereotopic ratio. After the work-up, the crude material still contained the remains of dicyclohexylurea (side product) and DMAP (by ^1H NMR analysis), however, their peaks did not interfere with the diastereotopic peaks as we intended to use them for determination of the diastereomeric ratio (*vide supra)*.



Scheme 16

Figure 6 shows that the two CH_2OMTPA signals δ 4.65-4.23 ppm exhibited by the bis-Mosher ester of racemic **75** are base-line separated in the region of δ 4.52-4.34 ppm. The two lower field doublet of doublets in the region of δ 4.65-4.52 ppm with baseline separation around δ 4.60-4.58 ppm are assigned to H_a and $H_{a'}$. These hydrogens are assumed to be split by the methyne proton (H_c ,$H_{c'}$) and (H_b , $H_{b'}$) of each diasteromeric CH_2OMTPA group. The other two doublet of doublets, expected for H_b and $H_{b'}$ do not show up as neatly as the H_a ($H_{a'}$) peaks due to overlapping in the region of δ 4.34-4.23 ppm; therefore, our estimation of the enantiomeric purity was based only on the H_a ($H_{a'}$) segments. Integration of H_a and $H_{a'}$ in the racemic mixture **75** gave 49.5% and 50.5% respectively, with a slight deviation from the theoretical 50:50 (acceptable between the error of detection).

Figure 7 shows the ^{1}H NMR spectra of the diasteromeric mixture of the bis-Mosher ester derivatives **75a** and **75b** derived from nonracemic diols. The precursor diols were obtained by the AD reaction (Method A), using $(DHQ)_2PYR$ and (DHQD)2PYR respectively. We can clearly observe the enrichment of one of the diastereotopic peaks. The enantiomeric purity of some nonracemic ether-linked glycerol lipids has also been evaluated in the same fashion.[156] Additional spectra corresponding to all bis-Mosher ester derivatives of 1,2-heptadecanediol run under different conditions are provided in section VII. It should be noted that ^{19}F NMR spectra of racemic and nonracemic bis-Mosher esters **75** did not give clean baseline separation.

As the determination of the ee% by Sharpless and co-workers[151] was obtained by using chiral, stationary-phase HPLC, we double-checked one of our samples under similar conditions[157] and did not find any variation from that obtained from the ^{1}H NMR analyses. Table 4 presents the results of the ee% evaluation for all our AD reaction attempts.

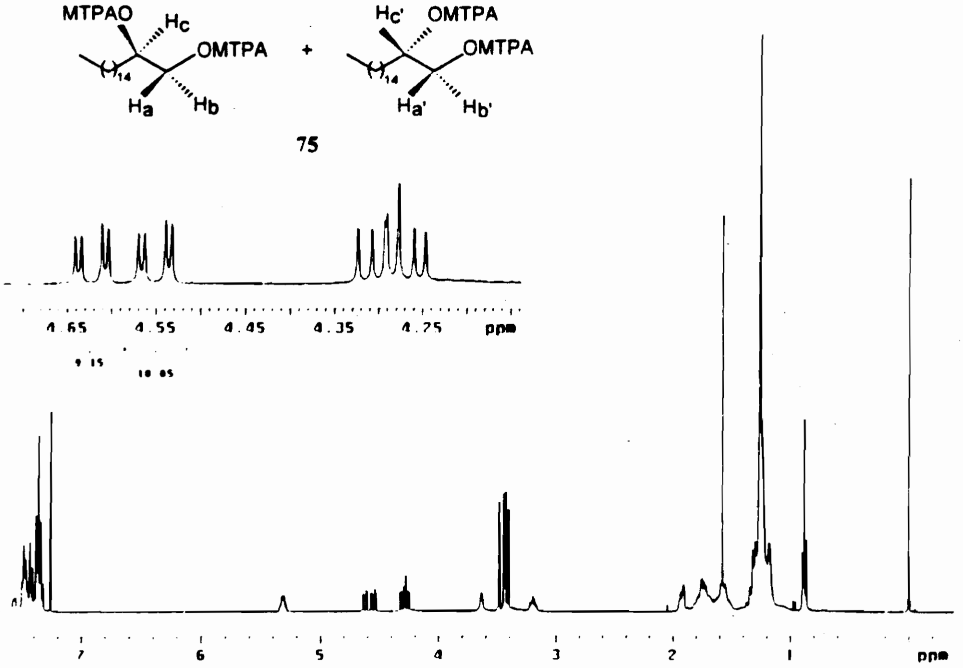


Figure 6. ^{1}H NMR Spectrum of the Diastereomeric Mixture **75**.

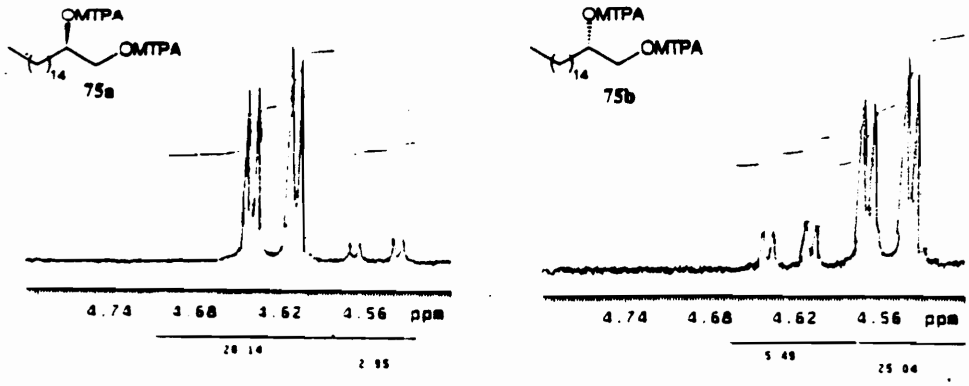


Figure 7. ^{1}H NMR Spectrum (expanded) of **75a** and **75b**.

Table 4. Enatiomeric Excess of Terminal Diols and Absolute Configuration.

Compound[a]	**Derivative Bis- MTPA**	**Method**	**ee% and configuration**[f]	**Reference**
50a[b]	**75a**	^{1}H-NMR	78%, *R*	This work
50b[b]	**75b**	^{1}H-NMR	63%, *S*	"
50a[c]	**75a**	^{1}H-NMR	74%, *R*	"
50b[c]	**75b**	^{1}H-NMR	64%, *S*	"
50b[c]	**75b**	HPLC [d]	64%, *S*	"
50d[b]	**75d**	^{1}H-NMR	73%, *R*	"
50e[c]	**75e**	^{1}H-NMR	69% *S*	"
$C_8H_{15}CH(OH)CH_2OH$	Bis MTPA-ester	HPLC[e]	89%, *R* ; 76%, *S*	148

[a] Compounds 50a and 50b were obtained using the $(DHQ)_2PYR$ and $(DHQD)_2$ PYR ligands, respectively. 50d and 50e were obtained using the ligands, $(DHQ)_2AQN$ and $(DHQD)_2AQN$.

[b] Diols were obtained using Method A.

[c] Diols were obtained using Method B.

[d] DNBPG J.T. Baker (25cm × 4.6 mm I.D.), 0.12% *i*-PrOH in hexanes 1 mL/min.

[e] Pirkle 1-A Ionic Spherical Silica (25cm × 10 mm I.D.) 0.5% *i*-PrOH in hexanes, 2.0 mL/min.

[f] The absolute configuration has been assigned according to previous results.[148]

As evidenced from the reaction conditions employed, variation of the reaction temperature did not affect the enantiomeric ratio between the limits of detection error. However, lower temperatures and more dilute concentrations are detrimental for reaction times. We do not have any explanation for the lower ee% obtained.

Recrystallization of the free diols with several solvent systems did not yield enantiomerically enriched material. Table 5 presents the ee% of recrystallized diols evaluated by ^{1}H NMR of the the bis-Mosher ester derivatives. Bis-2,4-dinitrobenzoyl derivatives were also prepared as for ee% enrichment, but we did not find an appropriate solvent with better crystallizing properties.

Table 5. Enantiomeric Excess of Diols after Recrystallization.

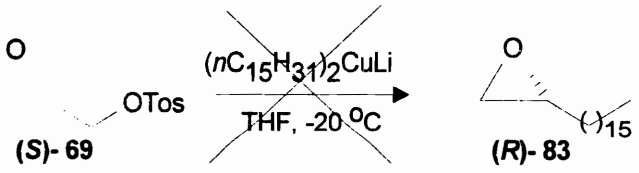
Compound[a]	**Solvent**	**Derivative Bis- MTPA ester**	**ee %**
50b	Toluene	**75c**	67
50b	MeOH:water, 9:1	**75c**	68
50b	Hexanes:EtOAc, 12:1	**75c**	72

[a] Compounds were obtained using Method A.

A recently published method to evaluate the ee % of diols has been reported by Resnick *et al.*[158] It consists of ^{1}H NMR determination of a diastereomeric mixture of cyclic boronate esters prepared by reacting the diol and an optically pure aryl boronic acid.[155,159] Certainly, it is a method that we should try in our future work because it offers the advantage of requiring only one equivalent of the boronic acid, whereas we require a large excess of the relatively expensive (*R*)-Mosher acid.

Approaches Toward the Synthesis of Optically Pure Epoxides from Glycidol and Glycerol derivatives

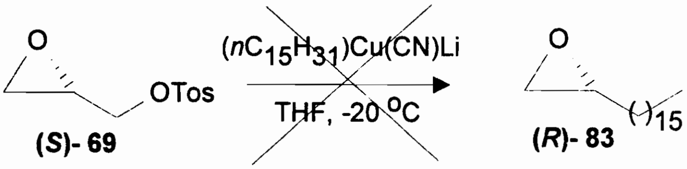
These approaches involved the long-chain alkylation of glycerol derivatives by using organocuprate and Gridnard reagents. (*R*)-glycidyl tosylate **(*R*)- 69** was prepared as previously described,[160] and then was added to an excess of lithium dialkylcuprate prepared from 1-bromopentadecane, *tert*-butyllithium and cupruous iodide in THF at -20°C. **Note**: We did not prepare di-(*n*-tetradecyl)copper lithium, because at the time of this work we did not have a supply of 1-bromotetradecane, and our primary goal was to check the feasability of the reaction.



Scheme 17

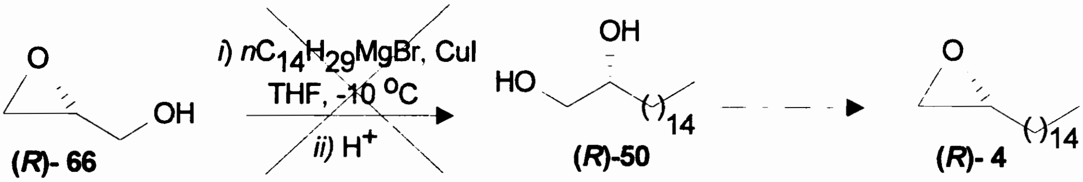
Once again the low solubility was a major drawback to executing the reported procedures. In general, organocuprates are very unstable and are made at low temperatures (-78 to -30 °C) with only a few exceptions at 0 °C.[161,162] For example, lithium dimethylcuprate-tributylphosphine complex is stable for 1.5 h at 0 °C, but the corresponding di-*tert*-butyl compound decomposes within 20 minutes at this temperature. We did not obtain the desired product, but rather a complex mixture that we were unable to identify by ^{1}H NMR.

As the reaction of epoxides are often more efficient with higher order cuprates,[158] we treated **(*R*)- 69** with di-(pentadecyl)cyanocuprate prepared following a modified procedure of Lipshutz.[158] We tried the preparation of the higher order organo cuprate at -20 °C in contrast to -70° C as in the literature, and under more dilute conditions. The higher order organocuprate was then added at -20 °C, and slowly warmed to room temperature, however, we did not observe any product.



Scheme 18

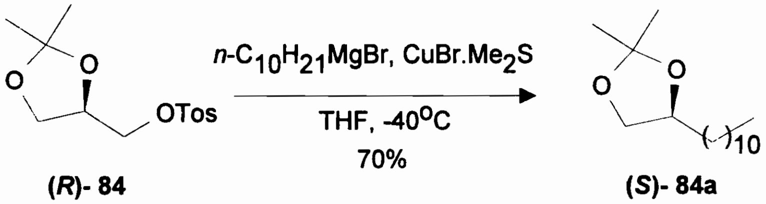
Efficient copper-catalyzed reactions of organolithiums and Grignard reagents to effect epoxide opening are reported.[163] Therefore, we tried the following reaction: (*R*)-glycidol **(*R*)- 66** was reacted with an excess of pentadecyllithium and tetradecylmagnesium chloride, respectively, in presence of catalytic amounts of cuprous iodide.



Scheme 19

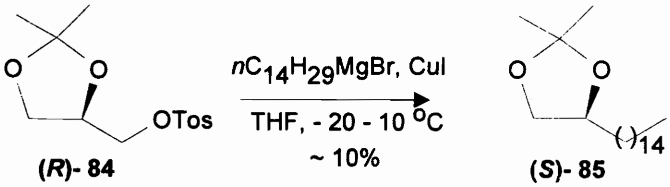
Under this basic condition, the first equivalent of the organometallic reagent is consumed as a base to deprotonate the free hydroxyl group. There is always the possibility of Payne rearrangement,[164] whereby the alcoholate displaces the epoxide internally to form a new 2,3-epoxy alcoholate. In the case of glycidol specifically, this rearrangement poses no problem since acemization does not occur. The second equivalent of the organometallic reagent should function as a nucleophile and preferentially open the epoxide *via* a C_3 attack to afford the terminal diol after acidic work-up, unfortunately our attempt was unsuccesful. The diol could have provided the desired epoxide.[148]

In response to these negative results with glycidol and glycidol tosylate, we opted to investigate alkylation of 2,2-dimethyl-1,3-dioxolane-4-ylmethyl-p-toluene sulfonate **84** which is commercially available in both enantiomeric forms. Due to their high expense, we prepared **(*R*)- 84** and **(*S*)- 84** following a reported procedure.[165] The copper(I) (CuI or CuBr) catalyzed reactions of long alkyl-chain Gridnard reagents with primary tosylates are reported in the literature.[166,167] Of particular interest, as reported by Chattopadhyay *et al,*[167] is the reaction of **(*S*)- 84** with decylmagnesium bromide to afford (*S*)-1,2-isopropylidenetridecane **(*S*)- 84a** in 70% yield (Scheme 20).



Scheme 20

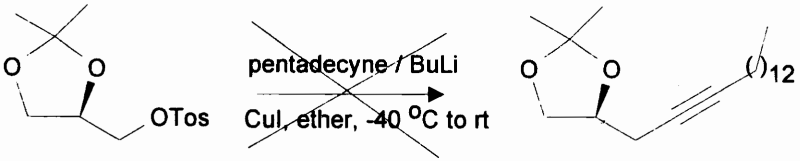
We attempted a similar reaction using tetradecylmagnesium chloride in the presence of a catalytic amount of CuI in anhydrous THF at -40 °C, but the slurry mixture of alkyl Grignard and CuI solidify prior to addition to the tosylate. Therefore, the temperature was raised to -10 °C to allow its transfer. The reaction gave the desired product (*S*)-1,2-*O*-isopropylidene heptadecane **(*S*)- 85**, but in low yield (≈10%). The major product observed was octacosane, the Wurtz coupled product.



O
O
OTos
(R)- 84
nC14H29MgBr, CuI
THF, - 20 - 10 °C
~ 10%
O
O
14
(S)- 85

Scheme 21

Trying to alleviate the recurring problem of low solubility, alkylation was attempted with a nucleophile containing an unsaturated alkyl chain. We prepared 1-pentadecynyllithium as reported,[168] and the alkylation was attempted in the presence of catalytic amounts of cuprous iodide, but the reaction did not afford any of the desired product.



O
O
OTos
(R)- 84
~~pentadecyne / BuLi~~
~~CuI, ether, -40 °C to rt~~
O
O
12
(S)- 84b

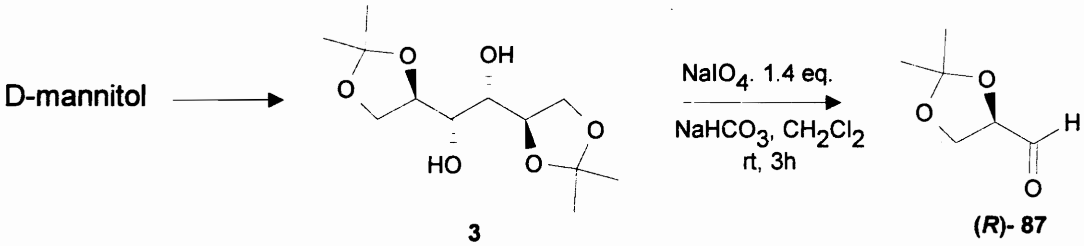
Scheme 22

III.2 Synthesis

Through out the course of our initial approaches, it became clear that low temperature reaction conditions were detrimental for the attachment of the long alkyl-chain to all three-carbon chirons employed. When the long alkyl-chain was part of the starting material as in 1-heptadecene used for the AD reaction, the result was a decrease of the enantiomeric purity of the product (*vide infra*).

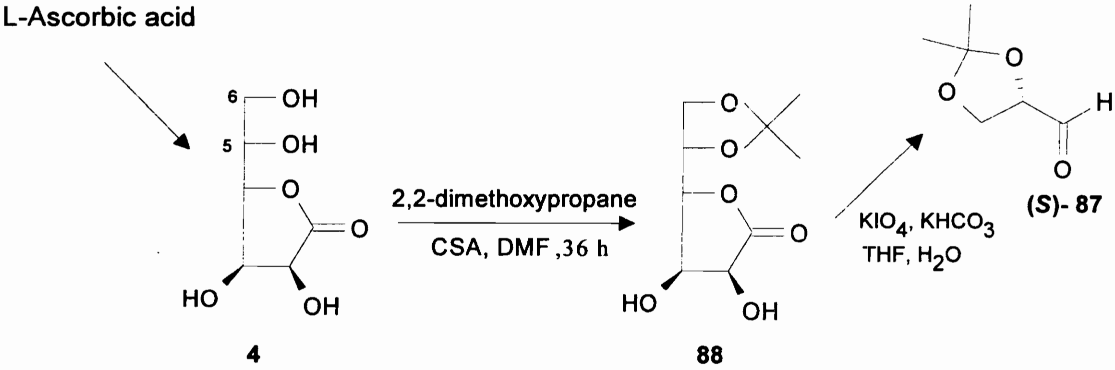
Many successful long-chain alkylations have been reported for the synthesis of sphingosines, involving the Wittig olefination as the key step.[169] Thus, we performed the Wittig olefination using enantiomerically pure (*R*)- and (*S*)-2,3-*O*-isopropylidene glyceraldehyde and commercially available tetradecyltri-phenylphosphonium bromide.

Despite the extensive use of the protected glyceraldehydes **(*R*)-87** and **(*S*)- 87** in organic synthesis,[170] neither enantiomer is commercially available. The compounds' tendency to polymerize on standing creates handling and storage problems. However, the syntheses of **(*R*)-87** [171,172,173] and **(*S*)- 87** [172,174,175] are well documented. Aldehyde **(*R*)-87** can be prepared from D-mannitol, a naturally occurring inexpensive sugar, in two steps (Scheme 23).[171]



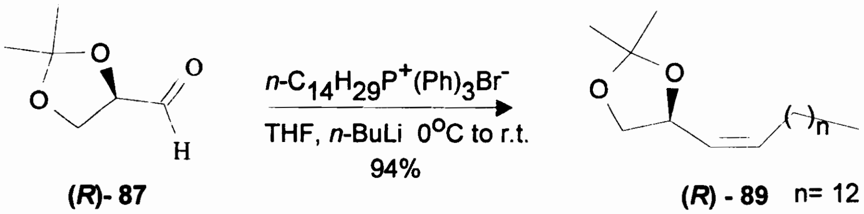
Scheme 23

Aldehyde **(*S*)- 87** was prepared from L-gulonic-γ-lactone **4**, a commercially available derivative of L-ascorbic acid, following an adapted published procedure.[176] The method involved selective protection of the 5,6 diol moiety with 2,2-dimethoxypropane in presence of camphorsulphonic acid (CSA) as the catalyst, followed by oxidation with KIO_4 in a buffered aqueous THF solution as shown below.



Scheme 24

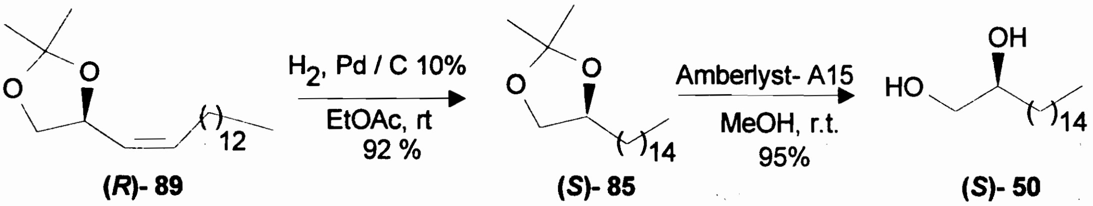
Wittig reactions with glyceraldehyde **87** have been reviewed.[167] In (*R*)- or (*S*)-2,3-*O*-isopropylidene glyceraldehyde, the chiral center of the dioxolane ring seems to play no part in the stereochemistry of the reaction and the *E/Z* ratio depends mainly on the nature of the phosphoylides and on the reaction conditions. In our case, the stereochemical outcome of the Wittig reaction does not represent a problem because hydrogenation of the double bond is the forward step (*vide supra*). The reaction temperature also plays a crucial role in the stereochemistry.[177] Higher temperatures favor the most stable *E*-olefin, whereas a higher ratio of the *Z*-olefin is obtained at low temperatures. As the *(E/Z)* ratio is irrelevant to our purposes, the reaction temperature factor was used to our advantage, enabling us to run the reaction at room temperature, after initial cooling at 0 °C (while adding the aldehyde). This "elevated" temperature should help in diminishing any problems with low solubility.


(*R*)- 87
n-$C_{14}H_{29}P^{+}(Ph)_3Br^{-}$
THF, n-BuLi 0°C to r.t.
94%
(*R*) - 89 n= 12

Scheme 25

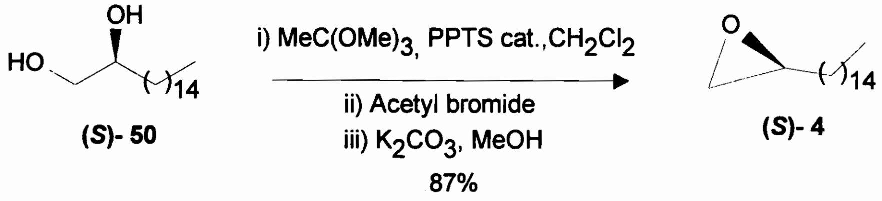
With the reaction conditions employed (salt free conditions), the Z-olefin was the major product as expected.[178,179] The *Z*:*E* ratio was 93:7 from the crude product, and 98:2 after purification.[180] Lower homologues of **(*R*)- 89**, such as (*R*)-1,2-di-*O*-isopropylidene-3-(*Z*)-tetradecene and (*R*)-1,2-di-*O*-isopropylidene-3-(*Z*)-undecene (n=9, n=6) prepared under similar conditions have also been reported with the *Z*-olefin as the major product.[181]

Catalytic hydrogenation of **(*R*)- 89** under atmospheric pressure afforded **(*S*)-85** in 95 % yield. Deprotection of the acetonide under acidic conditions yielded (*S*)-1,2-heptadecanediol in 95% after purification by column chromatography.


(*R*)- 89
H_2, Pd / C 10%
EtOAc, rt
92 %
(*S*)- 85
Amberlyst- A15
MeOH, r.t.
95%
(*S*)- 50

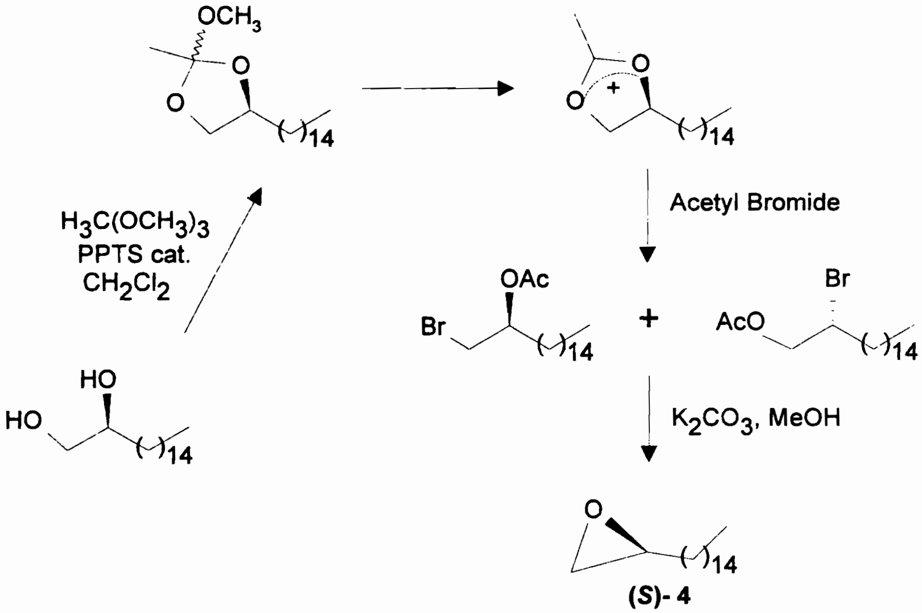
Scheme 26

The enantiomeric purity of ***(S)*- 50** was evaluated by ^{1}H-NMR analysis of the bis-Mosher ester derivative **75f** as described earlier in this section and by comparison with its racemate **75** (see Figure 6). No diastereotopic peaks were observed (see section VII). Therefore, the optical purity is at least 98% by the limits of detection. Compound ***(S)*- 50** was then converted into a terminal epoxide by an adapted "one-pot" procedure reported by Sharpless and Kolb.[7]



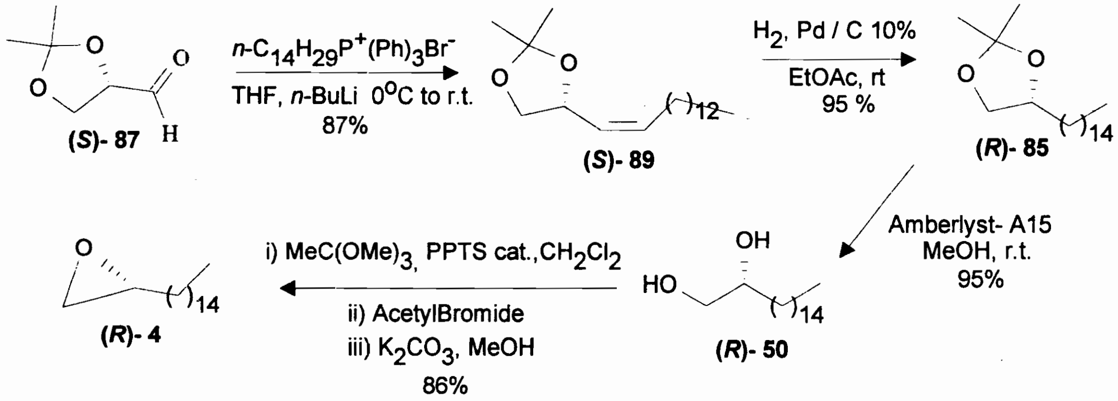
Scheme 27

Addition of trimethyl orthoacetate in presence of a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) to a solution of ***(S)*- 50** in dichloromethane forms an acetoxonium ion intermediate. Evaporation of the volatiles removes most of the methanol formed, however, a small amount of methanol is required to catalyze the subsequent transformation. The remaining methanol nucleophilically attacks acetyl bromide to generate HBr and methylacetate. The bromide liberated then opens the acetoxonium intermediate to yield a regioisomeric mixture of acetoxy bromides with inversion at the halide center. Following base mediated hydrolysis, and cyclization with a second inversion at the halide center gives epoxide ***(S)*- 4** with overall retention of configuration (Scheme 28). The regioselectivity of the acetoxy bromides is then, inconsequential.


OCH3
O
O
14
H3C(OCH3)3
PPTS cat.
CH2Cl2
HO
HO
14
O
O
+
14
Acetyl Bromide
OAc
Br
14
+
Br
AcO
14
K2CO3, MeOH
O
14
(S)- 4

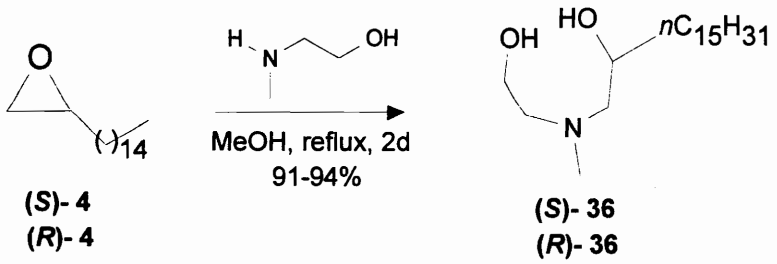
Scheme 28

The overall yield of (*S*)-1-oxiranepentadecane **(*S*)- 4** from (*R*)-glyceraldehyde acetonide **(*R*)- 87** is 72%. (*R*)-1-Oxiranepentadecane **(*R*)- 4**, was made in the same fashion starting from (*S*)-glyceraldehyde acetonide **(*S*)- 87** as shown in Scheme 29. The overall yield of **(*R*)- 2** was 68%. The bis-MTPA ester of diol **(*R*)- 50** obtained by this route was not prepared. Instead the optical purity was confirmed by evaluation of the optical rotation of **(*R*)- 50** and by comparison with its enantiomer **(*S*)- 50** obtained by the route shown in Scheme 26.



Scheme 29

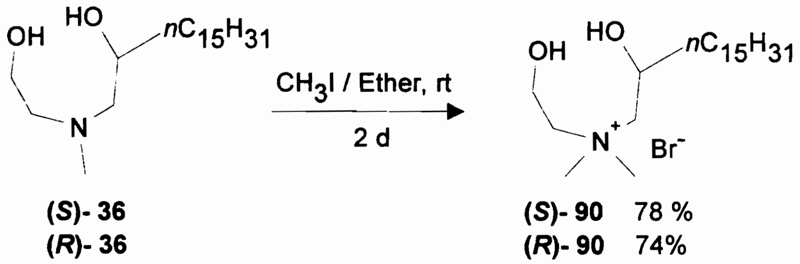
The epoxides **(*S*)- 4** and **(*R*)- 4** were independently treated with *N*-methylethanolamine in refluxing anhydrous methanol to provide aminodiols **(*S*)- 36** and **(*R*)- 36** in 91- 94 % yield. The crude materials were sufficiently clean for the following reaction as evidenced by their ^{1}H NMR spectra.[182]



Scheme 30

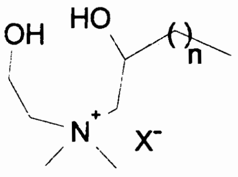
A portion of each enantiomer of **36** was used to prepare the quaternary ammonium salts **(*S*)- 90** and **(*R*)- 90**. The quaternization was readily accomplished with iodomethane in anhydrous ether. After two recrystallizations in EtOAc:CH_2Cl_2:

methanol (40:8:1) with moderate heating (< 45 °C), we obtained analytically pure samples in 78% and 74% yields respectively.



Scheme 31

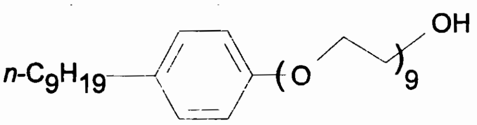
These compounds were synthesized in order to investigate any enantioselective activity in PKC inhibition and other biological activities (*vide supra*) in comparison with the activity exhibited by racemic mixtures of analogous compounds **(91 a-h)**.



91 a-h

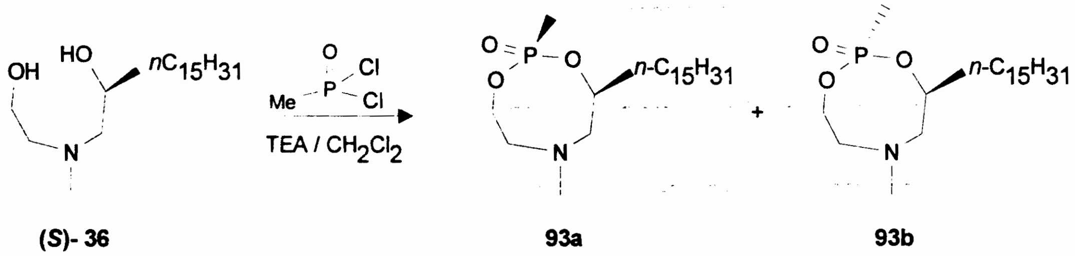
	n	X
a	11	Br
b	12	Br
c	13	Br
d	14	Br
e	15	Br
f	13	I
g	13	Cl
h	14	I

Racemates (**91 a-h**) show potent spermicidal activity[183] comparable to that of nonoxynol-9 **92**. Nonoxynol-9 is currently the most widely used spermicidal in the market, however, is not effective in reducing the risk of HIV infection in highly exposed women. Nonoxynol-9 also increases the risk of contracting genital ulcers.[184] In addition our lipids also inhibit a yeast *Candida albicans* that is responsible for most vaginal infections.[185] These compounds are currently undergoing vaginal irritation assays.



92

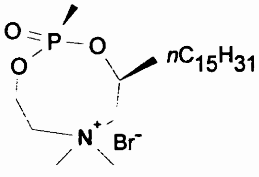
Continuing with the synthesis of PKC inhibitors, aminodiol **(*S*)- 36** was condensed with methylphosphonic dichloride to afford a diastereomeric mixture of (2*S*/4*S*) **93a** and (2*R*/4*S*) **93b** 6*N*-methyl-2-methyl-2-oxo-1,3-dioxa,4-pentadecyl-6-aza-2-phosphacyclooctane in 32 % and 19 % yield, respectively.



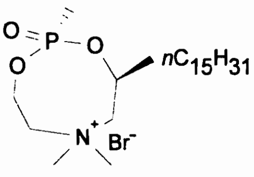
Scheme 32

In order to decrease the risk of polymerization, solutions of **(*S*)- 36** and methylphosphonicdichloride in dichloromethane were slowly added dropwise, independently and simultaneously, to a solution of triethylamine (TEA) in dichloromethane. The diastereomers **93a** (first fraction) and **93b** (second fraction) were readily separated by column chromatography on neutral alumina. Alternatively, they can also be separated on silica gel. The stereochemistry of these compounds has been assigned based on data obtained with their derivatives (*vide supra*).

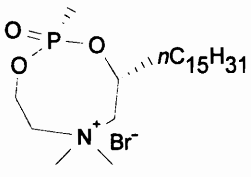
Conversely, the other pair of diasteromers **93c** and **93d** were obtained similarly from the corresponding aminodiol (***R***)- **36** in 33 % and 24 % yield, respectively. Finally, all four stereoisomers were quaternized with bromomethane in anhydrous ether to give compounds **1 a-d** in 60-65 % yield.



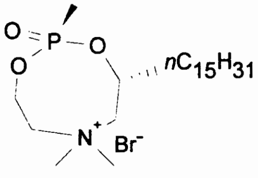
1a



1b

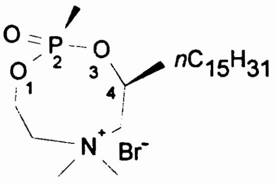


1c

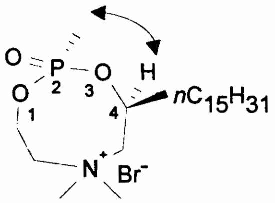


1d

Single crystal analysis of **1** (first fraction), as a racemate, obtained from racemic **93a** reveals that the methyl group on the phosphorus and the long alkyl chain are *cis* to each other.[1] Nuclear Overhauser Effect (NOE) differential experiments in d_6-DMSO also confirm the stereochemistry. Upon irradiation of the methyl group on phosphorus and the methine hydrogen at C4, stereoisomer **1b** shows a weak enhancement, whereas **1a** shows no enhancement at all. The small magnitude of the NOE effect observed is probably due to the conformational flexibility of the eight-membered ring in solution. Thus, on the basis of the aboved mentioned data and by comparison of the ^{1}H NMR spectra of **1a** and **1b** with those of **1c** and **1d** we have assigned their structures.



1a



1b

IV. BIOLOGICAL EVALUATION

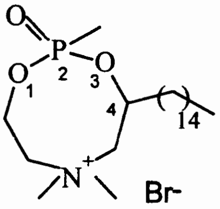
The motivation behind this project is the study of PKC inhibition. The *in vitro* assays of the four stereoisomers have been carried out by Dr. Curtis L. Ashendel at Purdue University. The data and the discussion are given below. Also presented are additional biological activitities involving processes regulated by lipids in which our compounds are currently being evaluated. These include spermicidal, anti-HIV, mycobactericidal and anti-cancer activities.

IV.1 Protein Kinase C Inhibition Results[186]

The four stereoisomers **1a-d**, and the enantiomers ***(S)***- and ***(R)***- **90** were assayed under the following conditions: All samples were dissolved in water at 10 mg/mL and assayed at 6 doses (five dilutions), and the doses range from 400 to 40 μg/mL. This assay condition differs from the one employed to test the diasteromers **2a-b**.[1]

The activity of PKC was assayed in a 50 μL reaction in the presence of 20 mM Tris-Cl buffer, pH 7.4, 0.1 mg/mL phosphatidylserine, 0.1 mM calcium chloride, 10 nM TPA, 10 mM magnesium chloride, 10 μg/mL leupeptin, 5 mM *p*-nitrophenylphosphate, 0.24 mg/mL lysine-rich histone, 0.2 mg/mL bovine serum albumin (BSA), and 20 μM[γ-^{32}P]ATP (ca 1000 cpm/pmol). The PKC used was a 1:1 mixture of mouse α and mouse β-II prepared separately by expressing in Sf9 cells and partial purification on DEAE-cellulose. The reactions were started by simultaneous addition of histone and ATP and allowed to proceed for 30 minutes at room temperature. The reactions were stopped by transferring 5 μL to phosphocellulose paper. The papers were washed in water, dried, cut into scintillation vials, and the reactivity bound to histone determined by scintillation counting. Results of duplicate assays were averaged and the assays lacking PKC activity subtracted. The results of the PKC inhibition assays are given in Table 6.

Table 6. PKC Inhibition Results.[a,b]



(2*S*/4*S*) 1a
(2*R*/4*S*) 1b
(2*R*/4*R*) 1c
(2*S*/4*R*) 1d

Sample	IC$_{50}$(μg/mL)	IC$_{50}$(μM)
1a	138	67
1b	98	47
1c	114	55
1d	67	32
(*S*)-90	118	56
(*R*)-90	110	52
Staurosporine 6	7.2 × 10^{-3}	

[a] Represents the average of duplicate results.
[b] The assays lacking PKC activity were subtracted

The IC_{50} values obtained for **1a-d** are about 10-fold higher than those of **2a-b**.[1] However, the assay conditions by which the racemates **2a-b** were tested are different from the latest employed. As the IC_{50} numbers are dependent of the assay conditions, further evaluation to relate the inhibition potency of these two series of compounds cannot be presented in this manuscript. Compounds **2a-b** will be re-assayed under the conditions employed for testing **1a-d** and the results will allow as to make the comparison in the future.

A major factor for the higher values is the increased concentration of PS (≈ 12 fold higher) in the new assay. As mentioned earlier, PS functions as a PKC activator,[47] therefore a higher concentration of the inhibitor is necessary to reach 50% of inactivation. Another important difference in the conditions is the use of a BSA solution. BSA is added as a stabilizer of PKC against the so-called "surface inactivation" and against inhibitors that are non-specific denaturants or reactive electrophiles. In the former assays (for **2a-b**), the delivery of the components was done in methanol.[1] The addition of an stabilizer is necessary because the samples were previously incubated in culture tubes for 10 minutes at 37 °C, however, now the assays are performed on well plates at room temperature and as a result the reactions will proceed more slowly. The presence of BSA may also account for the higher IC_{50}'s obtained. The high affinity of BSA toward highly hydrophobic agents and perhaps with our lipids may make them unavailable for inhibiting PKC in the assay.[186]

Staurosporine **6**, was run as standard giving $IC_{50} = 7.2$ ng/mL, which is two to four fold higher than the usual. It has been suggested[186] that the IC_{50} values of **1a-d** are high by a factor of two under these new conditions. From our data (Table 6), in reference to compounds **1a-d**, we do not observe any significant selective inhibition.

Compound **1d** was the most active (IC_{50} = 32 μM) being only about 2-fold more potent than the least active **1a** (IC_{50} = 67 μM). Compounds **1b** and **1d**, both of which bear a *trans* relationship at the stereocenters, were slightly more potent than **1a** and **1c** (their respective *cis* counterparts) by a factor of 1.4 and 1.7, respectively. This was an unexpected trend because it represents the reversal of what we previously observe with the racemates **2a-b** (*cis* more potent than *trans*). Currently, we do not have any explanation for these observations and must await the results of the assays on the racemates under these new conditions. In reference to compounds **(*S*)-** and **(*R*)-90** we do not observe any selectivity. The inhibition potency of these compounds are comparable to those of the stereosiomers.

IV.2 Miscellaneous Biological Activities

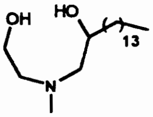
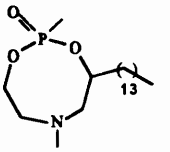
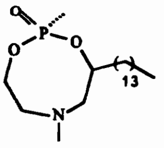
IV.2.1 Mycobactericidal Activity

Racemates **94**,[187] **95a**, and **95b**[188] show bactericidal activity against *Mycobacterium tuberculosis* H37Rv (*M. tuberculosis*) and *Mycobacterium avium* (*M. avium*). *M. tuberculosis* and *M. avium* have been increasing in frequency since the start of the AIDS epidemic. These opportunistic infections are responsible for the death of nearly 50% of AIDS patients.

The bioassays were run by Drs. Scott Franzblau and Anita Biswas from the Hanson Disease Center at Louisiana State University. The results are presented in Table 7. Two semisynthetic antibiotics, rifampin[189] and clarithromycin,[190] known to strongly inhibit these tuberculosis strains were run for comparison.

The activity of these antimycobacterial compounds is probably due to intercalation into the membranes, e.g., detergent-like activity, and/or the inhibition of fatty acid synthesis. Interestingly, the quaternary ammonium salts of the above mentioned agents are not active.[191] Recently, we have submitted optically pure analogues of the compounds shown in Table 7 for their testing.

Table 7. Minimum Inhibitory Concentration (MIC) of Some Amines.

Sample[a]	***M.tuberculosis*** **MIC (μM)**	***M.avium*** **MIC (μM)**
 **94**	39.6	79.2
 **95a**	133	133
 **95b**	133	133
Rifampin	0.20	----
Clarithromycin	----	5.3

[a] These compounds were made from commercially available (±)1-tetradecyloxirane employing the procedures reported in this manuscript for the preparation of the correspondent optically pure analogous compounds.

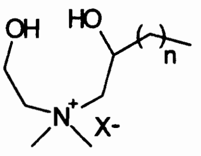
IV.2.2 Spermicidal and Anti HIV-Activities

The enantiomers **(*S*)-** and **(*R*)- 90** are expected to show spermicidal and anti - HIV activities as exhibited by the racemic mixtures of related compounds **91c** and **91g**. The enantiomers **(*S*)-** and **(*R*)- 90** have been sent to the Contraceptive Research and Development Program (CONRAD) for their evaluation.

Compound **91g** is as potent as nonoxynol-9 (N-9), the most widely used spermicide on the market. In general, our lipids exhibit comparable spermicidal and anti-HIV activity to that of N-9. The mechanism of action of N-9 is based primarily on its surfactant properties; it immobilizes sperm, bacteria, and viruses by disrupting cell membranes and viral envelopes.[192] In the spermicidal and anti-HIV tests, N-9 serves as the standard to which the candidate compounds are compared.

Table 8 and Table 9 show the spermicidal (Sander-Cramer assay)[193] and anti-HIV (Syncytium-forming assay)[194] activities of several lipoidal ammonium salts previously synthesized in our laboratories.[195] In the *in vitro* Sander-Cramer assay[193] the lowest concentration of a spermicide that trunk sperm motility within twenty seconds is found visually. Mixtures of semen-drug are tranferred to a microscope slide and examined to see if spermatozoa still exhibit progressive motility. In the syncytium-forming assay (*in vitro*), as described by Resnick *et al.*,[194] several experimental controls are run : *i*) virus/no spermicide, *ii*) virus/1% nonoxynol-9 (inactivation control), *iii*) no virus/spermicide (toxicity control), *iv*) no virus/no spermicide (cell control). The formation of giant syncytia (Multi-nucleated giant cells with more than five nuclei) occurs when cells expressing HIV interact with the CD4 (lymphocyte cell line) receptor, representing biologically active virus. Daily visual inspection of the samples is undertaken for seven days for syncytium formation. The HIV inoculation is then evaluated and endpoints titrations of infectious HIV can be performed.[194] The transmission of HIV can occur *via* a cell-free (HIV-1 IIIB) or cell-associated (H9/HIV-1 IIIB) route, and the assays are performed with different percentages of the drug under these two routes. The results obtained represent viral infectivity reduction (in logs). These data presented in table 8 and 9 have been summarized from data provided by CONRAD.

Table 8. Spermicidal Activity



91

	n	X
a	**11**	Br
b	**12**	Br
c	**13**	Br
d	**14**	Br
e	**15**	Br
f	**13**	I
g	**13**	Cl
h	**14**	I

Sample	Solvent	I_0 (mg/mL)	HSD(1/y)	MEC (mg/mL)
91c	H_2O	10	152.0 ± 37.2	0.112 ± 0.022
91g	H_2O	10	158.7 ± 48.8	0.163 ± 0.046
N-9, **92**	H_2O	10	178.7 ± 35.3	0.090+ 0.022

I_0 = Initial concentration
HSD = Highest Spermicidal dilution
MEC= Minimum Effective concentration
N-9 = Nonoxynol-9

Table 9. Anti-HIV Activity.

		Cell Free			Cell-Associated		
Sample	**Solvent**	**0.05%**	**0.01%**	**0.005%**	**0.05%**	**0.01%**	**0.005%**
91a	H_2O	>3.7	2.0	1.5	>4.5	<1.0	<1.0
91b	H_2O	>3.7	1.6	1.3	4.0	<1.0	<1.0
91c	H_2O	>3.7	3.0	2.3	3.2	3.0	<1.0
91f	H_2O	>3.7	2.3	2.1	>4.5	2.2	<1.0
91d	H_2O	>3.7	2.3	1.3	>4.5	<1.0	<1.0
91h	H_2O	>3.7	2.8	2.1	2.3	<1.0	<1.0
91e	H_2O	>3.7	2.0	1.6	>4.5	1.0	<1.0
N-9	PBS	>4.7	3.3	1.5	>4.5	2.0	1.4

N-9 = Nonoxynol-9
Results respresent viral infectivity reduction (in Logs)
PBS= Phosphate Buffer Solution
Spermicide Concentrations: 0.05%, 0.01%, 0.005%.

IV.2.3. Anti-Cancer Activity

Racemates **2a-b** have been evaluated *in vitro* in a disease-oriented primary antitumor screening test by the National Cancer Institute (NCI). There are six cell panels: Leukemia, non-small cell lung cancer, colon cancer, central nervous system (CNS) cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer. These cell panels cover a total of 60 cell lines against which the possible therapeutic agents are tested over a defined range of concentrations to evaluate the relative degree of growth inhibition or cytotoxicity. Complete information of the cell lines employed by the NCI has been described in detail elsewhere.[196,197]

Compound **2b** (*trans*) exhibited poor activity, and no further tests are pending. On the other hand, compound **2a** (*cis*) showed activity and selectivity against several different tumor cell lines and has been selected for further evaluation. The "dose-response curves" and the "mean graphs" data are of primary interest for most investigators. The following descriptions have been taken from recent reviews.[198] In the dose-response curves, the cell line subpanels are identified as the legends of each cell panel (labeled on top) plot. The "percentage growth" (PG) term, the meaning of the +50, 0, and -50 response reference lines and the calculated response parameters have been defined.[197,198,199] The 50 % growth inhibition parameter (GI_{50}) is reached when PG = 50. The "total growth inhibition" (TGI) or cytostatic level of effect is the drug concentration at PG = 0. The LC_{50} is the "lethal concentration" or net cell killing and is reached at PG = -50. The GI_{50}, TGI, and LC_{50} values are calculated by interpolation using the tested concentration that gives PG values above and below the respective reference value. The $\log_{10}$ of these parameters are printed with the mean graphs. A default value preceded by a "<" sign signifies that the "real" value is somewhat "less than" the lowest tested concentration, then a ">" sign preceding the default value signifies that the "real" value is somewhat "greater than" the number given. For any compound, the particular dose concentration employed can affect the extent of occurrence of "<" or ">" response parameter values. Therefore, further testing at different concentration ranges may be necessary depending upon the intended use of the data. Particularly in structure-activity studies, where both qualitative (e.g., profile of differential cytotoxicity) and quantitative (e.g., overall or panel-averaged potency) comparisons of compounds are desired.

The mean graphs depict the relative sensitivity of each cell line compared with the average sensitivity of all the lines. Depending on whether a cell line is either more or less sensitive than the average, graphic bars are projected to the right or left respectively and their lengths are proportional to the relative sensitivity of the cell lines. In this way, each agent can be represented by a characteristic "finger print" of cellular responsiveness.

The data given below are reproductions of some of the data provided by the National Cancer Institute Developmental Therapeutics Program (Experiment ID: 9509RS42) to Dr. Richard Gandour on November 1, 1995.

Table 10.- Mean Graph for **2a** Showing the Log$_{10}$ TGI / TGI for different cell lines.

Panel/Cell Line	Log$_{10}$ TGI
Leukemia	
CCRF-CEM	-4.96
HL-60(TB)	> -4.00
K-562	-4.32
MOLT-4	-4.55
RPMI-8226	-5.14
SR	-4.86
Non-Small Cell Lung Cancer	
A549/ATCC	-4.63
EKVX	-4.40
HOP-62	-4.81
HOP-92	-5.32
NCI-H226	-4.85
NCI-H23	-4.69
NCI-H322M	-4.58
NCI-H460	-5.53
NCI-H522	-4.90
Colon Cancer	
COLO 205	-5.47
HCC-2998	-5.48
HCT-116	-4.72
HCT-15	-4.60
HT29	-4.94
KM12	-5.43
SW-620	-4.57
CNS Cancer	
SF-268	-4.89
SF-295	-4.82
SF-539	-4.72
SNB-19	-5.00
SNB-75	-5.28
U251	-5.53
Melanoma	
LOX IMVI	-4.87
MALME-3M	-4.51
M14	-4.66
SK-MEL-2	-4.42
SK-MEL-28	-4.52
SK-MEL-5	-4.51
UACC-257	-4.51
UACC-62	-4.70
Ovarian Cancer	
IGROV1	-5.39
OVCAR-3	-4.69
OVCAR-4	-4.68
OVCAR-5	-5.36
OVCAR-8	-4.55
Renal Cancer	
786-0	-5.49
A498	-4.66
ACHN	-4.59
CAKI-1	-4.74
RXF 393	-5.56
SN12C	-4.80
TK-10	-5.35
UO-31	-4.56
Prostate Cancer	
PC-3	-5.39
DU-145	-4.61
Breast Cancer	
MCF7	-4.53
MCF7/ADR-RES	-4.41
MDA-MB-231/ATCC	-5.13
HS 578T	-4.59
MDA-MB-435	-4.53
MDA-N	-4.44
BT-549	-4.91
T-47D	-4.39
MG_MID	-4.83
Delta	0.73
Range	1.56

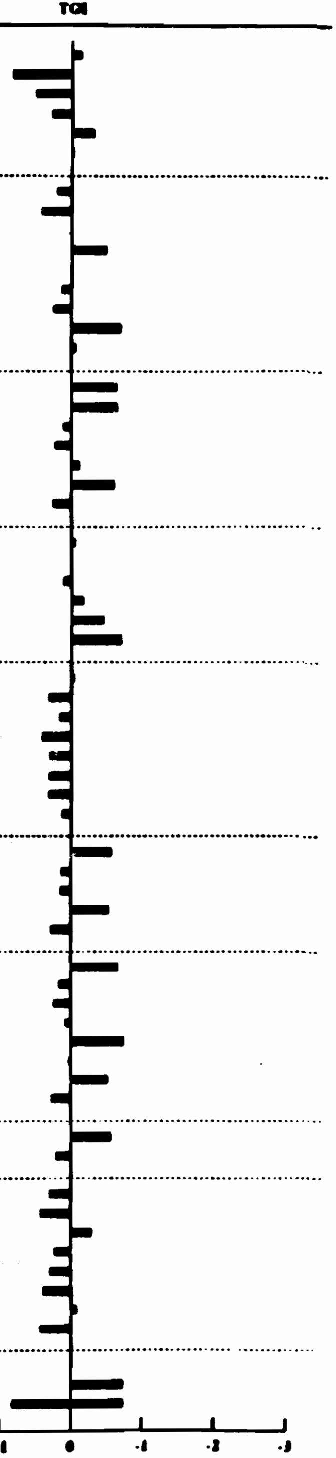


Table 11.- Mean Graph for **2a** Showing the Log$_{10}$ GI$_{50}$ /GI$_{50}$ for different cell lines.

Panel/Cell Line	Log$_{10}$ GI50
Leukemia	
CCRF-CEM	-5.63
HL-60(TB)	-5.80
K-562	-5.01
MOLT-4	-5.44
RPMI-8226	-5.66
SR	-5.52
Non-Small Cell Lung Cancer	
A549/ATCC	-5.13
EKVX	-4.74
HOP-62	-5.49
HOP-92	-5.84
NCI-H226	-5.49
NCI-H23	-5.31
NCI-H322M	-4.86
NCI-H460	-5.84
NCI-H522	-5.49
Colon Cancer	
COLO 205	-5.76
HCC-2998	-5.78
HCT-116	-5.35
HCT-15	-4.91
HT29	-5.55
KM12	-5.71
SW-620	-4.94
CNS Cancer	
SF-268	-5.45
SF-295	-5.39
SF-539	-5.28
SNB-19	-5.54
SNB-75	-5.79
U251	-5.82
Melanoma	
LOX IMVI	-5.51
MALME-3M	-4.81
M14	-5.01
SK-MEL-2	-4.73
SK-MEL-28	-4.80
SK-MEL-5	-4.89
UACC-257	-4.77
UACC-62	-5.13
Ovarian Cancer	
IGROV1	-5.77
OVCAR-3	-5.22
OVCAR-4	-5.26
OVCAR-5	-5.74
OVCAR-8	-5.04
Renal Cancer	
786-0	-5.76
A498	-5.31
ACHN	-4.95
CAKI-1	-5.43
RXF 393	-5.87
SN12C	-5.45
TK-10	-5.71
UO-31	-4.88
Prostate Cancer	
PC-3	-5.71
DU-145	-4.94
Breast Cancer	
MCF7	-4.94
MCF7/ADR-RES	-4.75
MDA-MB-231/ATCC	-5.56
HS 578T	-5.12
MDA-MB-435	-4.82
MDA-N	-4.75
BT-549	-5.43
T-47D	-4.76
MG_MID	-5.31
Delta	0.56
Range	1.14

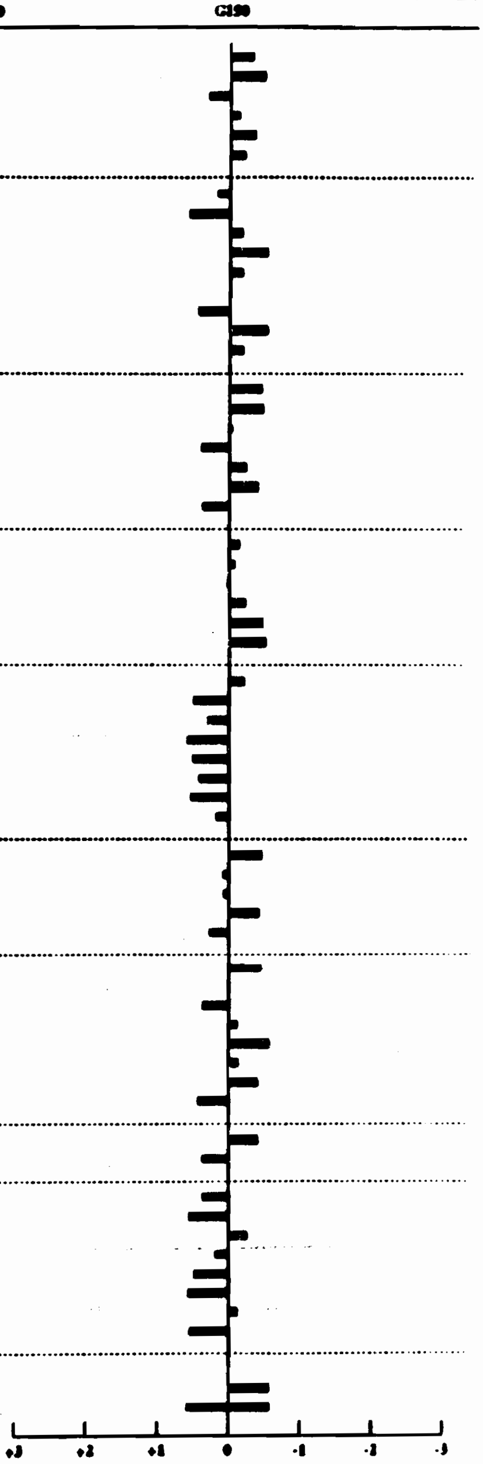
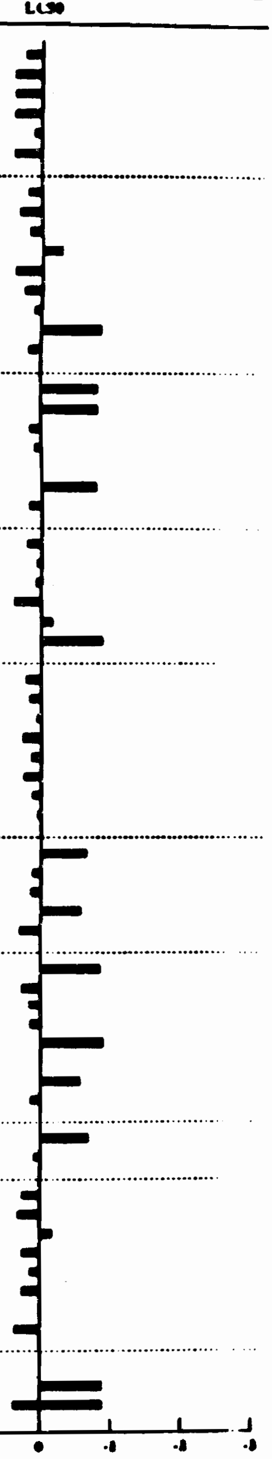


Table 12.- Mean Graph for **2a** Showing the $\text{Log}_{10}\,\text{LC}_{50}$ / LC_{50} for different cell lines.

Panel/Cell Line	Log_{10} LC50
Leukemia	
CCRF-CEM	-4.14
HL-60(TB)	> -4.00
K-562	> -4.00
MOLT-4	> -4.00
RPMI-8226	-4.27
SR	> -4.00
Non-Small Cell Lung Cancer	
A549/ATCC	-4.19
EKVX	-4.07
HOP-62	-4.21
HOP-92	-4.65
NCI-H226	> -4.00
NCI-H23	-4.14
NCI-H322M	-4.28
NCI-H460	-5.22
NCI-H522	-4.21
Colon Cancer	
COLO 205	-5.18
HCC-2998	-5.18
HCT-116	-4.22
HCT-15	-4.29
HT29	-4.38
KM12	-5.15
SW-620	-4.20
CNS Cancer	
SF-268	-4.17
SF-295	-4.31
SF-539	-4.31
SNB-19	> -4.00
SNB-75	-4.54
U251	-5.25
Melanoma	
LOX IMVI	-4.16
MALME-3M	-4.21
M14	-4.31
SK-MEL-2	-4.11
SK-MEL-28	-4.24
SK-MEL-5	-4.13
UACC-257	-4.26
UACC-62	-4.34
Ovarian Cancer	
IGROV1	-5.02
OVCAR-3	-4.26
OVCAR-4	-4.23
OVCAR-5	-4.95
OVCAR-8	-4.08
Renal Cancer	
786-0	-5.22
A498	-4.12
ACHN	-4.22
CAKI-1	-4.22
RXF 393	-5.25
SN12C	-4.37
TK-10	-4.94
UO-31	-4.24
Prostate Cancer	
PC-3	-5.06
DU-145	-4.29
Breast Cancer	
MCF7	-4.13
MCF7/ADR-RES	-4.07
MDA-MB-231/ATCC	-4.55
HS 578T	-4.13
MDA-MB-435	-4.24
MDA-N	-4.12
BT-549	-4.36
T-47D	-4.03
MG_MID	-4.38
Delta	0.87
Range	1.25



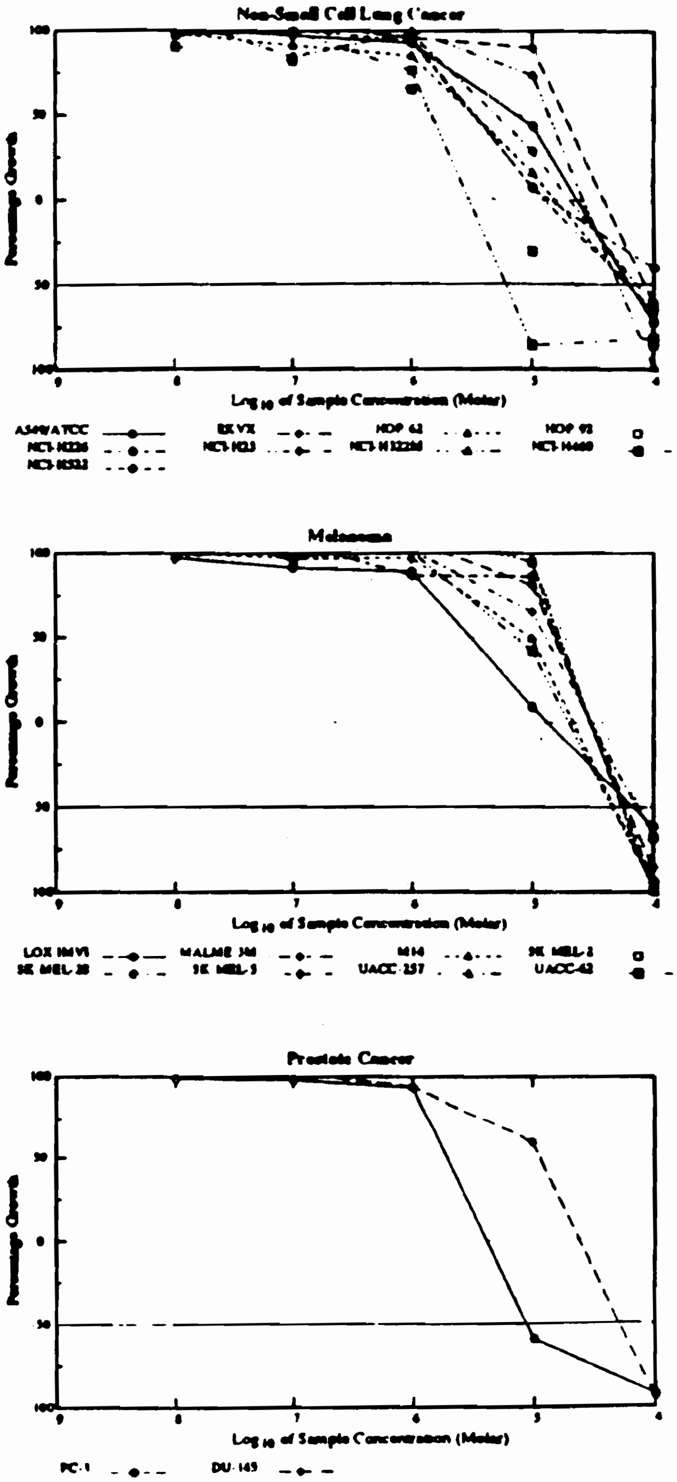


Figure 6.- Dose Response Curve for **2a** (Lung, Melanoma and Prostate Cancer).

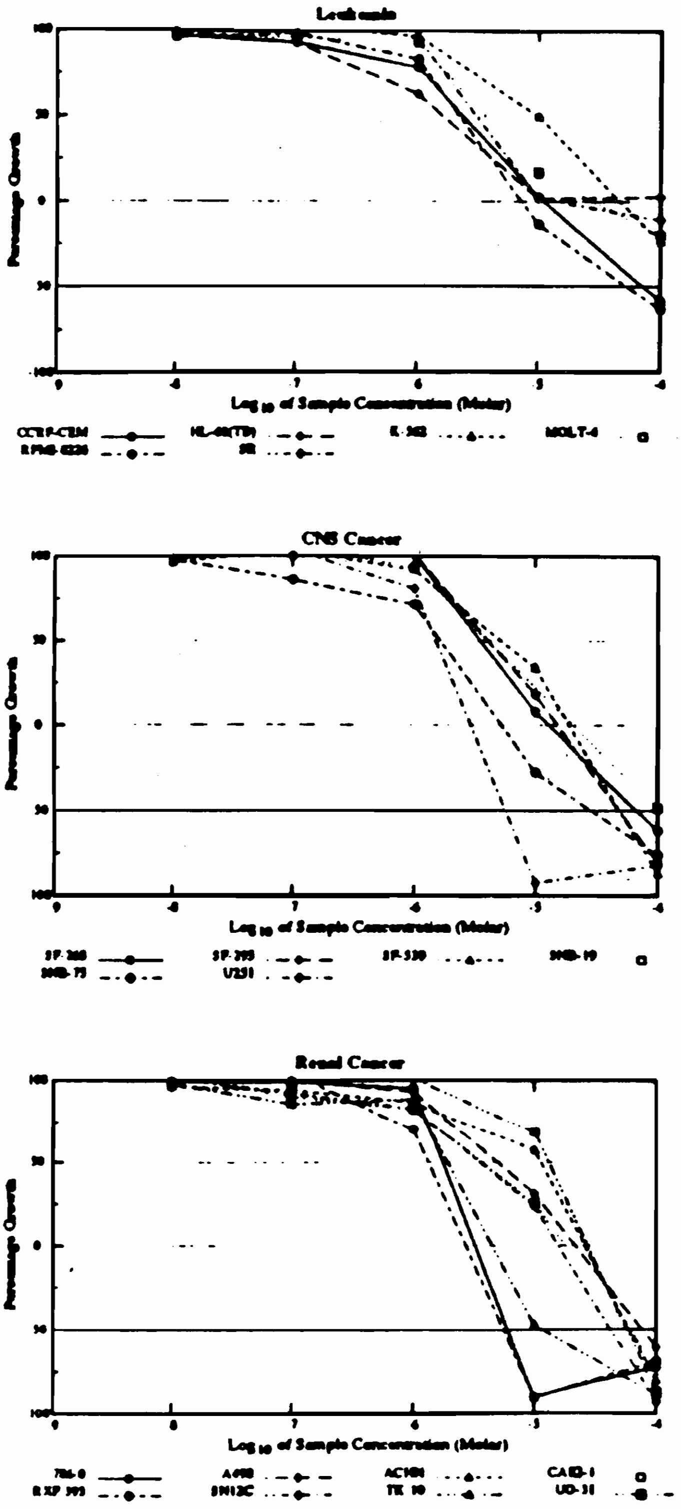


Figure 7.- Dose Response Curve for **2a** (Leukemia, CNS and Renal Cancer).

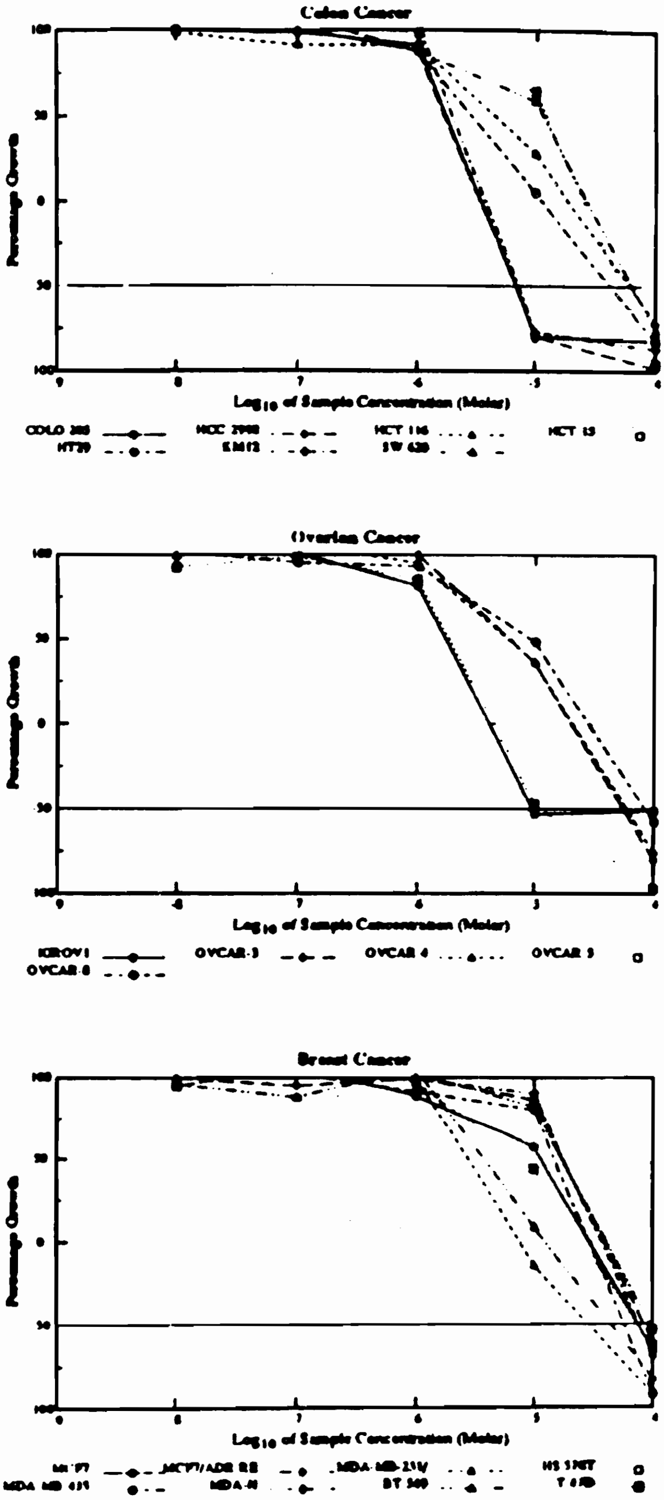


Figure 8.- Dose Response Curve for **2a** (Colon Ovarian and Breast Cancer).

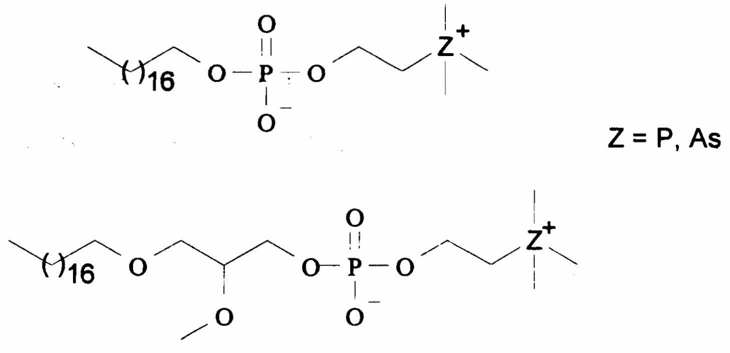
IV.3 FUTURE PERSPECTIVES : STRATEGIC AREAS OF RESEARCH INTEREST

In view of the PKC inhibition results (Table 6) and the potency trend observed, (*trans* **1b**, **1d** more potent than *cis* **1a**, **1c**) it should be interesting to extend the investigation toward computational studies of our inhibitors in relation to the phorbol ester pharmacophore by means of structure superimposition.

It has been suggested,[200] that the spatial arrangements may be responsible for PKC recognition. TPA, a regulatory domain activator of PKC has a high affinity for its binding site. Inhibitors of the regulatory domain of PKC compete for the phorbol ester/DAG binding site. In general , the affinity of these inhibitors are much smaller than that of TPA resulting in a poor binding in the presence of TPA. In our case, it may be possible that the methyl phosphonate group is functioning as the acetate group in TPA, whereas the long-alkyl chain is taking the role of the long-acyl group in TPA. If this is true, we could re-design the inhibitors by incorporating additional functionalities that could provide more recognizable sites to achieve a better fit in the binding site. One should be careful, however, because turning the inhibitor into an activator is a potential problem.

The next priority is to investigate the chain-length dependance of PKC towards our inhibitors. We shall find an optimal chain-length by making a series of lower and higher homologues of our existing prototypes molecules. In addition, analysis of different counter anions should provide further information; not only in terms of solubility, but also with implications to the inhibition potency. Another area that requires attention is the functionalization of the alkyl side-chain. In particular, it should be of great interest to explore the response of PKC towards unsaturated fatty acid chains such as oleyl or linoleyl containing one and two *cis*-double bonds respectively. There are several cases in which the addition of a *cis*-unsaturation or a structural feature that mimics a *cis*-double bond greatly enhances the enzyme response. Again, one should not overlook the possibility of turning an inhibitor into an activator.

Of equal importance is the necessity to investigate the therapeutic range, and tolerability of this new class of cyclic phospholipid analogues. Miltefosine (hexadecylphosphocholine) and edelfosine (2-*O*-methyl-1-*O*-octadecyl-*rac*-glyceryl-3-phosphocholine), two antineoplastic active phospholipids already in clinical use in Germany, have been reported to have undesirable side effects. The oral administration of these compounds to cancer patients was seriously hampered by side effects in the digestive tract (nausea, vomiting, diarrhea, loss of body weight).[105] Stekkar *et al.*[107] have suggested that the symptoms caused by miltefosine and edelfosine are reminiscent of those caused by phosphocholine and therefore alkylphospholipids not containing choline in their polar head should be better tolerated. This suggestion was supported act that replacement of *N* in the choline head groups by As or P lead to better tolerable but still pharmacologically active compounds.[201]



V. CONCLUSIONS

The main objective of this project, the synthesis and evaluation of PKC inhibitors **1a-d**, was accomplished. We did not observe any significant enantioselective inhibition. However, absolute inhibition constants were higher in comparison to those obtained for the racemates **2a-b**. Unfortunately at the present time we cannot compare the relative inhibition potency of these two series of compounds due to the different assay conditions employed. Therefore, it is still early to assume that the new compounds are less potent and confirm the therapeutic potential of these compounds as PKC inhibitors.

On the positive side, this work provides an efficient route to the syntheses of long-alkyl chain nonracemic epoxides in high overall yield and high enantiomeric purity from relatively inexpensive natural chirons. These epoxides are the key intermediates for the preparation of compounds **1a-d** and other valuable chiral lipids with different biological activities that includes spermicidal, anti-HIV, mycobactericidal, and anti-cancer activities.

VI. EXPERIMENTAL

General Procedures

THF and ether were freshly distilled from sodium/benzophenone. Dichloromethane, hexanes, benzene, and ethyl acetate were distilled from calcium hydride. Methanol was distilled from Mg/I_2. Triethylamine (TEA) was distilled over KOH pellets and stored under N_2 over KOH pellets. Anhydrous dimethylformamide (DMF) was purchased from Aldrich and used as received. All commercially available reagents were purchased from Aldrich unless otherwise specified. The chiral ligands used for the asymmetric dihydroxylation (AD) reaction were generously provided by Dr. K.B. Sharpless.

All nonhydrolytic reactions were carried out in an argon or nitrogen atmosphere with standard techniques for the exclusion of air and moisture. Glassware used for moisture sensitive reactions were heated under vacuum prior to use. Butyllithium was titrated with diphenylacetic acid in ethyl ether. Analytical thin layer chromatography (TLC) was run on silica gel (Whatman PE SILG/UV 250 μm) and visualized with phosphomolybdic acid (10% in ethanol) with subsequent heating unless otherwise noted. Preparative TLC was performed on Whatman K6F Silica gel 60 (layer thickness 250 M). Flash chromatography was performed on silica gel (Whatman 60 230-400 mesh). Alumina column chromatography was performed on Merck DC-Alufolien, Aluminiumoxid 60 F254 neutral (Typ E). Anhydrous $MgSO_4$ was used as the drying agent. Solutions were concentrated by rotary evaporation at reduced pressure.

Mass spectra were recorded on a FisonVG QUATRO. Infrared (IR) spectra were recorded on Perkin Elmer FT-IR spectrophotometer, Model 1 600. ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker WP-270 or Varian NR-400 instruments. ^{31}P NMR spectra were obtained on Varian NR-400 with phosphoric acid (83 %) as the external reference. 2D-NMR spectra were also recorded on Varian NR-400 instrument. Proton chemical shifts are reported in parts per million (ppm) relative to TMS as an internal reference (0.00 ppm) unless otherwise specified. Carbon chemical shifts are reported in ppm relative to the center line of the $CDCl_3$ triplet (77.00 ppm) unless otherwise noted. Assignments of the ^{1}H and ^{13}C NMR signals were made by comparison with similar known compounds and/or by their evaluation of their attached proton test (APT), ^{13}C, ^{1}H heteroatom correlation (HETCOR), total correlation TOCSY and nuclear overhauser effect (NOE) differential spectra. Observed coupling constants are not verified and are listed as J_{app}. Melting points were recorded on an Electrothermal IA 9200 Digital Melting Point apparatus, and are uncorrected. Optical rotations were recorded on a Perkin Elmer 241 digital polarimeter. Elemental analyses were performed by Atlantic MicroLab at Atlanta, Georgia. Experimental procedures and/or spectral data were reported for known compounds when modifications of the published procedures were employed.

(1-Bromomethyl)hexadecyl butanoate 72. 1-Bromo-2-hydroxy-heptadecane (0.70 g, 2.0 mmol) was dissolved in CH_2Cl_2 (45 mL). DMAP (0.04 g, 0.3 mmol) and TEA(0.36 mL, 2.6 mmol) were added. This solution was stirred at 5 °C for 1h under nitrogen. Butyric anhydride (0.40 mL, 2.5 mmol) was diluted in CH_2Cl_2 (5 mL) and added to the reaction over 5 min. The reaction was left running at rt overnight. The solvent was concentrated to yield an oil, which was diluted in hexanes (60 mL). HCl (1 N, 3 mL) was added and the phases separated. The organic phase was washed with sat. $NaHCO_3$ (3 mL) and then with water (10 mL). This phase was dried and concentrated to yield a pale yellow oil (0.84 g, 99%). R_f = 0.74 in (hexanes:EtOAc, 8:1). Purification of the crude material by flash chromatography with (hexanes:EtOAc, 20:1) afforded (0.76 g, 90%) of **72.** **^{1}H NMR (400 MHz, $CDCl_3$)** 5.01 (m, 1H, C$\underline{H}$), 3.52-3.40 (m, 2H, C$\underline{H}_2$Br), 2.31(t, 2H, J_{app} = 7.48 Hz, COC$\underline{H}_2$), 1.70-1.64 (m, 2H, $COCH_2C\underline{H}_2$), 1.69-1.64 (m, 2H, $C\underline{H}_2(CH_2)_{13}CH_3$), 1.25 (s, 26H, $(C\underline{H}_2)_{13}CH_3$), 0.96 (t, 3H, J_{app} = 7.48 Hz, $COCH_2CH_2C\underline{H}_3$), 0.87 (t, 3H, J_{app}= 7.02 Hz, $C\underline{H}_3(CH_2)_{14}$). **APT (100 MHz, $CDCl_3$)** 173.04 ($\underline{C}$O), 72.13 ($\underline{C}$H), 36.29 ($\underline{C}H_2$Br), 34.33, 32.532, 31.91, 29.68, 29.64, 29.59, 29.50, 29.41, 29.35, 29.28, 25.04, 22.68, 18.51 ($\underline{C}H_2$), 14.61 ($\underline{C}H_3$), 13.65 ($\underline{C}H_3$). **Anal. Calcd.** For $C_{21}H_{41}O_2Br$ C, 62.21; H, 10.19. **Found** C, 62.30; H, 10.24.

Attempted Enzymic Resolution of 72. In a separatory funnel, the solvents employed, $CHCl_3$ and ether, independently, were pre-washed with a buffer solution (pH= 7.0) made of Na_2HPO_4 and NaH_2PO_4 0.1M(8:5). The organic solvents collected after separation of phases are commonly called "wet" solvents.

Lipases[a]

a) Lipase EC 3.1.1.3 type VII from *Candida cylindracea* (860 units/mg) purchased from Sigma (0.75 mg).

b) Lipozyme IM from *Mucor miehei* lipase, immobilized on a ion-exchange resin purchased from Novo Nordisk Bioindustrial INC (30 mg).

c) Lipase PS-30 from *Pseudomonas cepacia* (25 mg).

d) and **e)** *Pseudomonas fluorescence*, SAM 2 (31.2 units/mg) purchased from Fluka (28 mg).

[a] Lipases a-c were generously provided by Dr. T. Hudlicky.

The enzymes **a-e** were suspended on Celite (*ca* 100 mg) to provide samples **1-5** respectively. Samples **1-4** were washed with "wet" chloroform. Sample **5** was washed with "wet" ether. Additional "wet" $CHCl_3$ (10 mL) was added to all samples, except sample **5** in which "wet" ether (10 mL) was added. A solution of **47** (0.75 g, 1.8 mmol) in "wet" $CHCl_3$ (50 mL) was divided into five aliquots (0.15 g, 0.37 mmol) each. Four aliquots containing the substrates were added to samples **1-4**. The fifth aliquot was redissolved in "wet" ether (10 mL), and added to **5**. Samples were kept with constant stirring at rt. The reactions were monitored by TLC (hexanes:EtOAc, 8:1) each day for seven days. Only starting material was detected.

Pentadecanal 73. 1-Pentadecanol (5.25 g, 22.9 mmol) was dissolved in CH_2Cl_2 (160 mL). Pyridinium chlorochromate PCC (5.95 g, 27.6 mmol) was added in one portion. The reaction was stirred at rt for 8h. A dark precipitate formed. Solids were removed by filtration through a short path of silica gel.

Filtrate was washed with brine (70 mL), and the aqueous layer was further extracted with CH_2Cl_2 (*ca* 60 mL). The combined organic layers were dried and filtered through a short path of silica gel as many times as necessary to obtain a colorless filtrate. Concentration of the solvent gave a thick oil that turned into a white solid upon cooling to rt. $R_f = 0.53$ in (hexanes:EtOAc, 8:1). Average yield of several reactions (4.6 g, 88%). Pentadecanal was used without further purification for the preparation of (*E*)-octadec-2-en-1-ol **76**. **^{1}H NMR (270 MHz-$CDCl_3$)** σ 9.76 (t,1H, C$\underline{H}$O), 2.41 (m, 2H, -C$\underline{H}_2$CHO), 1.43 (m, 2H, C$\underline{H}_2$CH$_2$CHO), 1.25 (m, 22H, CH$_3$($\underline{C}$H$_2$)$_{11}$), 0.87 (t, 3H, C$\underline{H}_3$(CH$_2$)$_{11}$)

(*E*)-Octadec-2-en-1-ol 76. This compound was prepared as reported by Roush and Adams.[145]

(*E*)-2,3-Epoxyoctadecan-1-ol 77. This compound was prepared as reported by Roush and Adams.[145]

1,2-Octadecanediol 95. To a 0 °C solution of (*E*)-2,3-epoxyoctadecan-1-ol **71** (0.06 g, 0.2 mmol) and titanium isopropoxide (0.10 g, 0.35 mmol) in benzene (10 mL), lithium borohydride (0.02 g, 0.9 mmol) in THF (3 mL) was added. The reaction was stirred under nitrogen at -10 °C ~ -15 °C for 4 h and then it was stored in the freezer overnight. The next day, stirring continued until disappearance of the starting material. Work-up: The reaction was diluted with ether (*ca* 10 mL) and quenched with H_2SO_4 5% (*ca* 15 mL). The phases were separated and the aqueous layer was further extracted with ether. The combined organic layers were washed with $NaHCO_3$ sat. and then dried. Removal of the solvent provided a white gel R_f = 0.13 (hexanes:EtOAc, 2:1). Purification of this material by column chromatography with (hexanes:EtOAc, 2:1) afforded a white solid (0.050 g, 80%). GC-MS analysis revealed the presence of an isomeric mixture (1,2-heptadecanediol and 1,3-heptadecanediol). Attempts to separate this isomeric mixture were unfruitful.

Asymmetric dihydroxylation. The optical rotation of all samples obtained by the dihydroxylation reaction are not presented. The optical rotation data were below the detection limit of our instrument. The absolute configuration was assigned based on published data.[148] The enantiomeric excesses were evaluated by ^{1}H NMR on the bis-MTPA esters derivatives (See Table 4).

General procedures

Method A: For 1 mmol of olefin
For $(DHQ)_2PYR$ or $(DHQD)_2PYR$, 14 mg (1.7 % mol)
$K_3Fe(CN)_6$, 1000 mg (3.03 equiv.)
K_2CO_3, 430 mg (3.12 equiv.)
$K_2OsO_2(OH)_4$, 10 mg (0.25 % mol)
t-BuOH:water, 1:1, 14 mL
Concentration of olefin 0.007 M
Temperature: 0 °C to rt.

Method B For 1mmol of the olefin

For $(DHQ)_2PYR$ or $(DHQD)_2PYR$, 14 mg (1.7 % mol)

Same as Method A, but the temperature was 25 °C throughout the reaction.

MethodB For 2 mmol of the olefin

For $(DHQ)_2AQN$ or $(DHQD)_2AQN$, 18 mg (1.0 % mol)

$K_3Fe(CN)_6$, 1980 mg (3.00 equiv.)

K_2CO_3 , 840 mg (3.00 equiv.)

$K_2OsO_2(OH)_4$, 15 mg (0.20 % mol)

t-BuOH:water, 1:1, 28 mL

Concentration of the olefin 0.007 M

Temperature: rt

Preparation of the AD-mixes. The reagents, $K_3Fe(CN)_6$ (1000m g,3.030 equiv.), K_2CO_3 (430 mg, 3.12 equiv.), $(DHQ)_2PYR$ or $(DHQD)_2PYR$ (14 mg, 1.7 % mol), $K_2OsO_2(OH)_4$ (10 mg, 0.25 % mol) were placed together in a vial. The mixtures were denoted as $(DHQ)_2PYR$-ADmix and $(DHQD)_2PYR$-ADmix respectively, according to the chiral ligand employed for their preparation. The $(DHQ)_2AQN$ and $(DHQD)_2AQN$- AD mixes were prepared in the same way.

(*R*)-1,2-Heptadecanediol 50a, Method A. $(DHQD)_2PYR$-ADmix for 1 mmol of olefin was poured into *t*-BuOH:water, 1:1 (14 mL) and was vigorously stirred at rt until two clear phases were obtained (*ca* 15 min). At this stage the temperature was brought down to 0 °C and 1-heptadecene (0.24 g, 1.0 mmol) was added while maintaining constant stirring. An orange precipitate formed upon addition of the olefin. The temperature was mantained at 0 °C for 1 d and the reaction was monitored by TLC with (CH_2Cl_2: MeOH,15:1). Mostly starting material $R_f = 0.94$ was observed, and traces of product $R_f = 0.36$. The reaction was allowed to warm-up to rt and it was stirred for 2d until disappearance of the olefin. The reaction was quenched by addition of $NaHSO_3$ (1.5 g) over 20 min. Stirring continued for 2.5 h until complete separation of phases (clear top organic phase and blue bottom aqueous phase) was observed. These phases were separated and the aqueous layer was further extracted with CH_2Cl_2 (3 × 8 mL). The combined organic layers were dried and concentrated to yield a white residue, which was purified by column chromatography with (CH_2Cl_2:MeOH, 16:1). Yield (0.26 g, 95%) of **50a**. **Mp.** = 77.8-78.2 °C. Alternatively the product can be purified with (Hexanes:EtOAc, 8:1). **IR (KBr, cm^{-1})** 3483, 3400-3200b, 2916, 2849, 1470. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 3.71-3.66 (m, 1H, $C\underline{H}OH$), 3.67-3.63 (m, 1H, $C\underline{H}HOH$), 3.46-3.40 (m, 1H, $C\underline{H}HOH$), 2.25 (d, J_{app} = 4.3 Hz, 1H, $CHO\underline{H}$), 2.17 (t, J_{app} = 5.7 Hz, 1H, $CH_2O\underline{H}$), 1.43 (m, 2H, $C\underline{H}_2(CH_2)_{13}CH_3$), 1.26 (s, 26H, $(C\underline{H}_2)_{13}CH_3$), 0.88 (t, 3H, $(CH_2)_{13}C\underline{H}_3$). **APT (100 MHz, $CDCl_3$)** δ 72.32 ($\underline{C}HOH$), 66.83 ($\underline{C}H_2OH$), 33.2 ($\underline{C}H_2(CH_2)_{13}CH_3$), 31.91, 29.68, 29.65, 29.64, 29.58, 29.53, 29.34, 25.53, 22.67 ($(\underline{C}H_2)_{13}CH_3$), 14.09 ($(CH_2)_{13}\underline{C}H_3$).

(*S*)-1,2-Heptadecanediol 50b, Method A. The procedure is the same as for (*R*)-1,2-heptadecanediol with method A. A $(DHQ)_2$PYR-AD mix was employed instead. The yield recovered from 1 mmol of olefin after purification was0.26 g (95%) **Mp.** = 77.8-78.2 °C. Spectral data same as for **50a**.

(*R*)-1,2-Heptadecanediol 50a, Method B. The reaction was run with (DHQD)$_2$PYR-AD mix scale-up for (0.72 g, 3.0 mmol) of 1,2-heptadecene. The reaction was totally run at rt and went onto completion in 30 h. Yield after purification (0.80 g, 98 %). **Mp.** = 78 -78.5 °C. Spectral data, were the same as for **50a**.

(*S*)-1,2-heptadecanediol 50b, Method B. The reaction was run with (DHQ)$_2$PYR-AD mix scale-up for (0.72 g,3.0 mmol) 1,2-heptadecene. The reaction was totally run at rt and went onto completion in 30 h. The yield after purification was 0.80 g (98 %). **Mp.** = 78 -78.9 °C. Spectral data, were the same as for **50a**.

(*R*)-1,2-Heptadecanediol 50d, Method B. The reaction was run with (DHQD)$_2$AQN AD-Mix scaled up for (0.48 g, 2.0 mmol) of 1,2-heptadecene. The yield after purification was 0.43 g (79 %). Spectral data were the same as for **50a**.

(*S*)-1,2-heptadecanediol 50e, Method B. The reaction was run with (DHQ)$_2$AQN AD-Mix scaled up for (0.48 g, 2.0 mmol) of 1,2-heptadecene. The yield after purification was 0.45 g (82 %). Spectral data were the same as for **50a**.

Preparation of 3,3,3-trifluoro-2-methoxy-2-phenyl-propionic acid 1-(3,3,3-trifluoro-2-methoxy-2-phenyl-propionyloxymethyl)-hexadecanoyl esters 75, 75a-e. General procedure. Dichloromethane (2.5 mL) was added to dicyclohexyl carbodiimide (DCC) (0.085 g, 4.1 mmol) and (*R*)-methoxy-trifluoromethyl-phenylacetic acid (0.032 g, 1.4 mmol). A white solid formed inmediately. This mixture was stirred at rt under nitrogen for 30 min. *rac*-1,2-Heptadecanediol (0.012 g, 0.45 mmol) and DMAP (0.006 g, 0.5 mmol) were added to the mixture in one portion. The reaction was vigororously stirred for 15 h. Work-up: The white precipitate formed was collected by filtration and washed with hexanes (~ 2 mL). The filtrate was washed with 1 N HCl and with $NaHCO_3$ sat. The organic layer was separated, and the aqueous layer was further extracted with hexanes (2 × 2.5 mL). The combined organic layer was dried and concentrated to afford a turbid oil. This material was purified by column chromatography eluted with (hexanes:EtOAc, 12:1), $R_f = 0.28$ to give 0.026 g, (81%) of the entitled compound (as a diastereomeric mixture). **^{1}H NMR (400MHz, $CDCl_3$)** δ 7.59-7.33 (m, 20H, aromatics), 5.32 (m, 2H, H_c, $H_{c'}$) , 4.64-4.54 (m, 2H, H_a and $H_{a'}$), 4.32-4.25 (m, 2H, H_b and $H_{b'}$) 3.64, 3.48(S, 3H, $OC\underline{H}_3$), 3.44 (S, 3H, $OC\underline{H}_3$), 3.43 (S, 3H, $OC\underline{H}_3$), 3.41 (S, 3H, $OC\underline{H}_3$), 1.92 (m,2H),1.74 (m, 2H), 1.3 (m,48H , $(C\underline{H}_2)_{12}CH_3$), 0.88 (t, 6H, $(CH_2)_{12}C\underline{H}_3$).

(*R*)-2,3-*O*-Isopropylidene- D-glyceraldehyde (*R*)- 87. This compound was prepared according to the literature procedure.[171]

(*S*)-2,3-*O*-Isopropylidene- D-glyceraldehyde (*S*)- 87. This compound was prepared as reported in the literature.[176]

(*R*)-1,2-Di-*O*-isopropylidene-3-(*Z*)-heptadecene (*R*)- 89. Tetradecyl-triphenyl phosphonium bromide (97%) was purchased from Lancaster and kept under high vacuum for at least 4 h prior to use.

To a cold solution (0 °C, ice bath) of tetradecyltriphenylphosphonium bromide (42.5 g, 78.8 mmol) in THF (145 mL) vigorously stirred was added butyllithium (37 mL, 2.2 M in hexanes, 80 mmol) dropwise over a period of 15 min to yield a dark red solution. This solution was stirred at 0 °C for additional 15 min. Aldehyde **(*R*)- 87** (9.0 g, 68 mmol) was dissolved in 50 mL of THF and added to the red solution via cannula. After approximately 15 min. of the addition, a precipitate appeared. This mixture was stirred at 0 °C for 20 min. and then at rt for additional 16 h. Work-up: The precipitate was isolated by vaccuum filtration and washed with ether (*ca* 300 mL). The filtrate was quenched with a saturated aqueous solution of $NH_4^+Cl^-$ (200 mL) and water (200 mL), and the phases separated. The aqueous layer was further extracted with ether (2 × 75 mL). The combined organic solution was dried, filtered, and concentrated to yield a wet amber residue. The residue was purified by silica gel column chromatography eluted with (hexanes: EtOAc, 25:1) to yield 1,2-di-*O*-isopropylidene-3-heptadecene (20 g, 94%) as a pale yellow oil. The olefin was obtained mainly in the (*Z*) configuration, 98:2 (*Z*:*E*) after purification, by integration of the ^{1}H NMR olefinic peaks of the regions 4.9-4.8 and 4.5-4.4 ppm. **IR (neat, cm^{-1})** 3018, 2983, 2923, 2851, 1796 (*trans* CH=CH), 1659 (*cis* CH=CH). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 5.61 (m, 2H, $CH{=}C\underline{H}(CH)_{12}CH_3$), 5.39 (m, 1H, $C\underline{H}{=}CH(CH_2)_{12}CH_3$), 4.9 (m, 1H, $C\underline{H}$), 4.05 (dd, 1H, $OC\underline{H}(H)$, J_{app} = 6.8 Hz), 3.5 (m, 1H, $OCH(\underline{H})$), 2.1 (m, 2H, $OCH{=}CHC\underline{H}_2$), 1.41 (s, 3H, CCH_3), 1.37 (s, 3H, CCH_3), 1.25 (s, 22H, $(C\underline{H}_2)_{11}CH_3$), 0.87 (t, 3H, $(C\underline{H}_2)_{11}CH_3$. **^{13}C NMR (100 MHz, CDCl$_3$)** δ 135.18 ($CH{=}\underline{C}H(CH_2)_{12}CH_3$), 126.95 ($\underline{C}H{=}CH(CH_2)_{12}CH_3$), 108.97 $\underline{C}(CH_3)_2$, 71.98 ($O\underline{C}H\text{-}CH{=}CH$), 69.44 $O\underline{C}H_2$, 31.89 ($CH{=}CH\underline{C}H_2$), 29.65 $C\underline{C}H_3$, 29.63 $C\underline{C}H_3$, 29,60, 29.56, 29.43, 29.33, 29.16, 27.73, 26.76, 25.98, 22.66

($\underline{C}H_2)_{11}CH_3$, 14.08 $(CH_2)_{12}\underline{C}H_3$. **MS** 310 ($M^+$), 295(22), 289(5), 265(6), 252(14), 208(12), 108(48), 97(89), 83(95), 72(99), 55(100). **Anal. Cald.** for $C_{20}H_{38}O_2$: C, 77.36; H, 12.34. **Found** C, 77.62; H, 12.35.

(*S*)-1,2-Di-*O*-isopropylidene-3-(*Z*)-heptadecene (*S*)- 89. This compound was prepared following the procedure above reported for its enantiomer **(*R*)- 89.** All the reagent amounts were proportionally scaled-down accordingly for (*S*)-2,3-*O*-isopropylidene-d-glyceraldehyde (4.70 g, 35.8 mmol) to afford the entitled compound in 9.7 g (87 %) after purification by column chromatography. Mainly in the (*Z*) configuration, 94:6 (*Z/E*). Spectral data were the same as for **(*R*)- 89.**

(*S*)-1,2-Di- *O*-isopropylidene heptadecane (*S*)- 85. To a solution of **(*R*)- 89** (19.8 g, 63.7 mmol) in 800 mL of degassed EtOAc was added 3 g of 10% Pd/C. The flask was capped with a rubber septum and equipped with a balloon in the end of a needle. Argon and vacuum were applied consecutively for 3 times. Hydrogen was flushed until the balloon was inflated. The reaction was run at rt with constant stirring for 2-3 d. The reaction was monitored by TLC, co-running both the starting material and product. Both materials have the same R_f's values (hexanes:EtOAc 9:1, $R_f = 0.6$). However, when stained with a solution of $KMnO_4$ only the starting material gives a yellow spot at rt subsequent heating develops the product as another single yellow spot. After the reaction completion, the solid was filtered over a short path of Celite under vacuum and washed with EtOAc. The filtrate was concentrated to give a thick cloudy liquid 18.28 g (92 %) which started crystallizing at rt. **Mp.** = 34.1-34.4 °C. $[\alpha]^{22}_D = +11.8$ (c =2.6 in $CHCl_3$). **IR (KBr, cm^{-1})** 2981, 2920, 2849, 1473, 1462, 1381, 1368, 1245, 1223, 1165,

1107. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 4.03 (m, 2H, OC$\underline{H}$ and OC$\underline{H}_2$), 3.49 (m, 1H, OC$\underline{H}_2$), 1.64 (m, 2H ,C$\underline{H}_2$$(CH_2)_{13}CH_3$), 1.51-1.44 (m, 2H, ,C$\underline{H}_2$$(CH_2)_{12}CH_3$), 1.4 (s, 3H, CC$\underline{H}_3$), 1.34 (s, 3H, CC$\underline{H}_3$), 1.24 (s, 24H, (C$\underline{H}$)$_{12}$$CH_3$), 0.87 (t, 3H, $(CH_2)_{12}$C$\underline{H}_3$). **APT (100 MHz, $CDCl_3$)** δ 108.54 ($\underline{C}(CH_3)_2$) 76.16 ($\underline{C}$H) , 69.53 ($\underline{C}H_2O$), 33.57 ($\underline{C}H_2(CH_2)_{13}CH_3$), 26.93 (C$\underline{C}H_3$), 25.75 (C$\underline{C}H_3$), 31.90, 29.66, 29.65, 29.63, 29.62, 29.55, 29.49, 29.34, 22.67 (($\underline{C}H_2)_{12}CH_3$), 14.09 ($(CH_2)_{12}\underline{C}H_3$). **Anal. Calcd.** for $C_{20}H_{40}O_2$: C,76.86; H,12.90. **Found** C, 76.82; H, 12.92. **MS (EI$^+$)** 299(7), 298(57), 297(100, M$^+$- 15), 125(19), 111(78), 101(83), 97(94), 83(98), 69(95), 55(94).

(*R*)-1,2-Di-*O*-isopropylidene heptadecane (*R*)- 85. The procedure is the same as for the preparation of its enantiomer **(*S*)- 85**, adapted for (9.50 g, 3.06 mmol) of (*S*)-1,2-Di-*O*-isopropylidene-3-(*Z*)-heptadecene **(*S*)- 89**. The yield was 8.6 g (95 %) of **(*R*)- 85** after purification. **Mp.** = 34.3-34.6 °C. $[\alpha]^{22}_D$ = -12.2 (c=1.4 in $CHCl_3$). **Anal. Calcd.** for $C_{20}H_{40}O_2$: C, 76.86; H, 12.90. **Found** C, 76.73; H, 12.84. Spectral data were the same as for **(*S*)-85**.

(*S*)-1,2-Heptadecanediol (*S*)- 50. To a solution of (*S*)-1,2-Di-*O*-isopropylidene heptadecane (17.5 g, 55.9 mmol) **(*S*)- 85** in 1.2 L of MeOH, Amberlyst-A15 (6.5 g) was added. Mixture stirred for 2 d at rt during that time the solution became cloudy. The reaction was monitored by TLC (CH_2Cl_2:MeOH 16:1) until disappearance of starting material. Diol gives a single spot R_f = 0.24. The product was purified by column chromatography on silica gel (hexanes:EtOAc 12:1, 5:1, 1:2) to yield a white shinning crystalline product (14.4 g, 95.0%). **Mp.** = 80.9-81.3 °C, $[\alpha]^{22}_D$ = -7.85 (c = 0.8 in MeOH). **Anal. Calcd.** for $C_{17}H_{36}O_2$: C, 74.94; H, 13.32. **Found** C, 74.98; H, 13.24. Spectral data were the same as for **50a**.

***(R)*-1,2-Heptadecanediol *(R)*- 50**. The procedure is the same as for the deprotection of its enantiomer **(*S*)- 50**, adapted for a smaller amount of (*R*)-1,2-Di- *O*-isopropylidene heptadecane **(*R*)- 85**. (8.40 g, 27.2 mmol). The yield was 7.04 g (95 %). **Mp.** = 81-81.3 °C $[\alpha]^{22}_{D}$ = +8.15 (c = 0.8 in MeOH). **Anal. Calcd.** for $C_{17}H_{36}O_2$: C, 74.94; H, 13.32. **Found** C, 75.02; H, 13.25. Spectral data were the same as for **50a**.

***(S)*-1-Pentadecyloxirane *(S)*- 4.** (*S*)-1,2-Heptadecanediol (5.0 g, 18 mmol) and PPTS (0.46 g, 1.8 mmol) were placed in a vessel. Dichloromethane (300 mL) added (not all the solid dissolved) and mixture stirred at rt under Argon for 10min. Trimethyl orthoacetate (2.8 mL, 22 mmol) was added via syringe and the solution was stirred for 1 h. TLC was checked in (hexanes :EtOAc 8:1). Solvent was concentrated to yield a wet white solid which was taken up in 180 mL of dichloromethane. Acetyl bromide (1.70 mL, 22.9 mmol) was added via syringe. The solution turned yellow but it gradually decolorized with time. The reaction was stirred at rt under Argon for 1.5 h. The solvent was concentrated to give a pale amber oil which was taken up in 100 mL of methanol. K_2CO_3 (4.05 g, 29.2 mmol) was added and mixture stirred at rt for 1 d. TLC checked (hexanes:EtOAc 15:1, R_f= 0.27). The reaction was quenched with of a saturated solution of $N^+H_4Cl^-$ (140 mL) and extracted with dichloromethane. The combined organic layers were dried and the solvent was concentrated to yield a white solid. The product was purified by column chromatography on silica gel

(Hexanes:EtOAc 25:1) to give pure epoxide (4.1 g, 87%). $[\alpha]^{22}_D$ = -5.4 (c =3.5 in $CHCl_3$). **IR (KBr, cm^{-1})** 2923, 2853 1466, 1467. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 2.89 (m, 1H), 2.74 (m, 1H, C$\underline{H}$), 2.46 (dd, 1H, OC$\underline{H}_2$), 2.46 (dd, 1H, J_{app} = 2.3, 2.7 Hz, OC$\underline{H}_2$), 1.51 (m, 2H, C$\underline{H}_2$(CH$_2$)$_{13}$CH$_3$), 1.44 (m, 2H, C$\underline{H}_2$(CH$_2$)$_{12}$CH$_3$), 1.26 (m, 24H, (C$\underline{H}_2$)$_{12}$CH$_3$), 0.87 (t, 3H, (CH$_2$)$_{12}$C$\underline{H}_3$). **^{13}C NMR (100 MHz, CDCl$_3$)** 52.38 ($\underline{C}$H), 47.10 (O$\underline{C}$H$_2$), 32.48 ($\underline{C}$H$_2$(CH$_2$)$_{13}$CH$_3$), 31.91(($\underline{C}$H$_2$(CH$_2$)$_{12}$CH$_3$) , 29.69, 29.67, 29.66, 29.63, 29.55, 29.44, 22.35, 25.95, 25.93, 22.67 ($\underline{C}$H$_2$)$_{12}$CH$_3$, 14.09 (CH$_2$)$_{12}\underline{C}$H$_3$. **Anal. Calcd.** for $C_{17}H_{34}O$: C, 80.24; H, 13.47. **Found** C, 80.23; H, 13.42. **MS (CI)** 255 (M$^+$ + 1), 235(20), 125(30), 111(60), 97(99), 83(100).

(*R*)-1-Pentadecyloxirane (*R*)- 4. The procedure is the same as for the preparation of its enantiomer **(*S*)- 4** adapted for a smaller amount of (*R*)-1,2-heptadecanediol (4.60 g, 16.8 mmol). The yield was 3.7 g (86 %). $[\alpha]^{22}_D$ = +5.43 (c =1.4 in $CHCl_3$) **Anal. Calcd.** for $C_{17}H_{34}O$: C, 80.24; H, 13.47. **Found** C, 80.38; H, 13.38. Spectral data were the same as for **(*S*)- 4.**

(*S*)-*N*-(2-Hydroxyethyl)-*N*-(2-hydroxyheptadecyl)-methylamine (*S*)- 36. *N*-methylaminoethanol (1.40 mL, 16.9 mmol) was added to a solution of (*S*)-1-pentadecyl oxirane (4.32 g, 16.9 mmol) in MeOH (250 mL). The reaction was refluxed for 2 d under nitrogen. TLC of the product was checked on neutral alumina and developed with I_2 [hex: EtOAc (2:3), R_f = 0.46]. Evaporation of the solvent gave a white solid (5.4 g, 98%) which was used for the next reaction without further purification. **IR (KBr, cm^{-1})** 3600-3150b, 2917, 2848, 1473, 1085, 1031. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 3.66 (m, 3H,C$\underline{H}$OH, *N*C$\underline{H}_2$), 2.70-2.48 (m, 2H, $NC\underline{H}_2CH_2OH$), 2.40-2.36 (s, 3H, N-$C\underline{H}_3$), 1.50-1.36 (m, 2H, $C\underline{H}_2(CH_2)_{13}CH_3$), 1.26 (s, 26H, $(C\underline{H}_2)_{13}CH_3$), 0.88 (t, J_{app} = 7 Hz, 3H, $C\underline{H}_3$). **^{13}C NMR (100 MHz, CDCl$_3$)** δ 67.6 ($\underline{C}H$), 64.15 ($\underline{C}H_2OH$), 59.66 ($N\underline{C}H_2CH_2OH$), 59.40 ($N\underline{C}H_2$), 42.42 N-$\underline{C}H_3$, 34.92 ($\underline{C}H_2(CH_2)_{13}CH_3$), 31.92, 29.77, 29.69, 29.66, 29.61, 29.35, 25.63, 22.60 ($CH_2(C\underline{H}_2)_{13}CH_3$), 14.11 ($\underline{C}H_3$). $[\alpha]^{22}_{D}$ = + 27.69 (c = 0.8 in CHCl$_3$). **Mp.** = 42.4-43.5 °C. This compound did not give satisfactory alemental analysis according to the ACS requirements (≤ 0.4 %). **Anal. Cald.** For $C_{20}H_{43}NO_2$: C, 72.89; H, 13.15; N,4.25. **Found** C, 71.45; H, 13.05; N, 4.28.

(*R*)-*N*-(2-Hydroxyethyl)-*N*-(2-hydroxyheptadecyl)-methylamine (*R*)- 36. The procedure is the same as for the preparation of its enantiomer adapted for (5.00 g, 18.1 mmol). The crude yield was 5.2 g (91 %). Spectral data were the same as for **(*S*)- 36.** $[\alpha]^{22}_{D}$ = - 26.56 (c= 0.9 in CHCl$_3$). **Mp.** = 41.7-42.6 °C

(*S*)-*N*,*N*-Dimethyl-N-(2-hydroxyethyl)-*N*-(2-hydroxyheptadecyl) ammonium iodide (*S*)- 90. Iodomethane (0.76 mL, 12 mmol) was added in one portion to a solution of **(*S*)-36** (0.80 g, 2.4 mmol) in ether (35 mL). The reaction was run in the dark and stirred for 3 d at rt giving an unsoluble white powder. The solvent was concentrated to yield crude product (1.1 g, 96%) which was recrystallized from EtOAc:CH$_2$Cl$_2$:MeOH (20:8:0.5) with moderate heating (*ca* 45 °C). The yield after two recrystallyzations was 0.9 g (78 %). **Mp.** = 73.7- 74.0 °C. $[\alpha]^{21}_{D}$ = +11.55 (c = 0.9, CHCl$_3$). **IR (KBr, cm^{-1})** 3326, 2917, 2848, 1469, 937, 719. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 4.4-4.3 (m, 1H, $C\underline{H}$), 4.2-4.1 (m, 2H, $NC\underline{H}_2CH_2OH$), 3.9-3,8 (m, 3H, $C\underline{H}_2OH$, $NC\underline{H}_2$), 3.70-3.6 (m, 1H, $NC\underline{H}_2$), 3.46 (s, 6H, $NC\underline{H}_3$, $NC\underline{H}_3$), 1.6-1.4 (m, 2H, $C\underline{H}_2(CH_2)_{13}CH_3$, 1.26 (s, 26H, $(C\underline{H}_2)_{13}CH_3$), 0.87 (t, 3H, $(CH_2)_{13}C\underline{H}_3$) **APT (100 MHz, $CDCl_3$) δ** 70.1 ($N\underline{C}H_2$), 66.7 ($\underline{C}H_2OH$), 65.69 ($\underline{C}H$), 55.98 ($N\underline{C}H_2CH_2OH$), 53.98 ($N\underline{C}H_3$), 53.74 ($N\underline{C}H_3$), 35.88, 31.91, 29.72, 29.66, 29.62, 29.55, 29.37, 25.17, 22.67 ($(\underline{C}H_2)_{13}CH_3$ 14.10 $(CH_2)_{13}\underline{C}H_3$. **MS ($CI^+$)** 331(cation -$CH_3$, (45)), 299(30), 142(25), 88(100), 58(30). **Anal. Calcd.** for $C_{21}H_{46}INO_2$: C, 53.49; H, 9.83; N, 2.97. **Found** C, 53.57; H, 9.77; N, 2.98.

(*R*)-*N*,*N*-Dimethyl-N-(2-hydroxyethyl)-*N*-(2-hydroxyheptadecyl) ammonium iodide (*R*)- 90. The procedure is the same as for the preparation of its enantiomer **(*S*)- 90** scaled down for (0.38 g, 1.2 mmol). The yield after two recrystallizations was 0.4 g (74 %) **Mp.** = 73.7-74.3 °C. $[\alpha]^{21}_{D}$ = -11.89 (c =0.37, $CHCl_3$). Spectral data were the same as for **(*S*)- 90**. **Anal. Calcd.** for $C_{21}H_{46}INO_2$: C, 53.49; H, 9.83; N, 2.97. **Found** C, 53.22; H, 9.75; N, 2.93.

Preparation of the Cyclic Phosphorus Compounds.

To triethylamine (8.96 mL, 64.3 mmol) in CH_2Cl_2 (300 mL) were added simultaneously aminodiol **(*S*)- 36** (5.30 g, 16.1 mmol) in CH_2Cl_2 (125 mL) and methylphosphonic dichloride(3.8 g, 23 mmol) in CH_2Cl_2 (125 mL) over a period of 6 h. After the addition was over the reaction was stirred overnight at rt. The solvent was evaporated, and the residue was taken up in ether (200 mL), filtered, and discarded. The filtrate was concentrated and the residue was purified by column chromatography on neutral alumina with EtOAc:hexanes (1:1). Two diastereomers were collected. First fraction, diasteromer **(93a)** 2.04 g; second fraction, diasteromer **(93b)** 1.18 g. The total yield was 51 %.

Diasteromers **93c** (first fraction) and **93d** (second fraction) were prepared similarly from aminodiol **(*R*)- 36** (5.00 g, 15.2 mmol) to yield **93c** (2.00 g) and **93d** (1.45 g). The total yield was 57 %.

(2*S*/4S)-6-*N*-methyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphocyclooctane (93a). $[\alpha]^{22}_{D} = +6.12$ (c = 1.3, $CHCl_3$). **IR(KBr, cm^{-1})** 2915, 2848, 1455, 1312, 1231 (P=O), 1083, 1033, 962, 908. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 4.42 (m, 1H), 4.25 (m, 1H), 3.75 (m, 1H), 2.9 (m, 1H), 2.78 (m, 2H), 2.76-2.51 (m, 1H), 2.55 (s, 3H, N-$C\underline{H}_3$), 1.42 (d, 3H, J_{app}=18 Hz, P-$C\underline{H}_3$), 1.26 (s, 26H, $(C\underline{H}_2)_{13}CH_3$), 0.87 (t, 3H, J_{app} = 7 Hz, $(CH_2)_{13}C\underline{H}_3$). **$^{13}C$ NMR (100 MHz, $CDCl_3$)** δ 76.69 (d, J_{app} = 7.5 Hz, $\underline{C}H$), 65.59 (d, J_{app} = 8.4 Hz, $NCH_2\underline{C}H_2$), 62.71 ($N\underline{C}H_2CH$), 58.68 ($N\underline{C}H_2CH_2$), 42.14 N-$\underline{C}H_3$, 32.88 (d, J_{app} = 9.1 Hz, $\underline{C}H$ $_2(CH_2)_{13}CH_3$), 31.89, 29.66, 29.62, 29.53, 29.47, 29.43, 29.33, 25.43, 22.66 $(\underline{C}H_2)_{12}CH_3$, 14.09 $(CH_2)_{14}\underline{C}H_3$, 12.36 (d, J_{app} = 155.5 Hz, P-$\underline{C}H_3$. **^{31}P NMR (400 MHZ, $CDCl_3$)** δ 26.81. **MS(CI^+)** 390(100, M^+), 294(15), 96(12), 85(12). This compound did not give satisfactory elemental analysis according to the ACS requirements. **Anal. Calcd.** for $C_{21}H_{44}NO_3P$: C, 64.75; H, 11.38; N, 3.59. **Found**: C, 62.45; H, 10.91; N, 3.52. Its derivative **1a**, passed elemental analysis.

(2*R*/4S)-6-*N*-methyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphocyclooctane (93b). Mp. = 45.1-48.2 °C. $[\alpha]^{21}_{D} = +12.10$ (c = 1.5 in $CHCl_3$). **IR(KBr, cm^{-1})** 2918, 2848, 1465, 1317, 1251(P=O), 1083, 930, 814.

^{1}H NMR (400 MHz, CDCl$_3$) δ 4.39-432 (m, 1H), 4.16-4.08 (m, 1H), 3.91-3.87 (m, 1H), 2.95-2.89 (m, 1H), 2.76-2.61 (m, 3H), 2.53 *N*-C$\underline{\mathrm{H}}_3$, 1.48 (d, 3H, J_{app} = 16.4 Hz, P-C$\underline{\mathrm{H}}_3$), 1.6-1.4 (m, 2H), 1.25 (s, 26H, (C$\underline{\mathrm{H}}_2$)$_{13}$CH$_3$)) 0.88 (t, 3H, (CH$_2$)$_{14}$C$\underline{\mathrm{H}}_3$). **APT(100 MHz, CDCl$_3$)** δ 77.96 (d, J_{app} = 8.39 Hz, ($\underline{\mathrm{C}}$H)), 65.33, (d, J_{app} = 7.63 Hz, (*N*CH$_2\underline{\mathrm{C}}$H$_2$)), 62.53 (*N*$\underline{\mathrm{C}}$H$_2$CH), 55.78 (*N*$\underline{\mathrm{C}}$H$_2$CH$_2$)), 45.55 (*N*-$\underline{\mathrm{C}}$H$_3$), 33.72 (d, J_{app} = 6.9 Hz, $\underline{\mathrm{C}}$H$_2$(CH$_2$)$_{13}$CH$_3$), 31.9($\underline{\mathrm{C}}$H$_2$(CH$_2$)$_{12}$CH$_3$), 29.67, 29.63, 29.60, 29.54, 29.45, 29.40, 29.34, 25.71, 22.67 ($\underline{\mathrm{C}}$H$_2$)$_{12}$CH$_3$, 14.13 (CH$_2$)$_{12}\underline{\mathrm{C}}H_3$, 11.41 (d, J_{app}= 148.02 Hz, P-$\underline{\mathrm{C}}$H$_3$). **^{31}P NMR (40 MHz, CDCl$_3$)** δ 27.96. **Anal. Calcd.** for $C_{21}H_{44}NO_3P$: C, 64.75; H, 11.38; N, 3.59. **Found**: C, 64.63; H, 11.29; N, 3.66.

(*2R*/4*R*)-6-*N*-methyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphocyclooctane (93c). Preparation and spectral data is the same as for its enantiomer **93a**. $[\alpha]^{21}_{\mathrm{D}}$ = - 5.75 (c = 1.2, CHCl$_3$). This compound did not give sastifactory elemental analysis.according to the ACS requirement. **Anal. Calcd.** for $C_{21}H_{44}NO_3P$: C, 64.75; H, 11.38; N, 3.59. **Found**: C, 63.01; H, 11.24; N, 3.55. The derivative of this compound **1d**, however, passed elemental analysis.

(*2S*/4*R*)-6-*N*-methyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphocyclooctane (93d). Preparation and spectral data is the same as for its enantiomer **93b**. $[\alpha]^{21}_{\mathrm{D}}$ = -12.54 (c =1.10 in CHCl$_3$). **Anal. Calcd.** for $C_{21}H_{44}NO_3P$: C, 64.75; H, 11.38; N, 3.59. **Found**: C, 64.84; H, 11.35; N, 3.65.

(*2S*/4S)-6-*N,N*-methyl-2-dimethyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphocyclooctane bromide (1a). Compound **(93b)** (0.48g 1.2 mmol) was dissolved in ether (30 mL). The reaction flask was protected from light. Bromomethane was bubbled through for 30 min and then stirred for 5 d at rt. Additional bromomethane was bubbled through on the third day. A white precipitate formed. The solvent was evaporated and the crude product was washed throughly with ether to yield 0.39 g (65 %) of the product. **Mp.** = 91.7-159.3 °C (liquid crystal formation and decomposition). $[\alpha]^{22}_{\mathrm{D}}$ = -7.96 (c = 0.59 in $CHCl_3$). **IR (KBr, cm^{-1})** 2918, 2845, 1472, 1318, 1245 (P=O), 1077, 977, 917, 810. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 4.84 (m, 1H, C$\underline{\mathrm{H}}$), 4.64 (m, 1H, *N*CH$_2$C$\underline{\mathrm{H}}_2$), 4.55-4.35 (m, 2H, *N*CH$_2$C$\underline{\mathrm{H}}_2$, *N*C$\underline{\mathrm{H}}_2$), 4.25-4.12 (m,2H, *N*C$\underline{\mathrm{H}}_2$, *N*C$\underline{\mathrm{H}}_2$), 3.93-3.80 (m, 1H, *N*C$\underline{\mathrm{H}}_2$), 3.89 (s, 3H, *N*-C$\underline{\mathrm{H}}_3$), 1.71 (m, 2H, $\underline{\mathrm{C}}\mathrm{H_2(CH_2)_{13}CH_3}$), 1.61 (d, J_{app} = 18 Hz, 3H, P-C$\underline{\mathrm{H}}_3$), 1.44 (m, 2H, $\mathrm{C\underline{H}_2(CH_2)_{12}CH_3}$), 1.25 (s, 24 H, $\mathrm{(C\underline{H}_2)_{12}CH_3}$), 0.87 (t, J_{app} = 7 Hz, 3H, $\mathrm{(CH_2)_{12}C\underline{H}_3}$). **APT (100 MHz, CDCl$_3$)** δ 71.14 (*N*$\underline{\mathrm{C}}H_2$), 69.23 (d, J_{app} = 7.63 Hz, ($\underline{\mathrm{C}}$H)), 64.22 (*N*$\underline{\mathrm{C}}$H$_2$), 60.09 (d, J_{app}= 7.79 Hz (*N*CH$_2$$\underline{\mathrm{C}}H_2$)), 56.55 (*N*$\underline{\mathrm{C}}H_3$), 50.93 (*N*$\underline{\mathrm{C}}H_3$), 34.94 (d, J_{app} = 6.9 Hz, ($\mathrm{\underline{C}H_2(CH_2)_{13}CH_3}$)), 31.98, 29.66, 29.62, 29.59, 29.53, 29.42, 29.32, 29.98, 24,88, 22.65 ($\mathrm{(\underline{C}H_2)_{14}CH_3}$), 14.08 ($\mathrm{(CH_2)_{14}\underline{C}H_3}$). 10.89 (d, J_{app} = 145.73, P$\underline{\mathrm{C}}$H$_3$). **^{31}P NMR (40 MHz, CDCl$_3$)** δ 33.62. **MS(EI$^+$)** 389 (cation -15), 375(10), 279(45), 192(30), 152(15), 96(50), 70(100), 58(99). **Anal. Calcd.** for $C_{22}H_{47}NO_3PBr$: C, 54.54; H, 9.77; N, 2.89. **Found**: C, 54.39; H, 9.72; N, 2.85.

***2R*/4S)-6-*N*,*N*-dimethyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphocyclooctane bromide (1b).** Compound **(93b)**, (0.33 g, 0.85 mmol) in ether (25 mL). The reaction flask was protected from light, and bromomethane was bubbled through for 30 min. Reaction was stirred for 5 d at rt. Additional bromomethane was bubbled through on the third day. The solvent was evaporated and the white solid washed throughly with cold ether to yield 0.26 g (63 %) of product. $[\alpha]^{22}_{D}$= -12.57 (c = 0.81 in $CHCl_3$) . **IR (KBr, cm^{-1})** 2918, 2845, 1464, 1317, 1252 (P=O), 1082, 1044, 996, 930, 901. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 4.70-4.59 (m, 1H, $C\underline{H}$), 4.60-4.40 (m, 2H, *N*$CH_2C\underline{H}_2$), 4.35-4.20 (m, 3H, *N*$C\underline{H}_2$, *N*$C\underline{H}_2$) 3.68-3.65 (m, 1H, *N*$C\underline{H}_2$), 3.80 (s, 3H, *N*-$C\underline{H}_3$), 3.75 (s, 3H, *N*-$C\underline{H}_3$), 1.85-1.65 (m, 2H, $C\underline{H}_2(CH_2)_{12}CH_3$), 1.64 (d, J_{app} = 17.6 Hz, 3H, P-$C\underline{H}_3$), 1.5-1.13 (m, 2H, $C\underline{H}_2(CH_2)_{12}CH_3$), 1.25 (s, 24H, $(C\underline{H}_2)_{12}CH_3$), 0.88 (t, J_{app} = 7.2 Hz, 3H, $(CH_2)_{12}C\underline{H}_3$). **APT (100 MHz, $CDCl_3$)** δ 73.06 (d, J_{app} = 7.6 Hz, ($\underline{C}$H)), 71.10 (*N*$\underline{C}H_2$), 63.51 (*N*$\underline{C}H_2$), 59.70 (d, J_{app} = 7.6 Hz (*N*$CH_2\underline{C}H_2$)), 58.23 (*N*-$\underline{C}H_3$) , 49.42(*N*-$\underline{C}H_3$) , 33.79 (d, J_{app} = 2.9 Hz , ($\underline{C}H_2)(CH_2)_{13}CH_3$)), 31.90, 29.68, 29.66, 29.59, 29.53, 29.41, 29.34, 29.22, 25.17, 22.66 ($\underline{C}H_2$'s), 14.09, $(CH_2)_{13}\underline{C}H_3$) , 10.79 (d, J_{app} = 141.2 Hz, P-$\underline{C}H_3$). **^{31}P NMR (40 MHz, $CDCl_3$)** δ 31.8. **MS** **Anal. Calcd.** for $C_{22}H_{47}BrNO_3P$: C, 54.54; H, 9.77; N, 2.89. **Found**: C, 54.58; H, 9.82, N, 2.98.

(2*R*/4S)-6-*N,N*-methyl-2-dimethyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphocyclooctane bromide (1c). Preparation and spectral data same as for its enantiomer **1a**. $[\alpha]^{22}_{D}$ = +8.46 (c = 0.52 in $CHCl_3$) **Anal. Calcd.** for $C_{22}H_{47}BrNO_3P$: C, 54.54; H, 9.77; N, 2.89. **Found** C, 54.43; H, 9.75; N, 2.82.

(2*S*/4S)-6-*N,N*-dimethyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphocyclooctane bromide (1d). Preparation and spectral data same as for its enantiomer **1b**. **Mp.** = 94.8-162 ^{0}C (liquid crystal formation and decomposition). $[\alpha\ ]^{22}_{D}$= + 12.95 (c=0.78, $CHCl_3$) **Anal. Calcd.** for $C_{22}H_{47}BrNO_3P$: C, 54.54; H, 9.77; N, 2.89. **Found** C, 54.38; H, 9.75; N, 2.80.

VII. SPECTRA

Page

1) (1-Bromomethyl)hexadecyl butanoate **72**

^{1}H NMR.. 120

APT.. 121

2) Pentadecanal **73**

^{1}H NMR 122

3) (*R*)-1,2-Heptadecanediol **50a**

^{1}H NMR 123

^{13}C NMR 124

IR 125

4) Bis-Mosher ester of racemic **75**

^{1}H NMR 126

5) Crude bis-Mosher esters **75a** and **75b** from diols obtained by Method A.

^{1}H NMR expanded 127

6) Crude bis-Mosher esters **75a** and **75b** from diols obtained by Method B.

^{1}H NMR expanded 128

7) Crude bis-Mosher ester **75c**

^{1}H NMR expanded

Top: Recrystallized from toluene 129

Middle: Recrystallized from methanol:water .... .129

Bottom: Recrystallized from hexanes:ethylacetate 129

8) Bis-Mosher ester **75d** and **75e**

^{1}H NMR expanded 130

9) (*R*)-1,2-Di-*O*-isopropylidene-3-(*Z*)-heptadecene ***(R)*- 89**

^{1}H NMR 131

^{13}C NMR 132

IR, Mass Spectrum 133

10) (*S*)-1,2-Di-*O*-isopropylideneheptadecane ***(S)*- 85**

^{1}H NMR 134

APT 135

IR 136

11) Crude bis-Mosher ester **75f**

^{1}H NMR 137

12) (*S*)-1-Pentadecyl oxirane ***(S)*- 4**

^{1}H NMR 138

^{13}C NMR 139

IR, Mass Spectrum 140

14) (*S*)- N-(2-Hydroxyethyl_-N-(2-hydroxyheptadecyl)-methylamine **(S)- 36**

^{1}H NMR 141

^{13}C NMR 142

IR 143

15) (*S*)-*N,N*-Dimethyl-*N*-(2-hydroxyethyl)-*N*-(2-hydroxyheptadecyl)-ammonium iodide ***(S)*- 90**

^{1}H NMR 144

APT 145

IR, Mass Spectrum 146

16) (2*S*/4*S*)-6-*N*-Methyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphacyclooctane **93a**

^{1}H NMR 147
^{13}C NMR 148
IR, Mass Spectrum 149

17) (2*R*/4*S*)-6-*N*-Methyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphacyclooctane **93b**

^{1}H NMR 150
APT 151
IR, Mass Spectrum 152

18) (2*S*/4*S*)-6-*N*-Methyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphacyclooctane **93a** and (2*R*/4*S*)-6-*N*-Methyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphocyclooctane **93b**

^{31}P NMR 153

19) (2*S*/4*S*)-6-*N,N*-Dimethyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphacyclooctane bromide**1a**

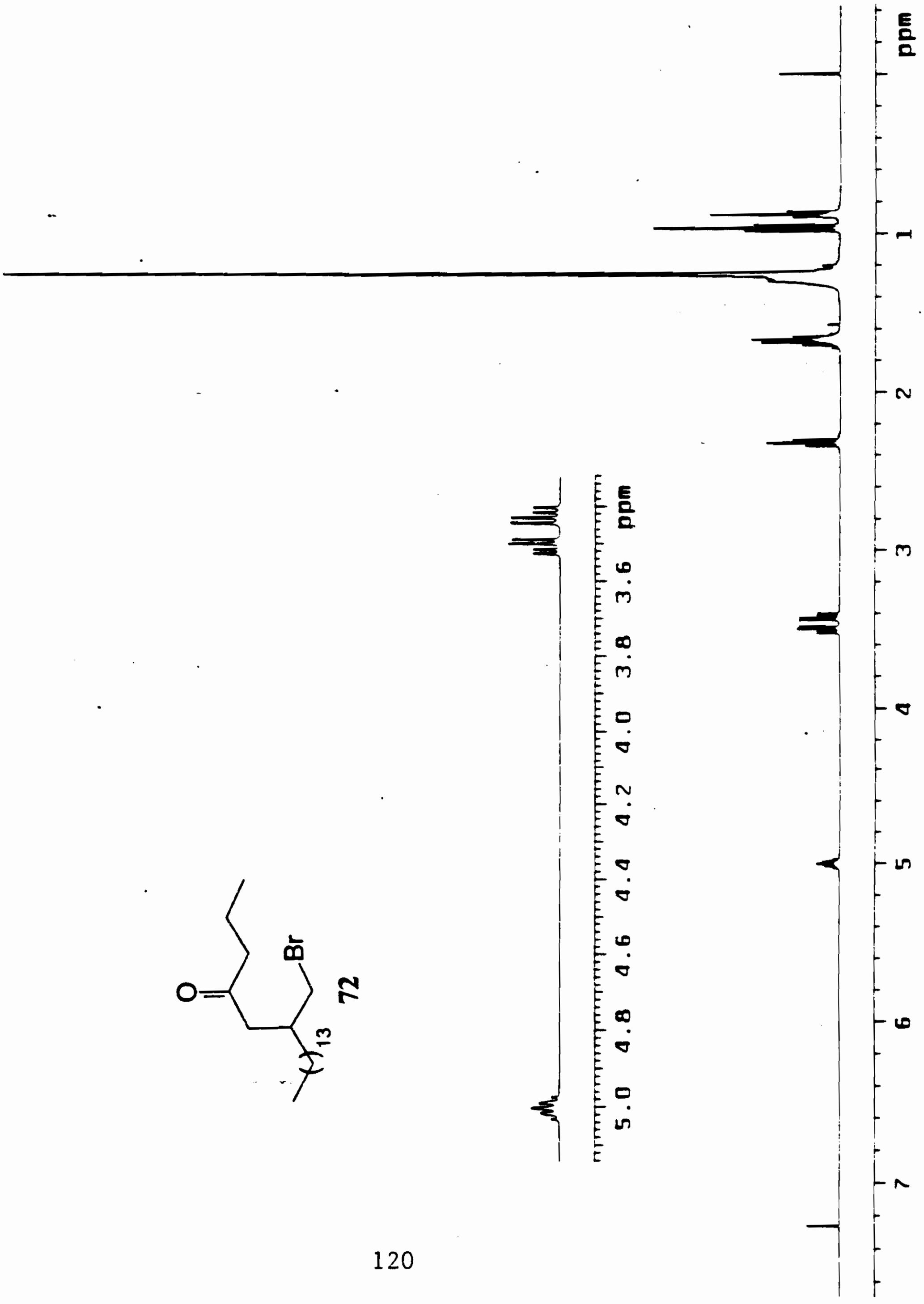
^{1}H NMR 154
APT 155
IR, Mass Spectrum 156

20) (2*R*/4*S*)-6-*N,N*-Dimethyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphacyclooctane bromide**1b**

^{1}H NMR 157
APT 158
IR, Mass Spectrum 159

21) (2*S*/4*S*)-6-*N,N*-Dimethyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphocyclooctane **1a** and (2*R*/4*S*)-6-*N,N*-Dimethyl-2-methyl-2-oxo-1,3-dioxa-4-pentadecyl-6-aza-2-phosphocyclooctane **1b**

^{31}P NMR 160



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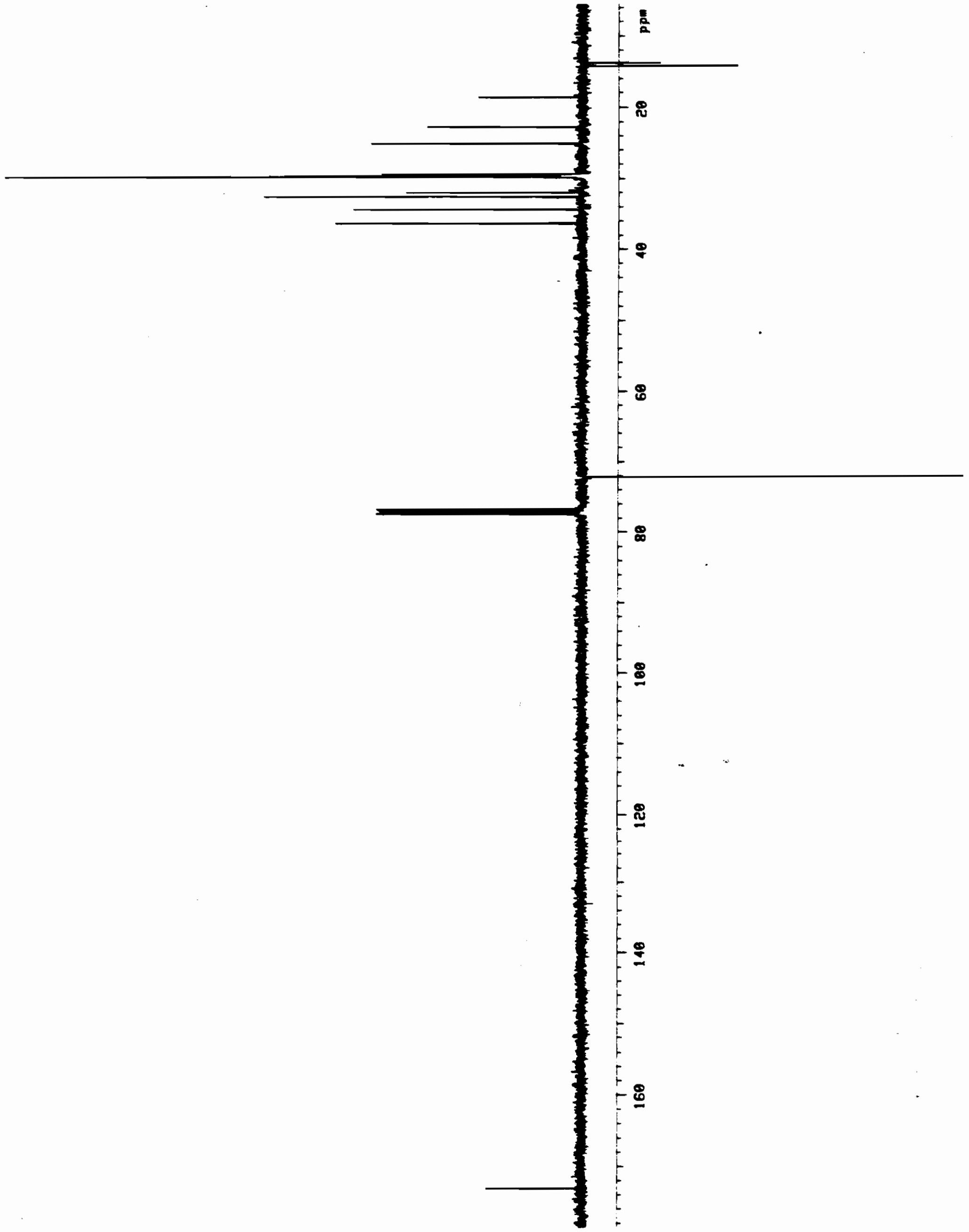
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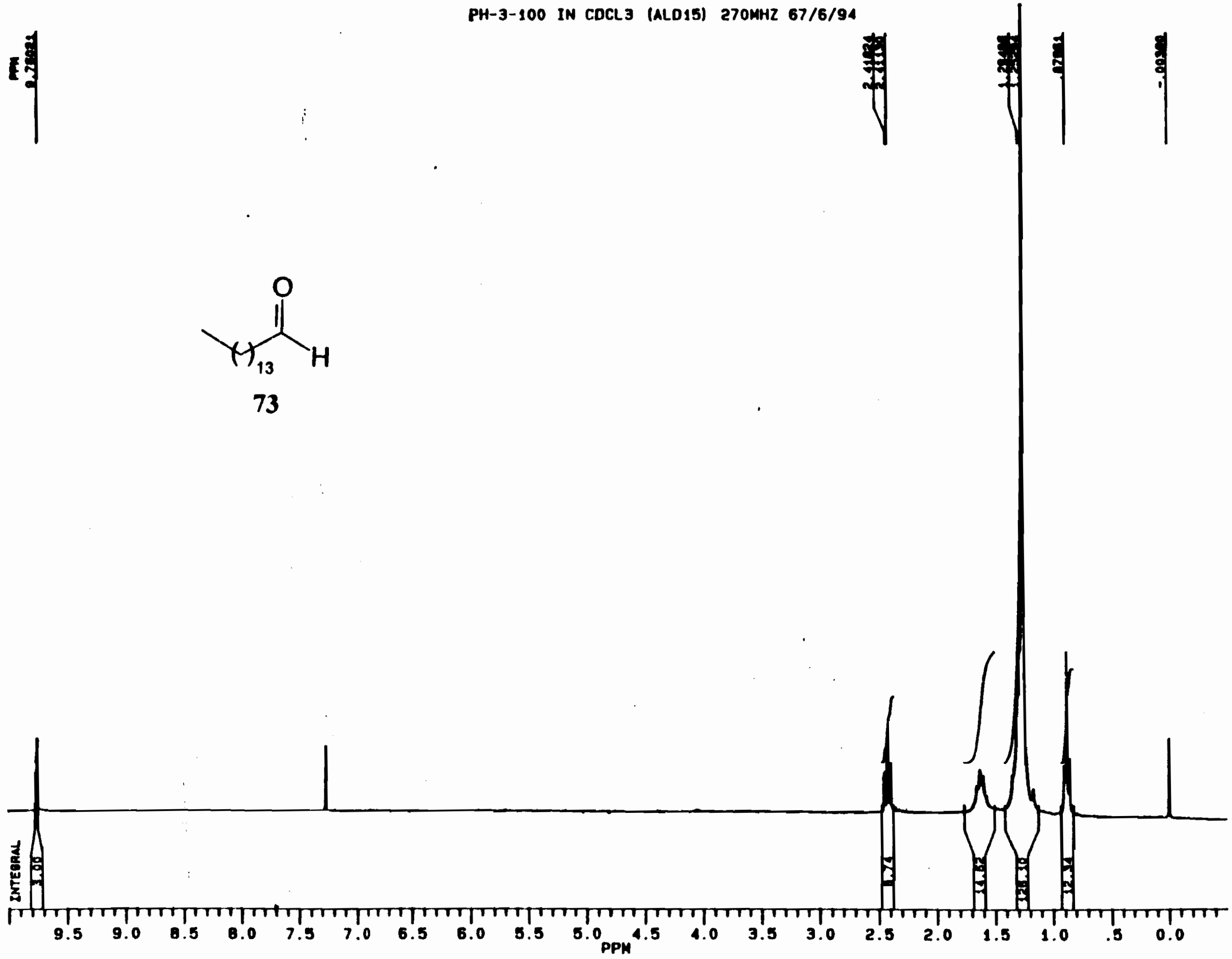

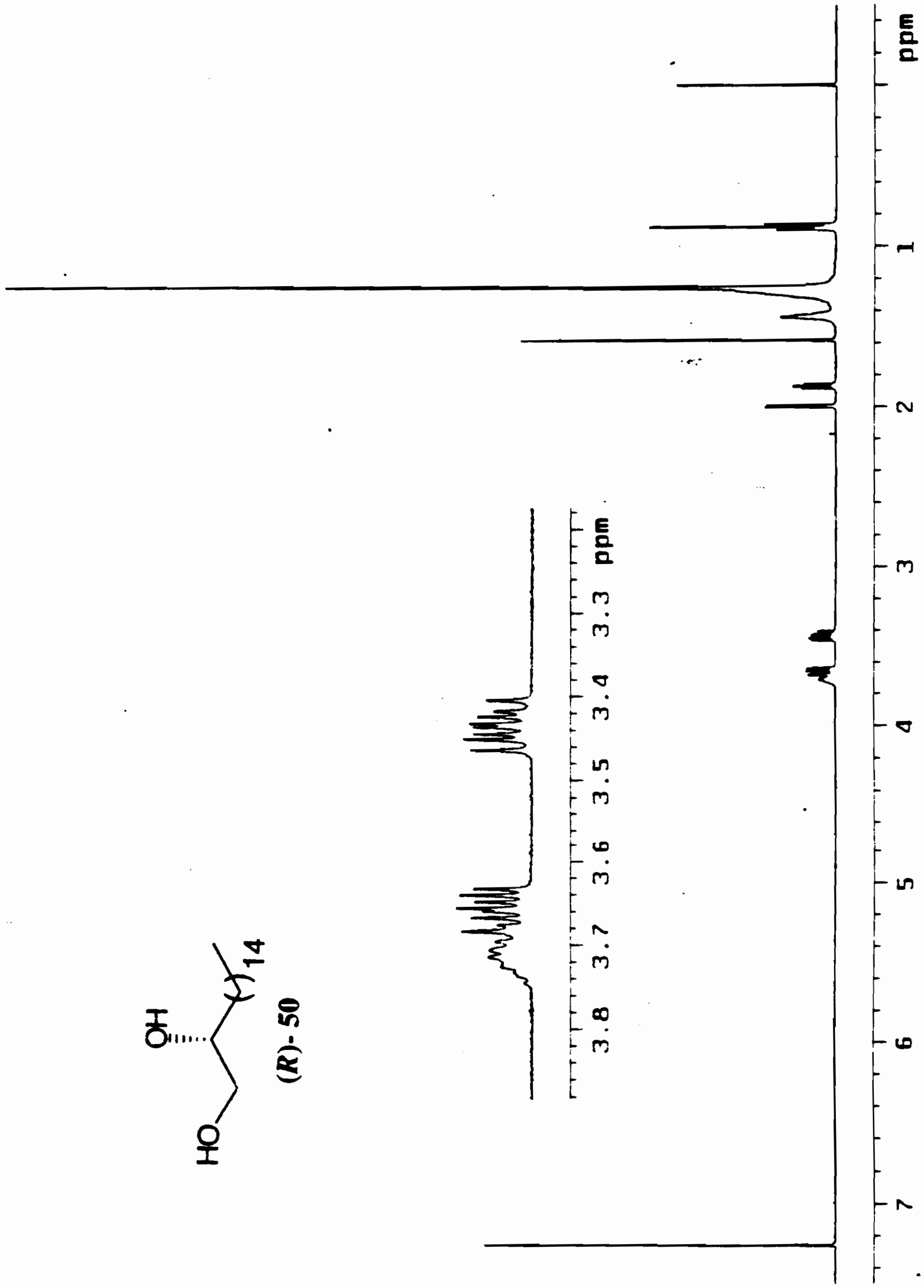

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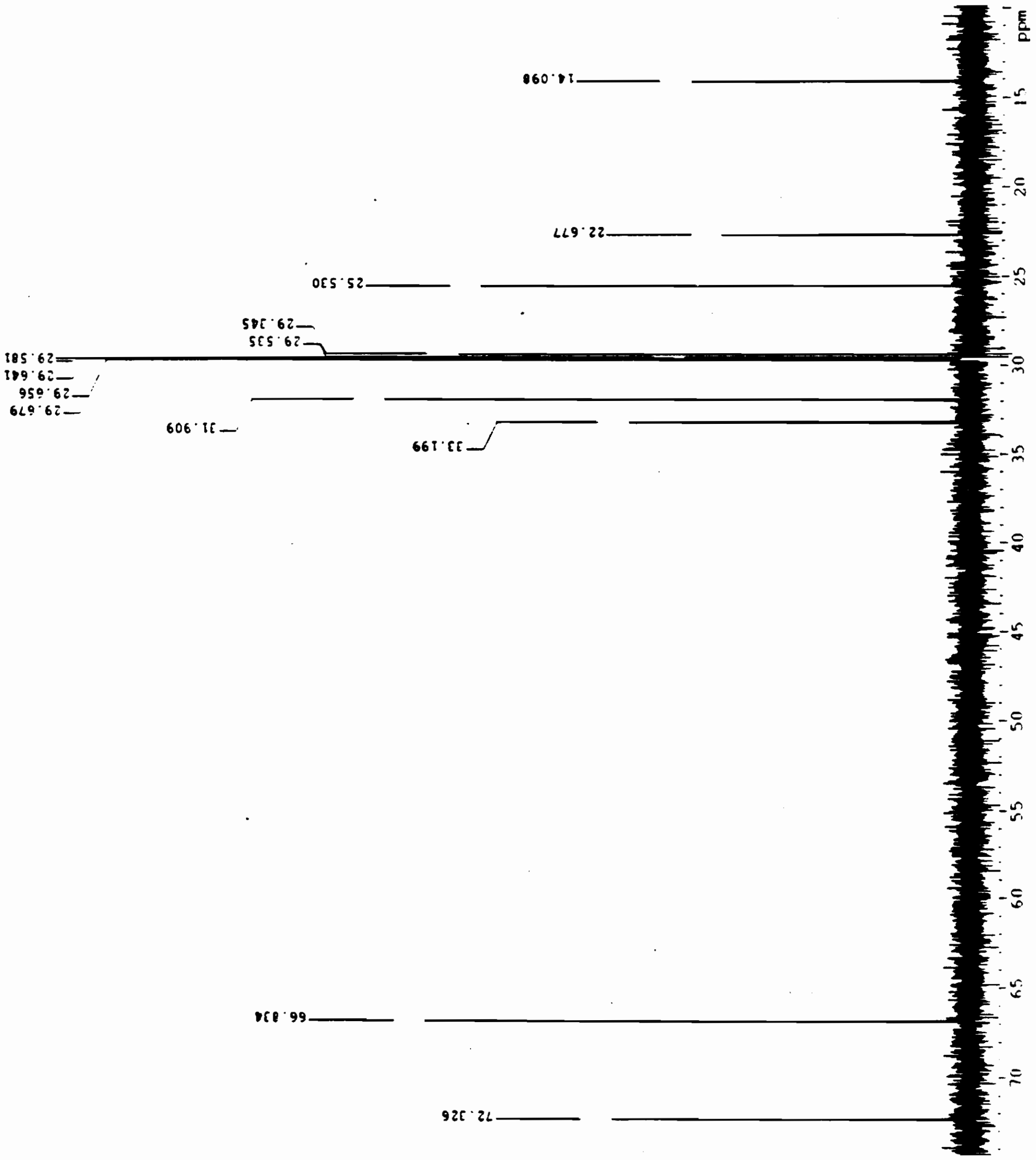
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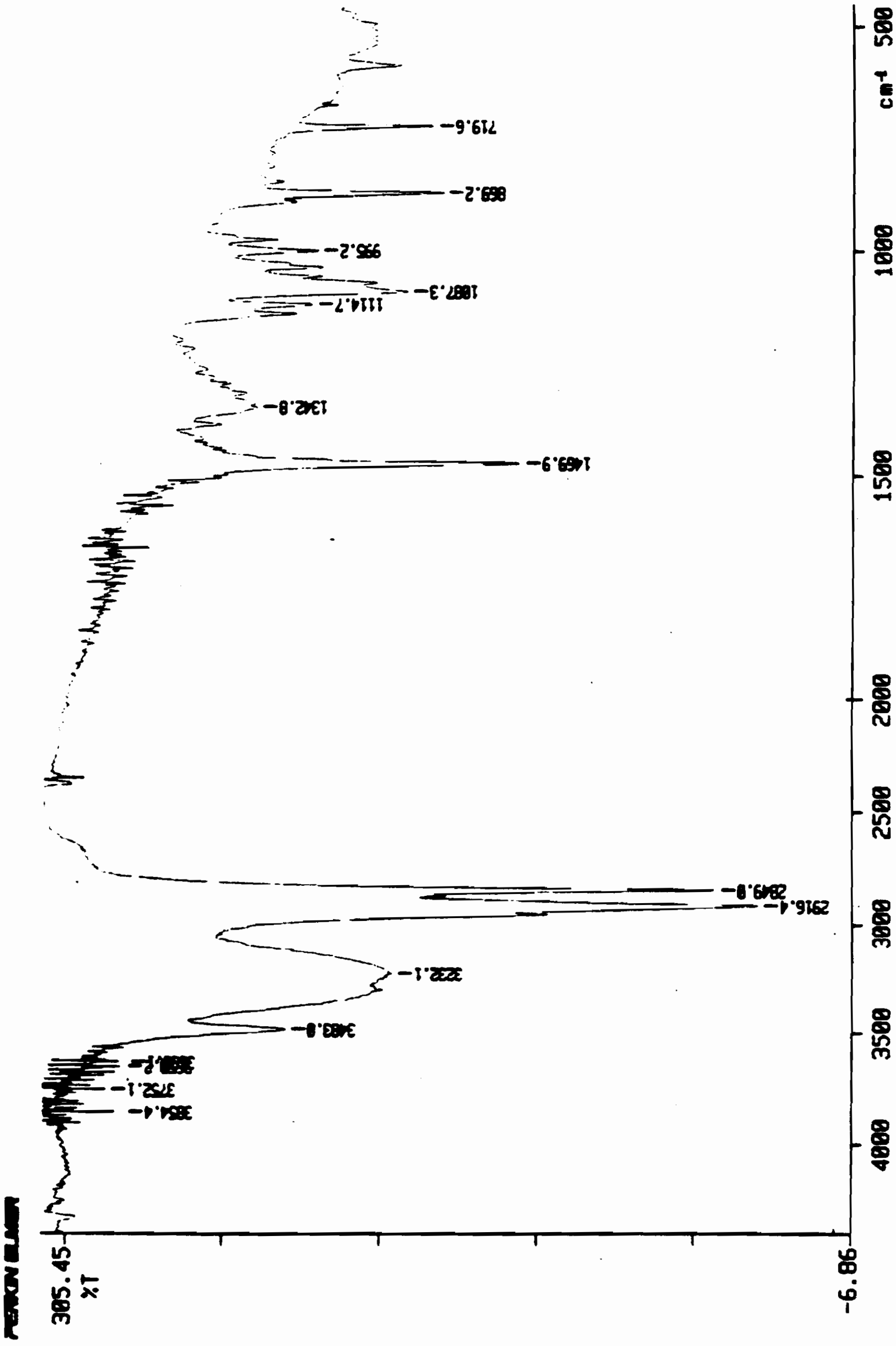
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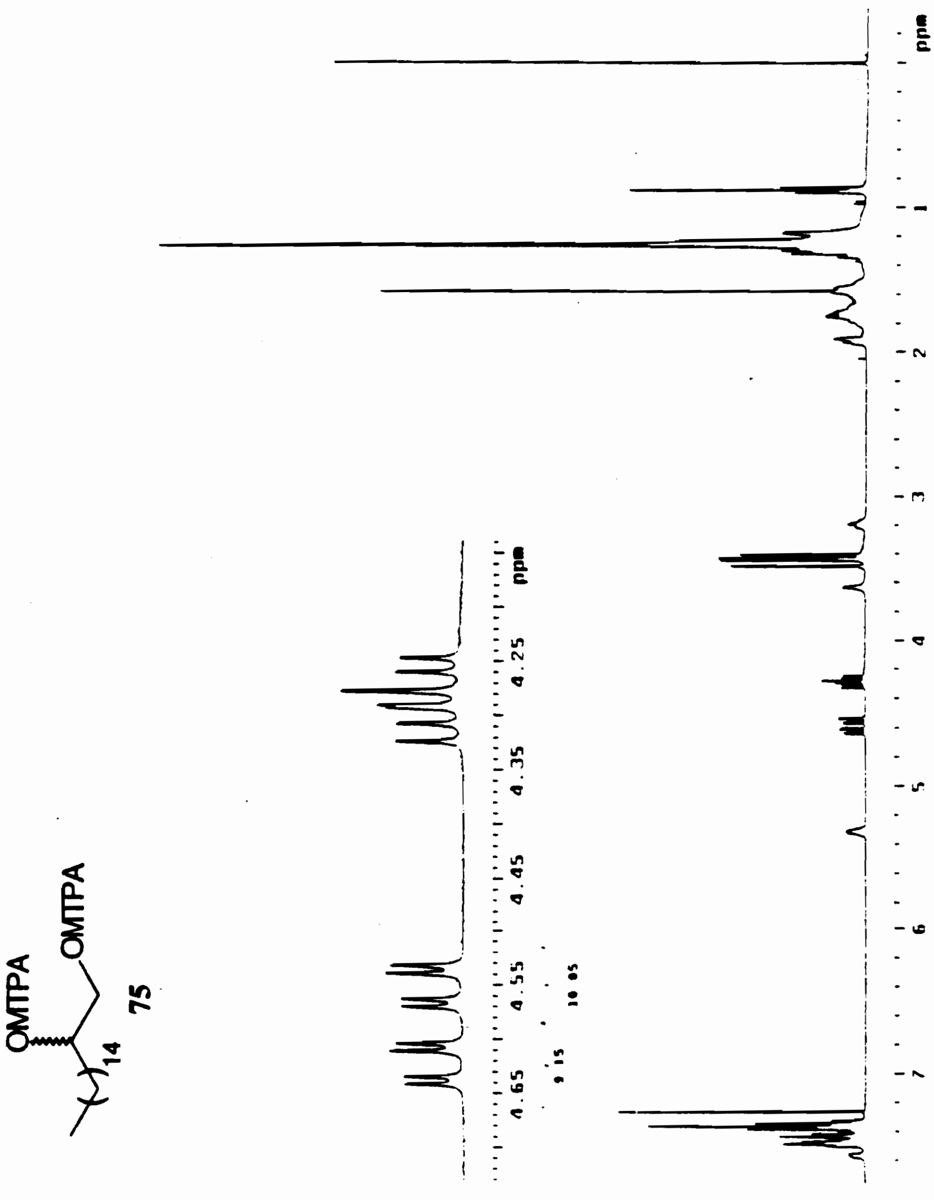
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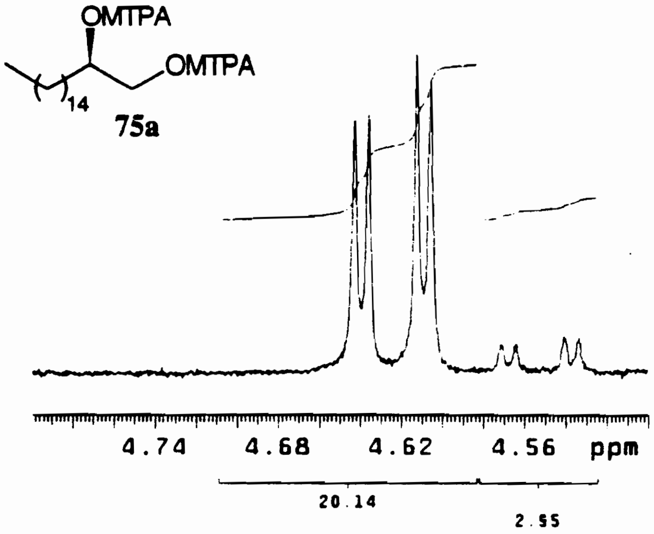
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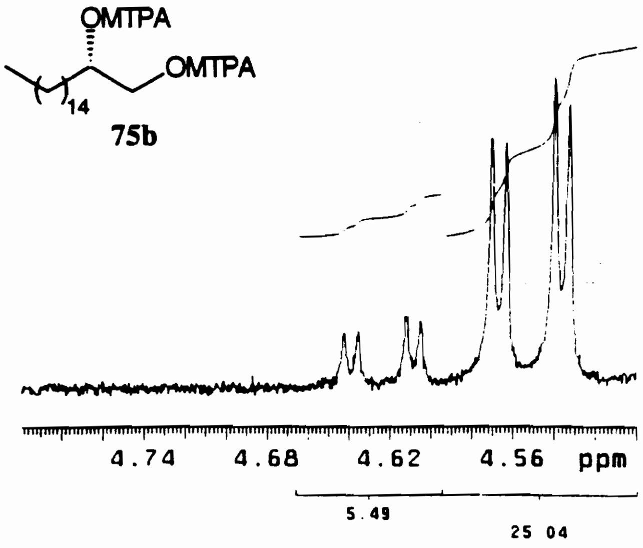
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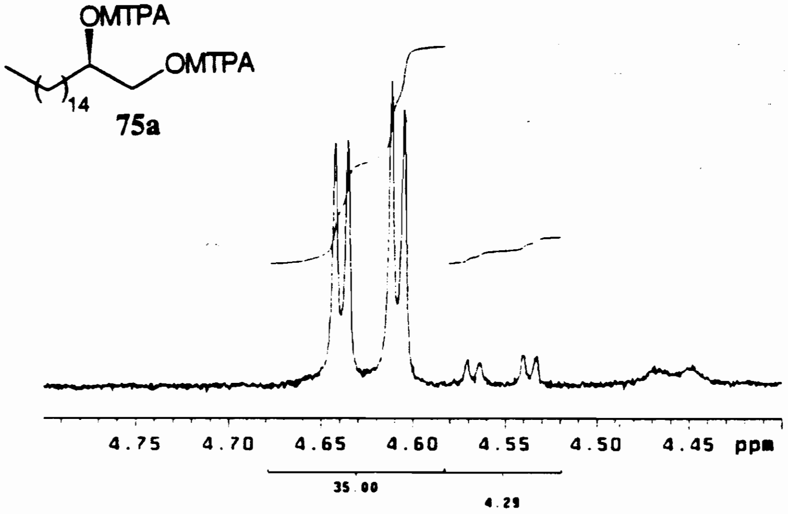

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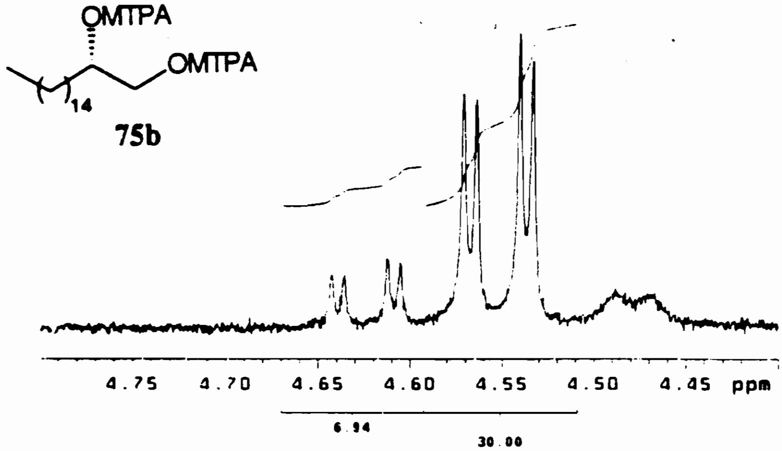

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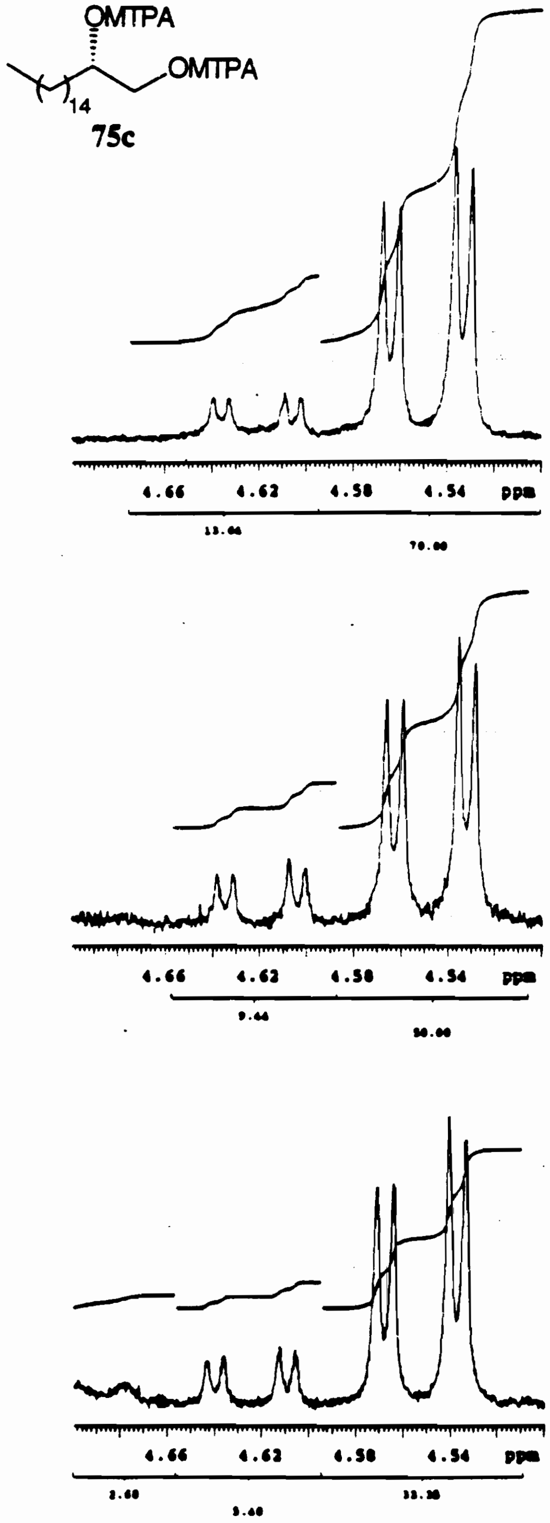
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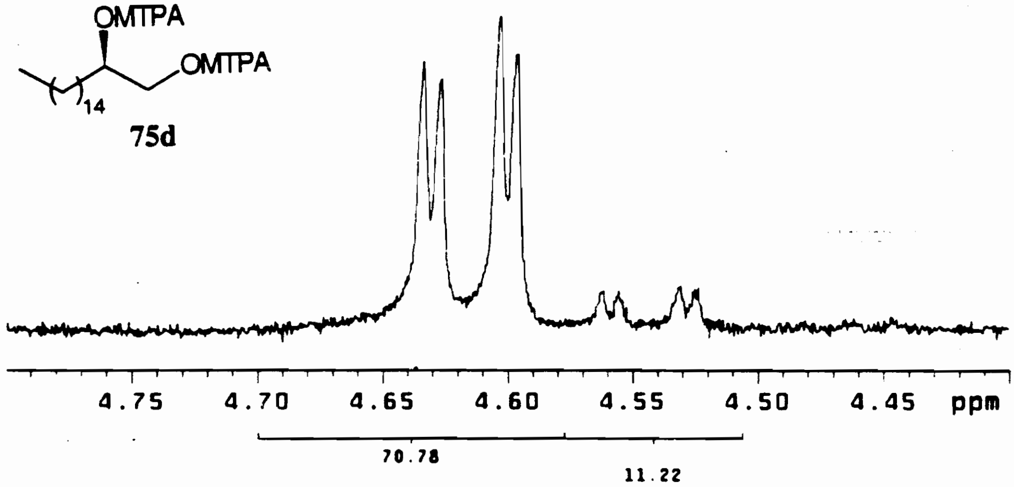
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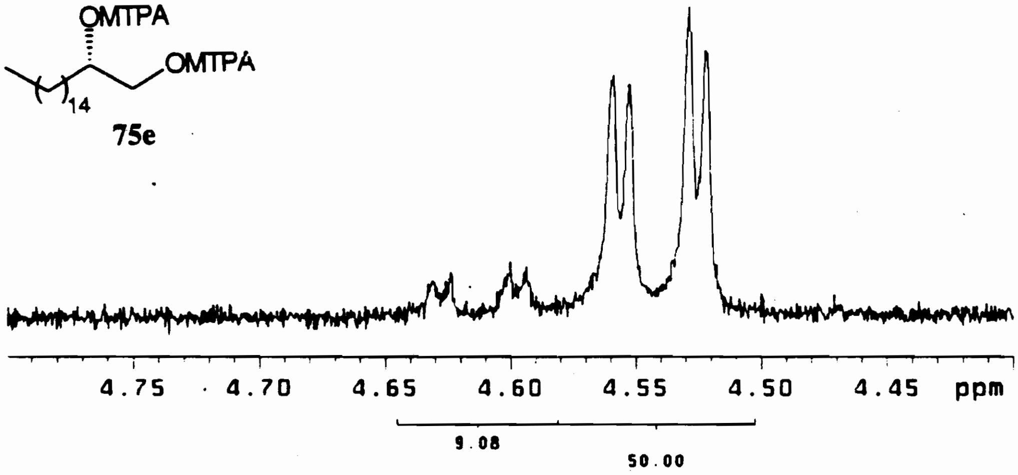
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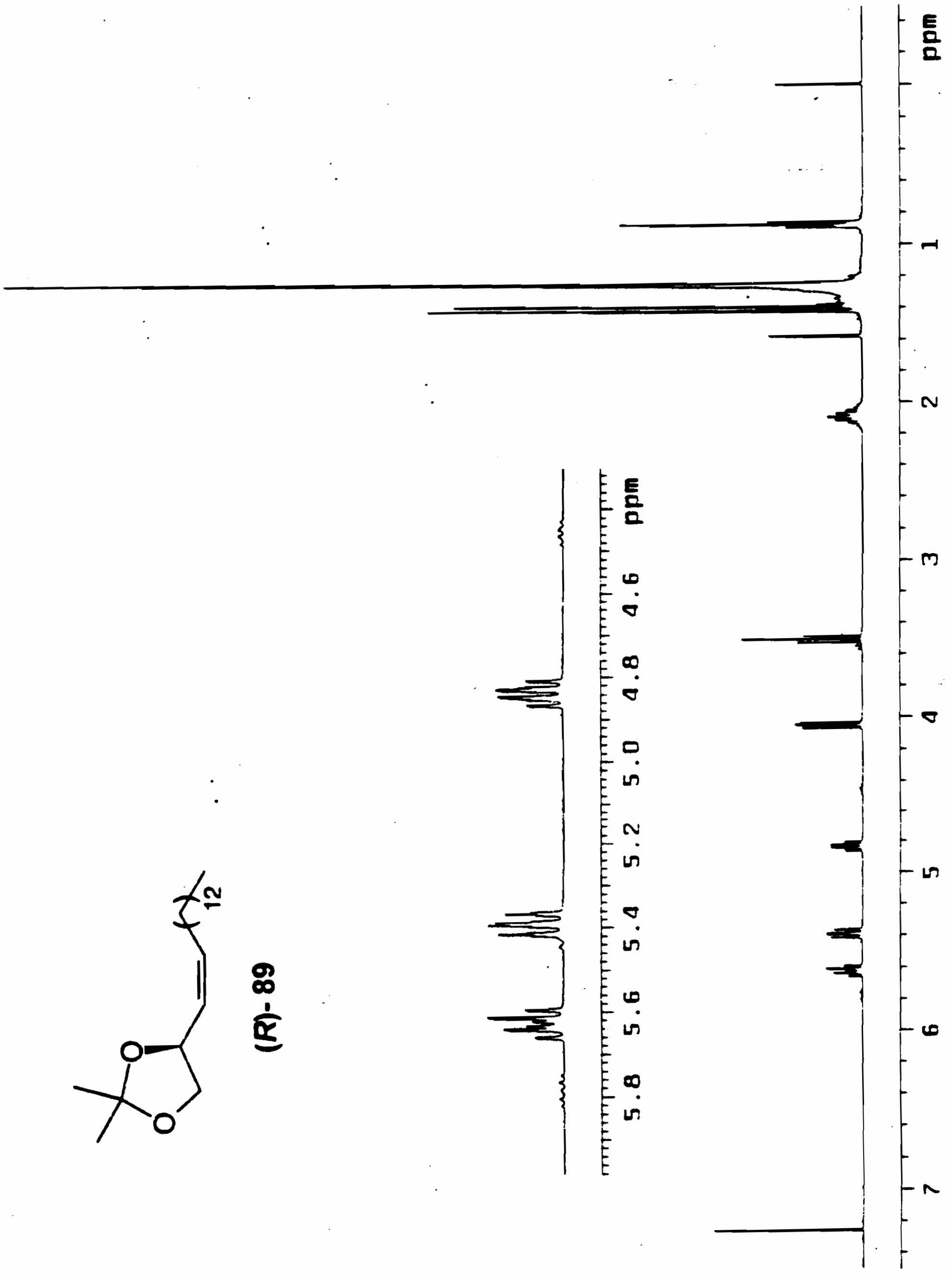

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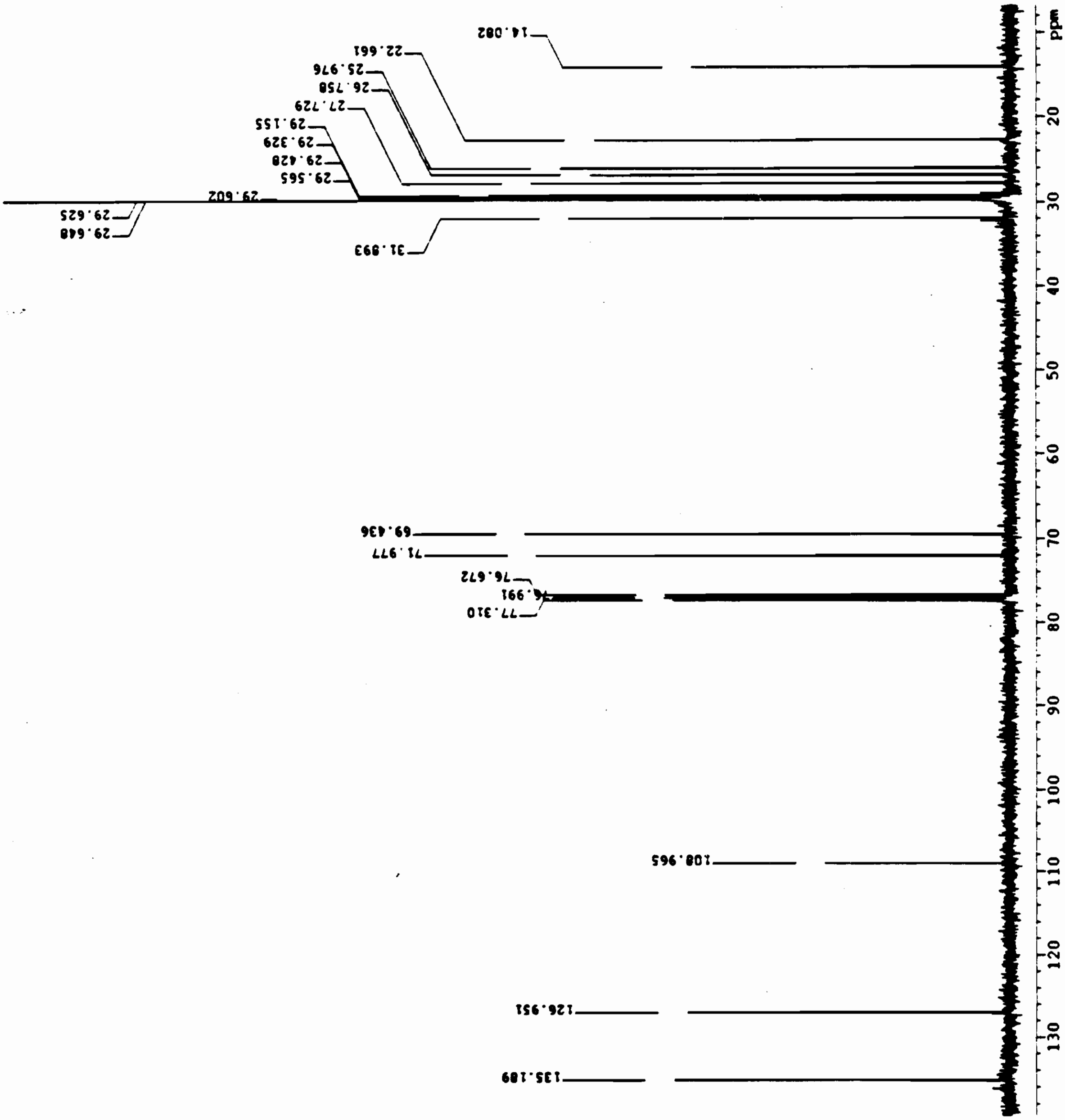
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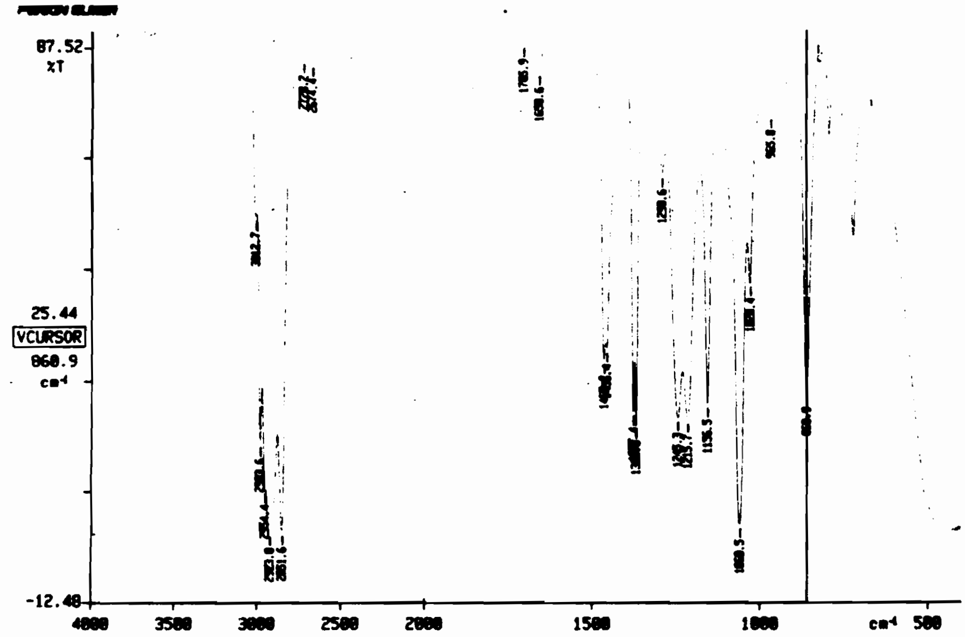

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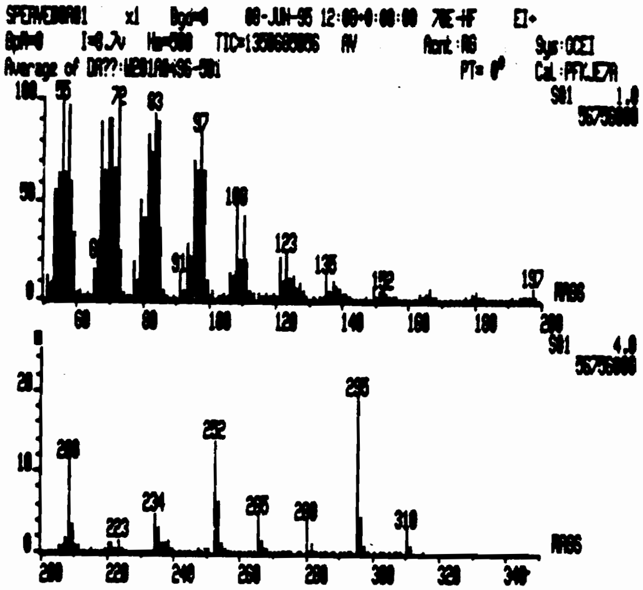

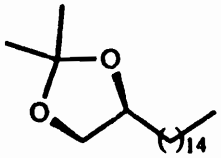

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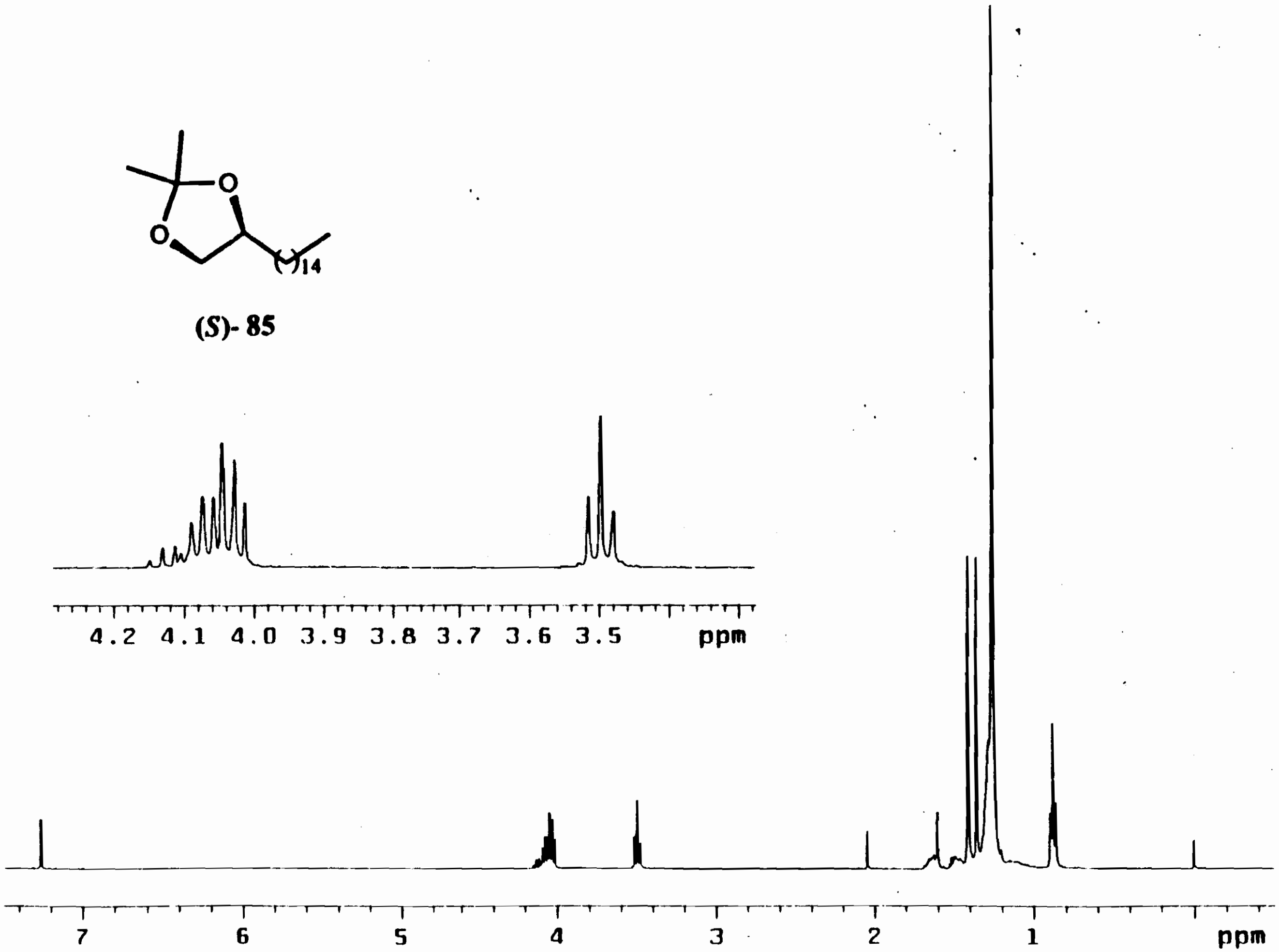

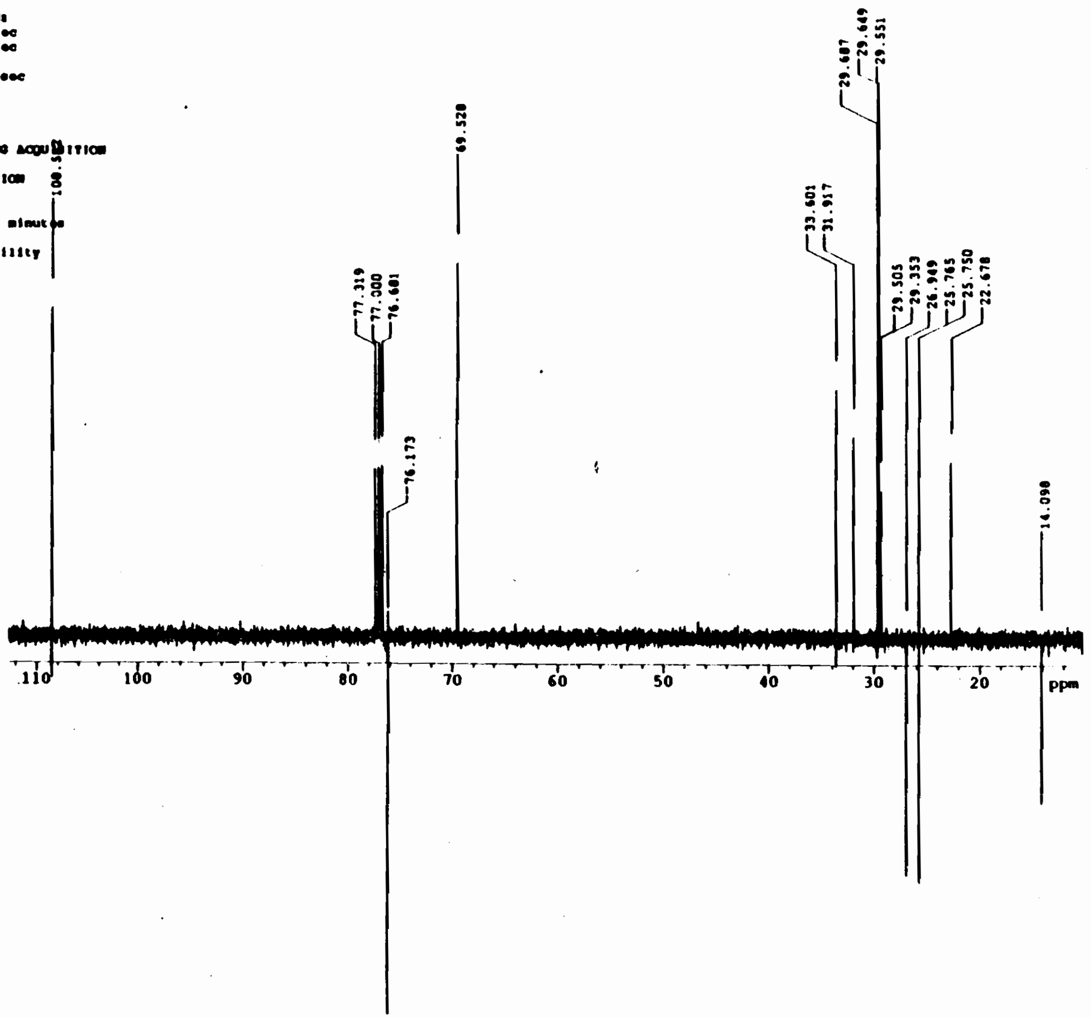

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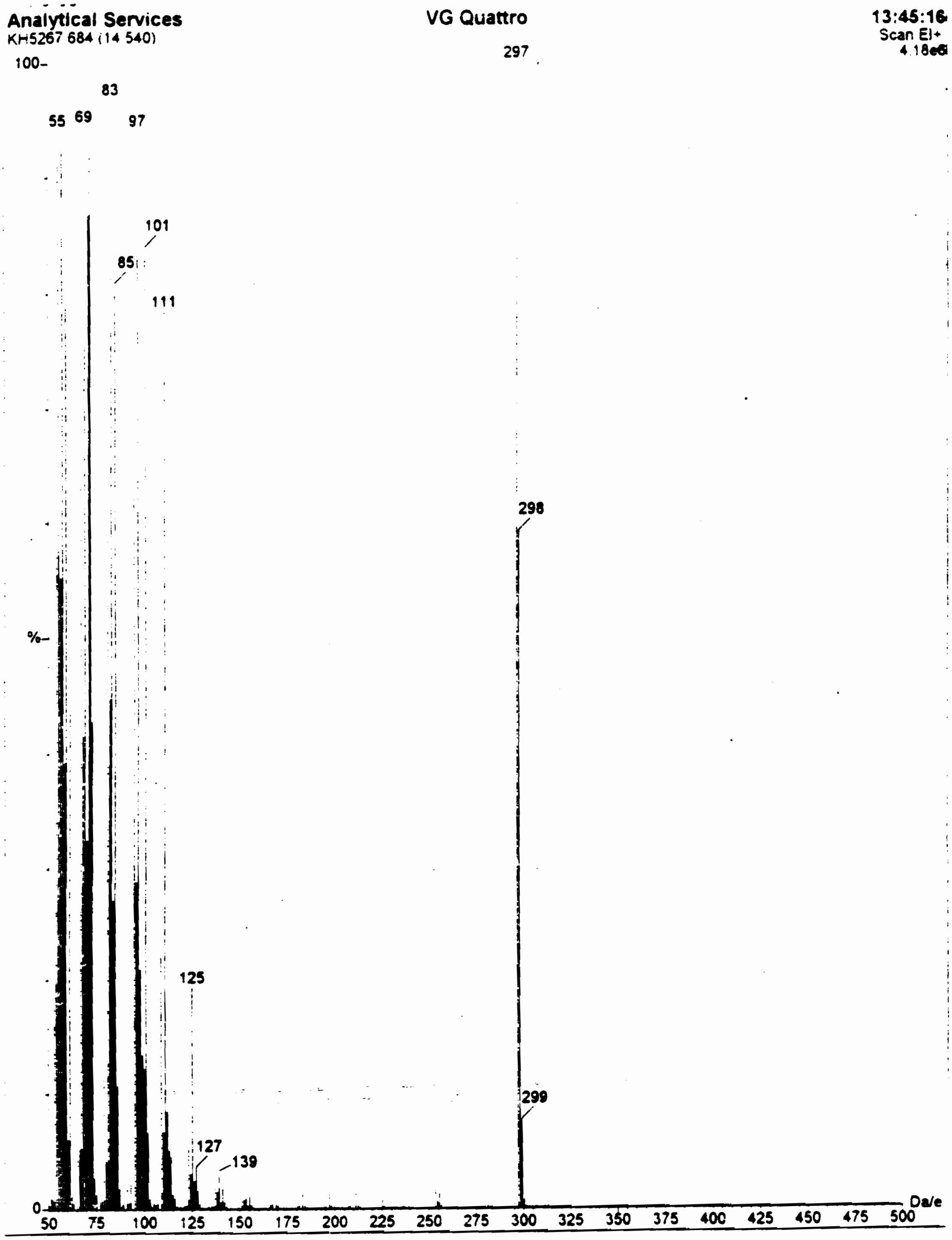
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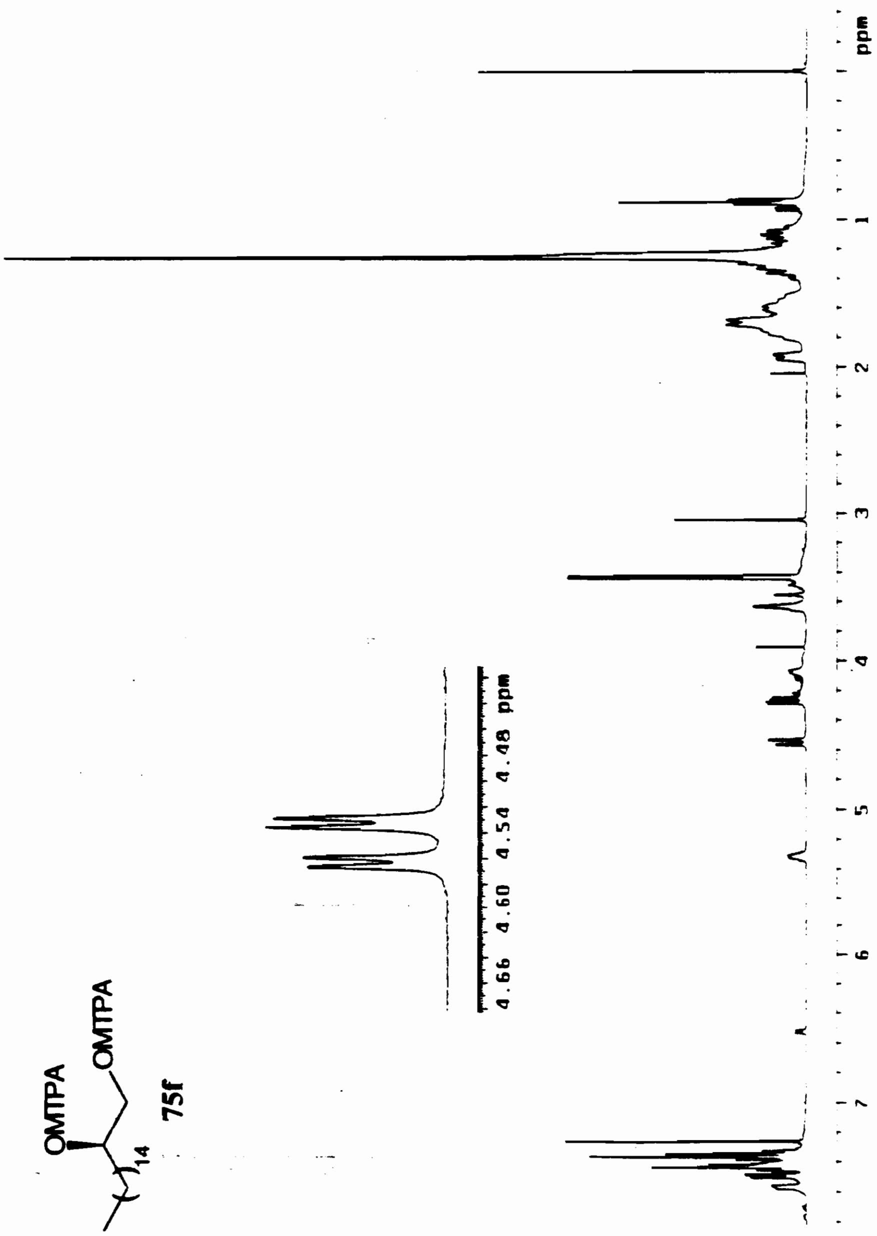

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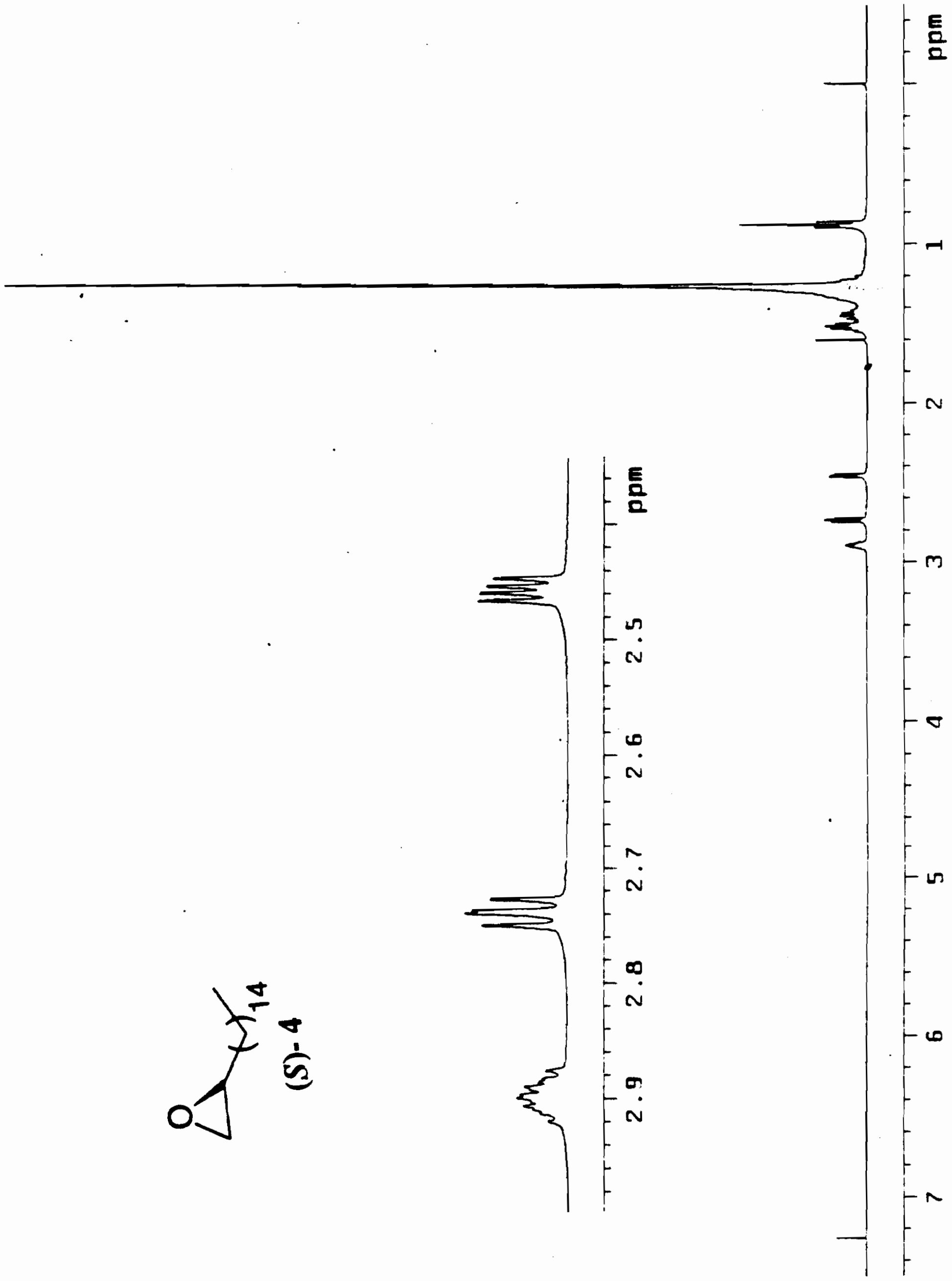

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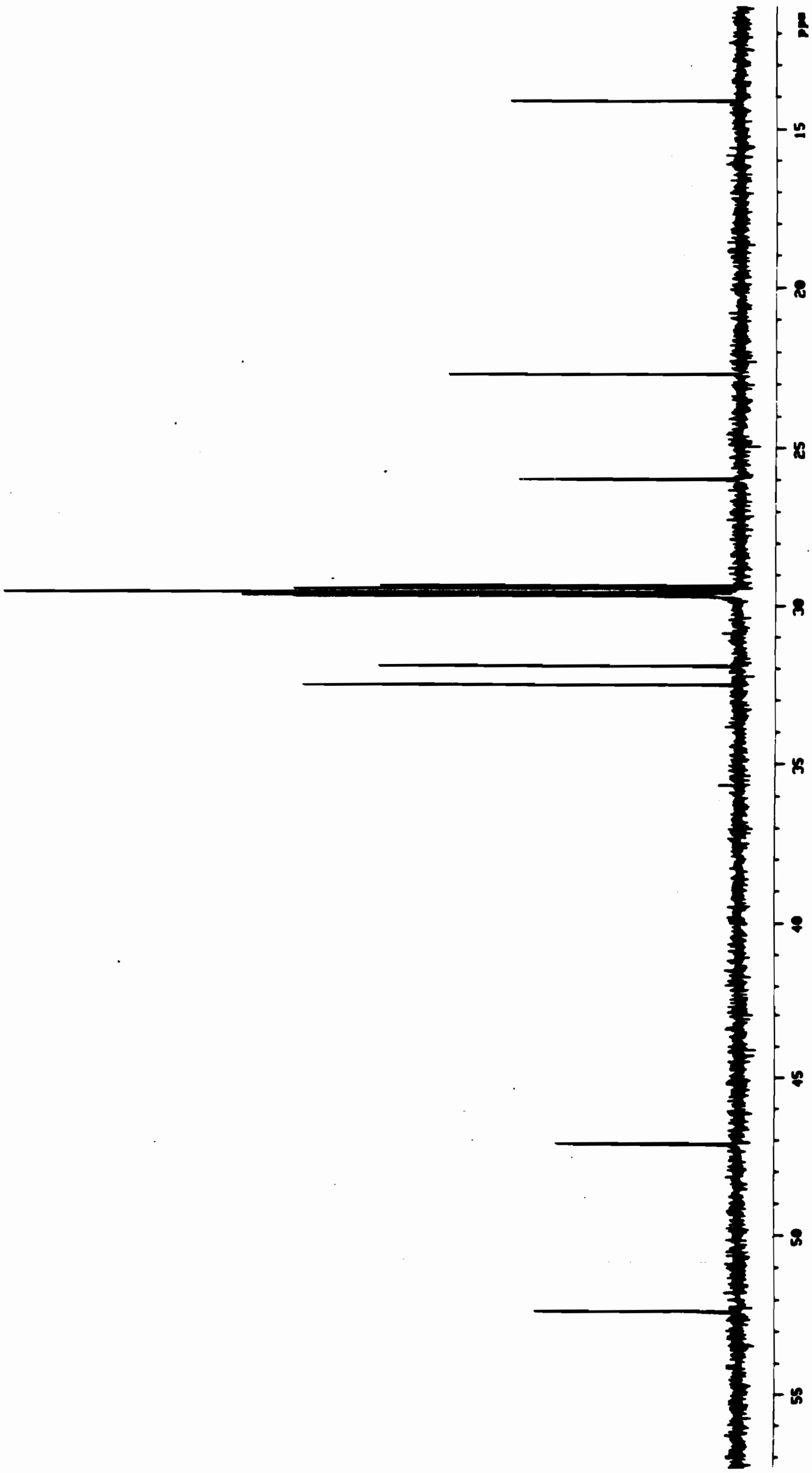

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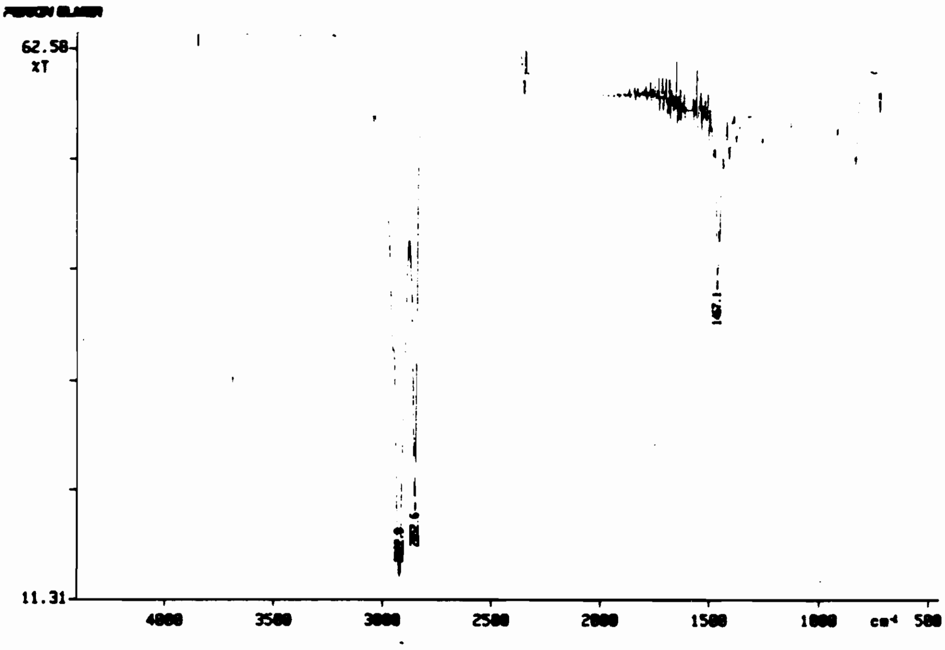
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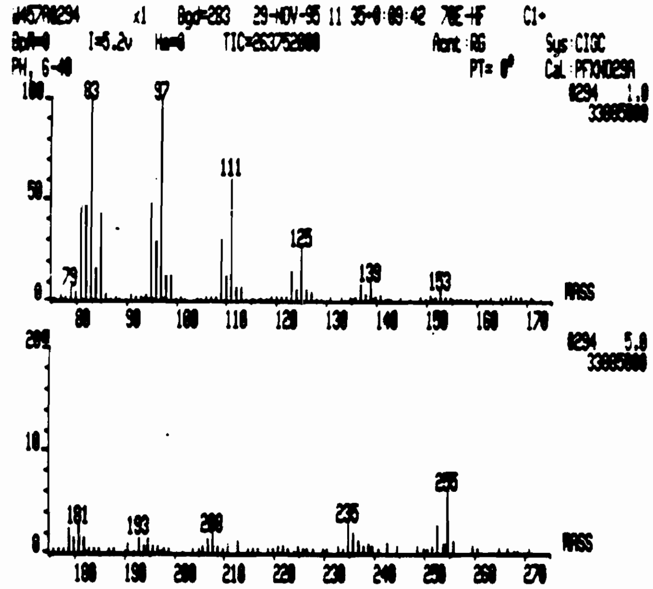
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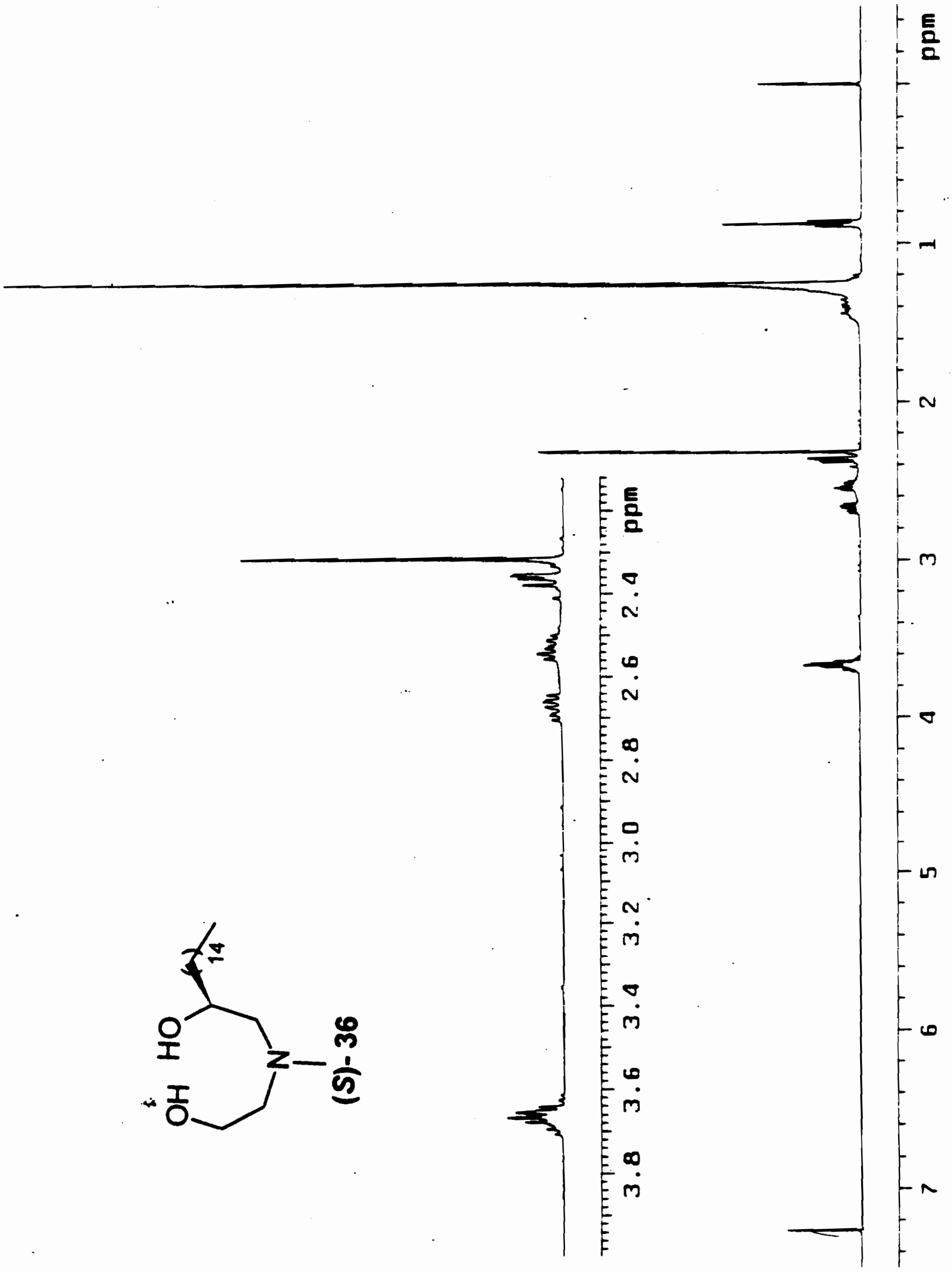
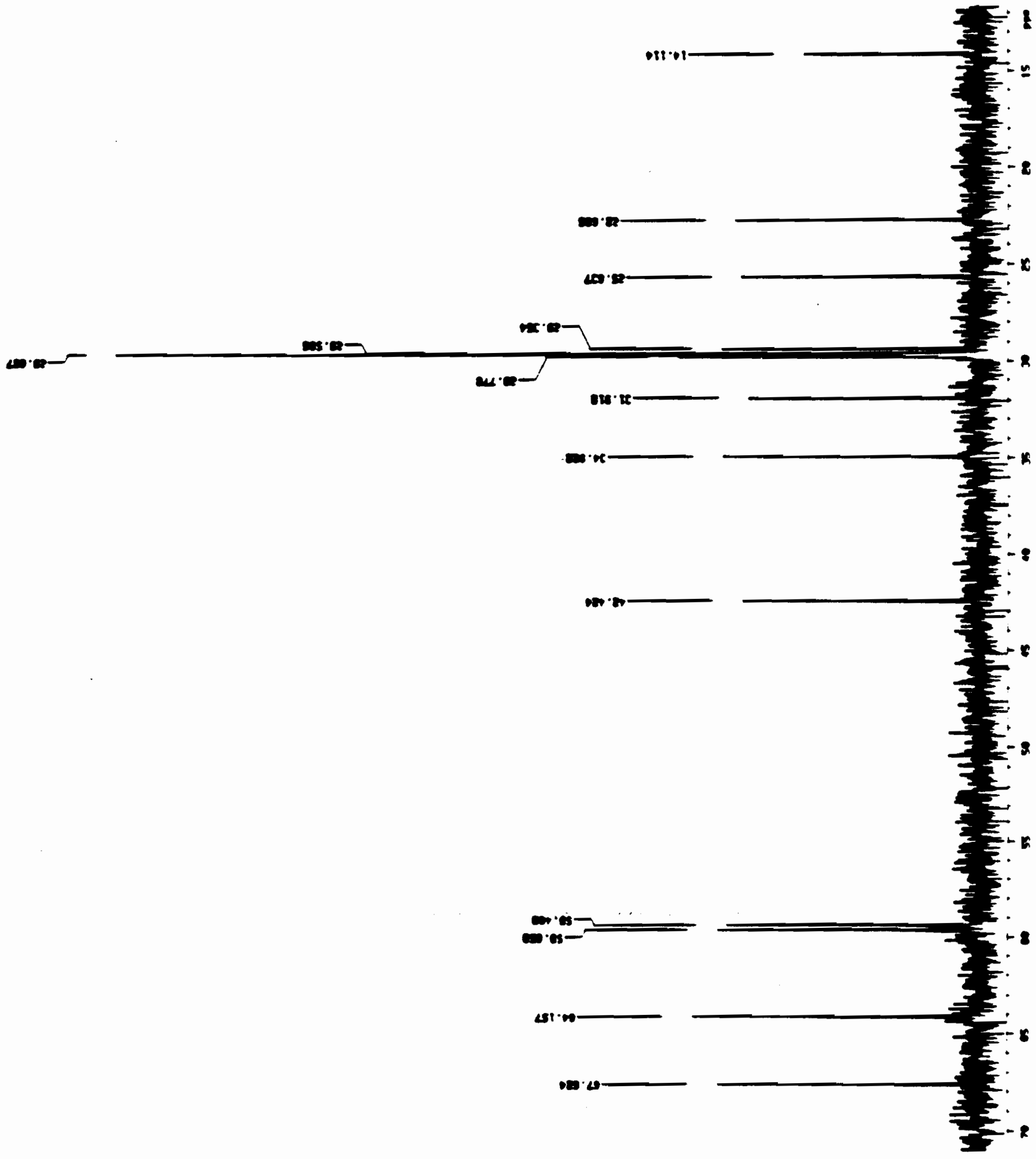
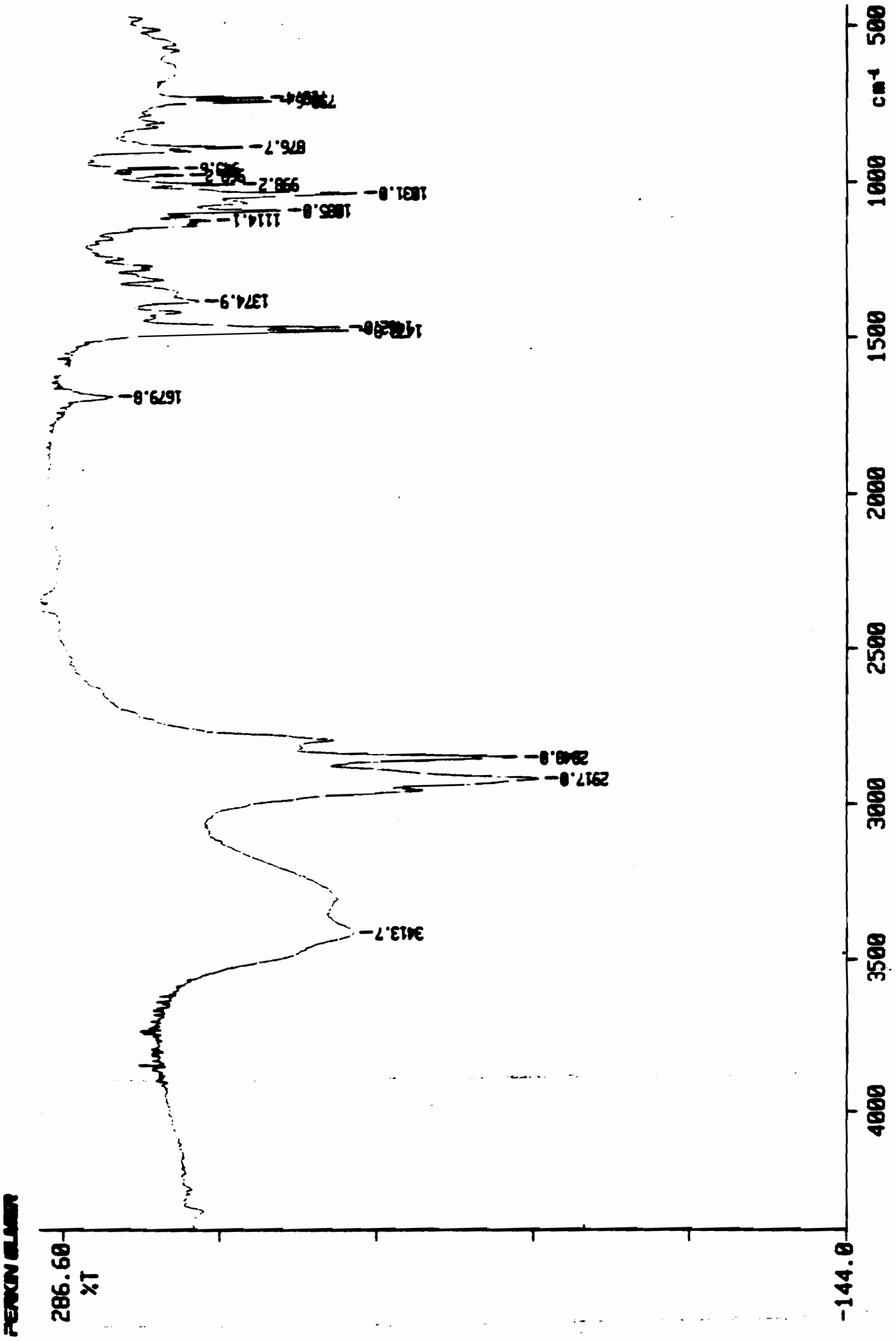
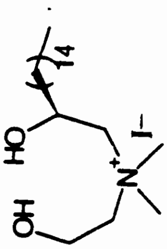
190
200
210
220
230
240
250
260
270



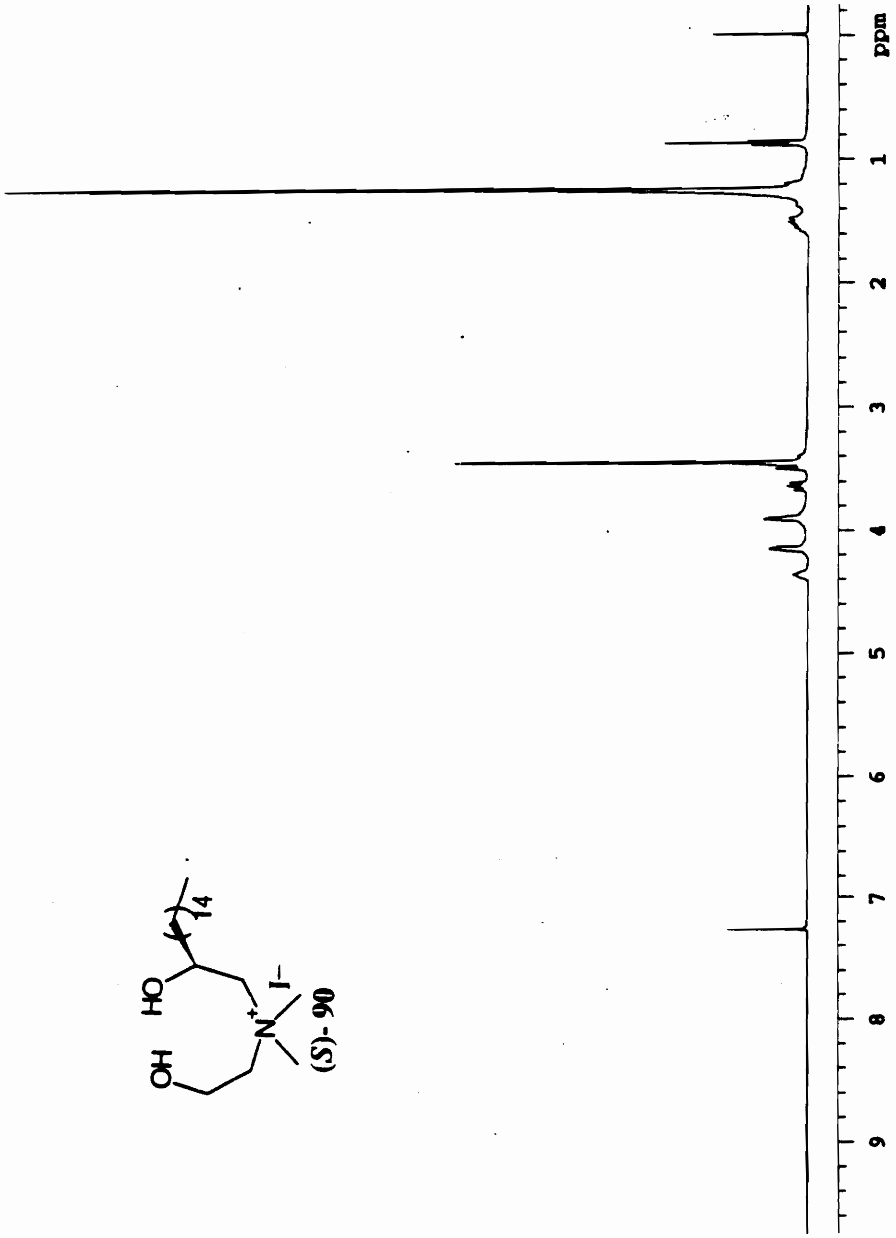
(S)-36

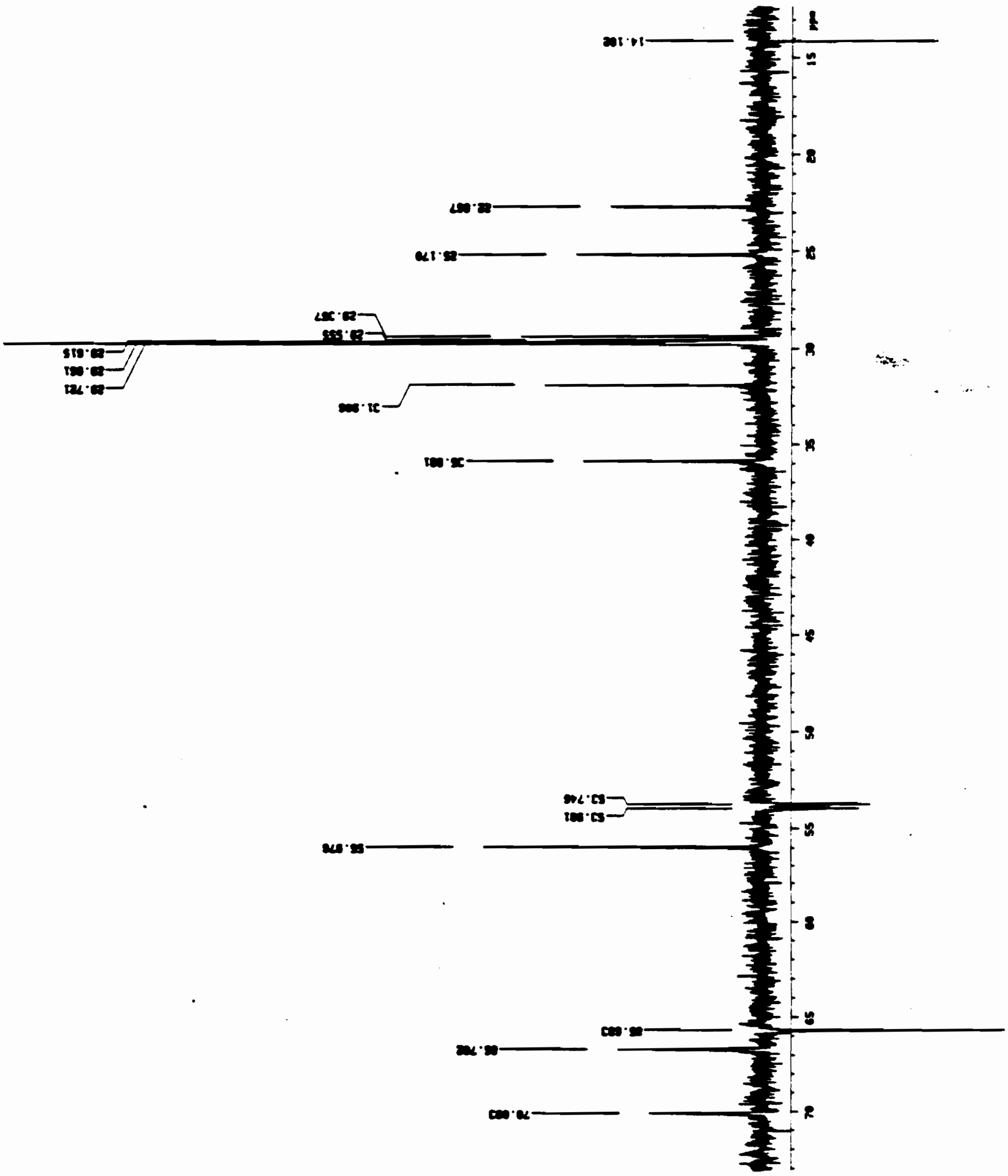

ppm
15
20
25
30
35
40
45
50
55
60
65
70
14.114
22.688
25.637
29.364
29.598
29.657
29.778
31.918
34.088
42.484
58.408
58.688
64.157
67.024

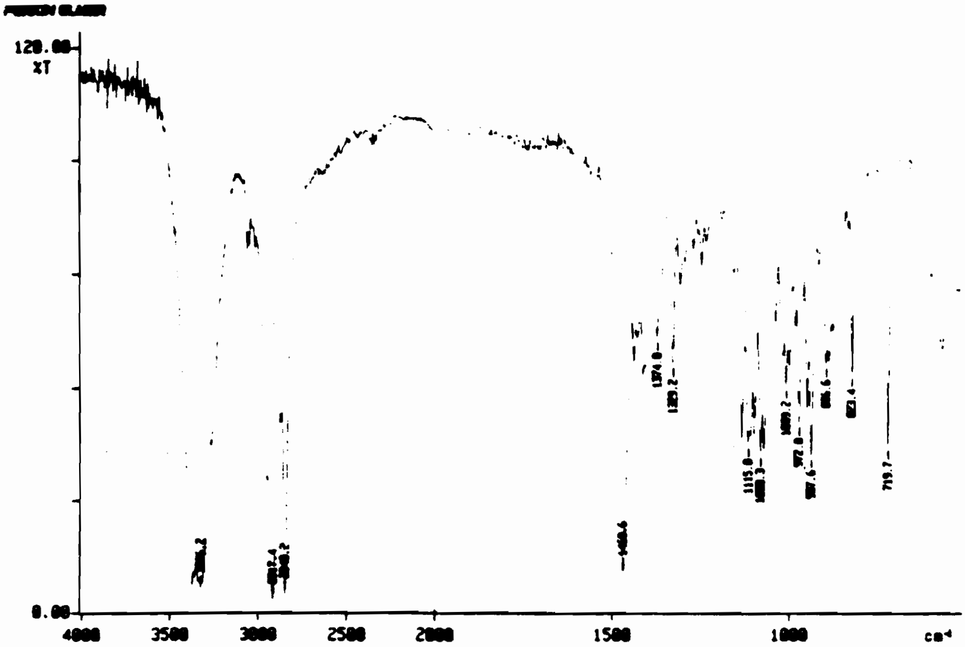

PERKIN ELMER
286.60
%T
-144.0
4000
3500
3000
2500
2000
1500
1000
500
cm-1
3413.7
2917.0
2848.0
1679.0
1374.9
1114.1
1085.0
1031.0
998.2
876.7



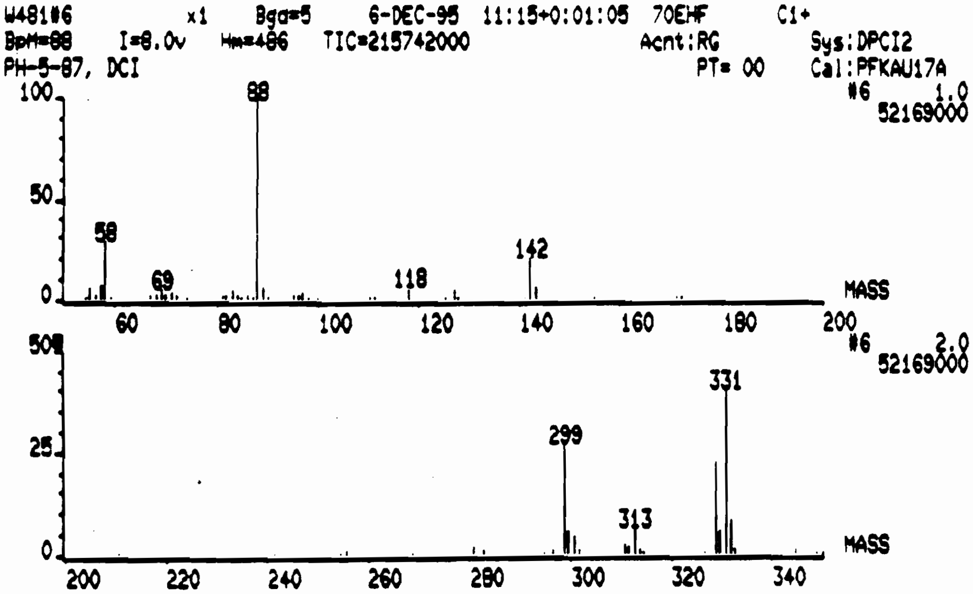
(S)-90

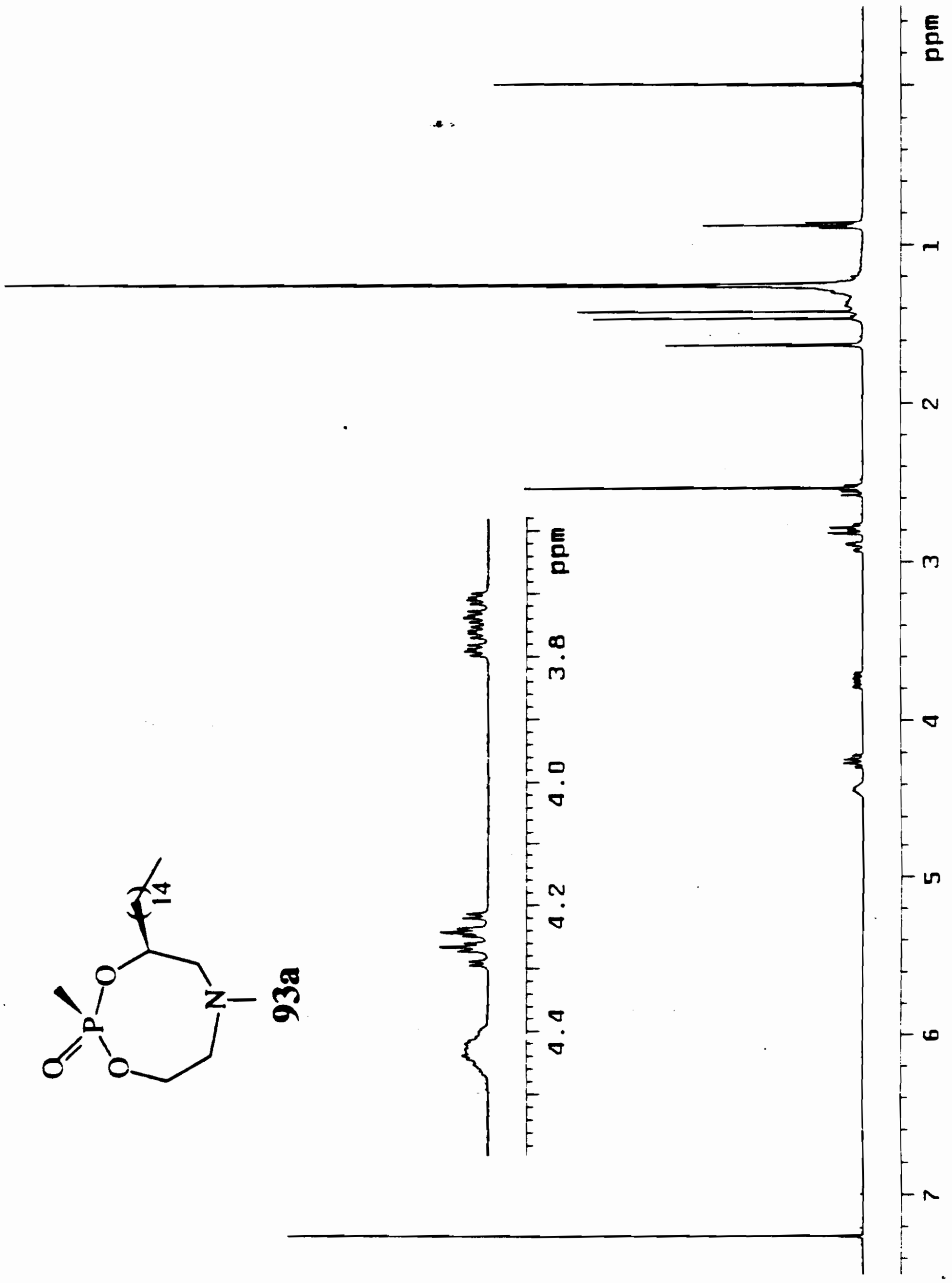



ppm
15
20
25
30
35
40
45
50
55
60
65
70
14.108
22.687
25.170
29.367
29.555
29.615
29.661
29.721
31.906
35.881
53.746
53.881
55.876
65.883
66.798
70.083

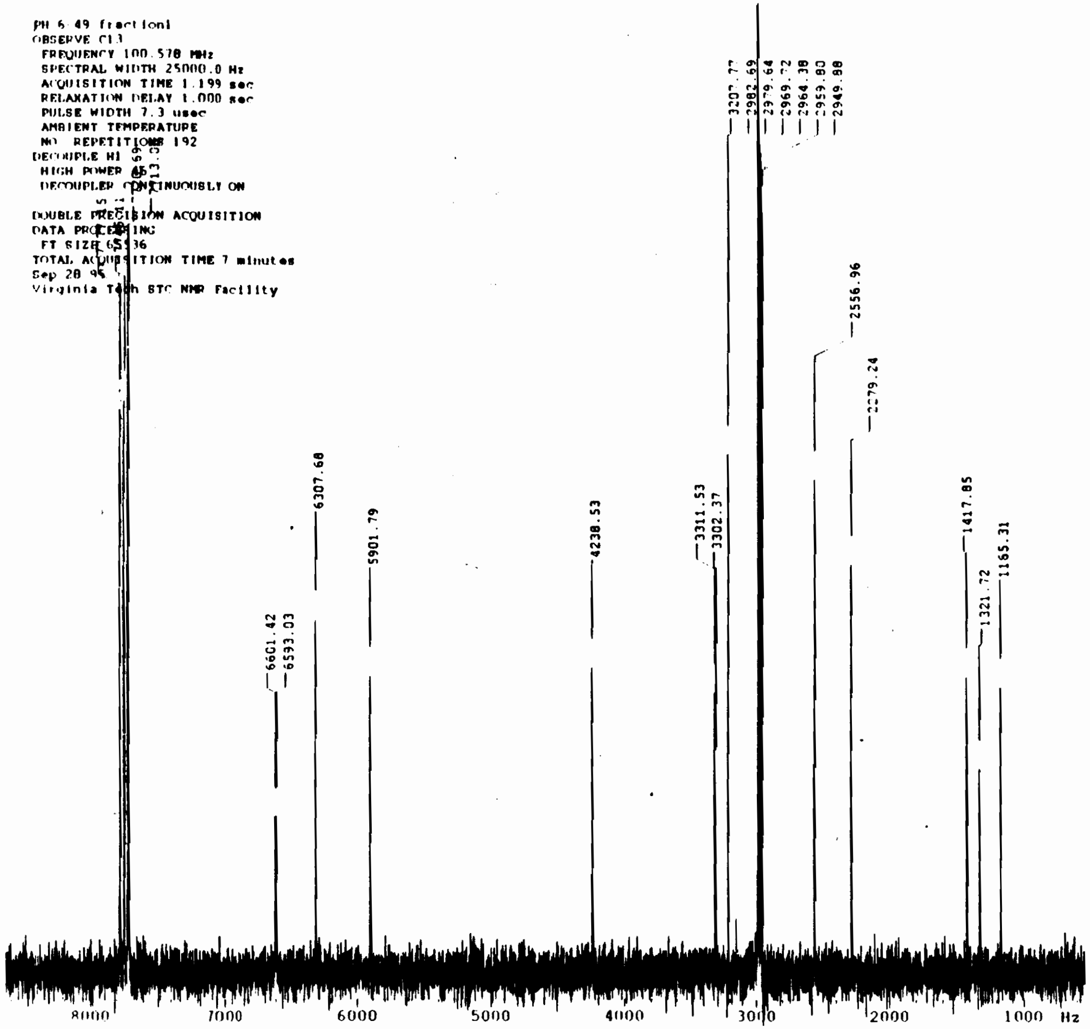


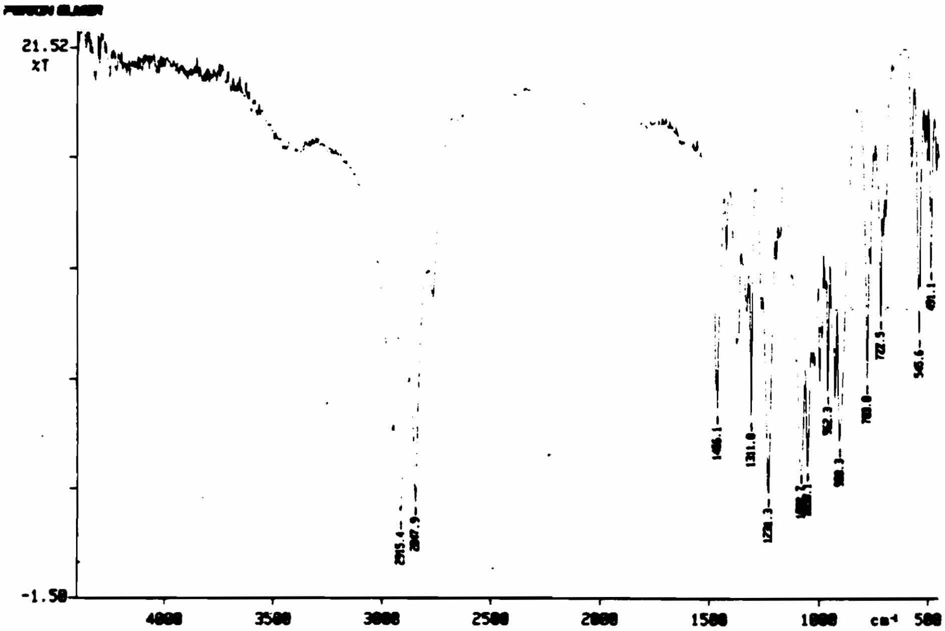
95/10/25 15:30
X: 1 scan, 4.0cm-1

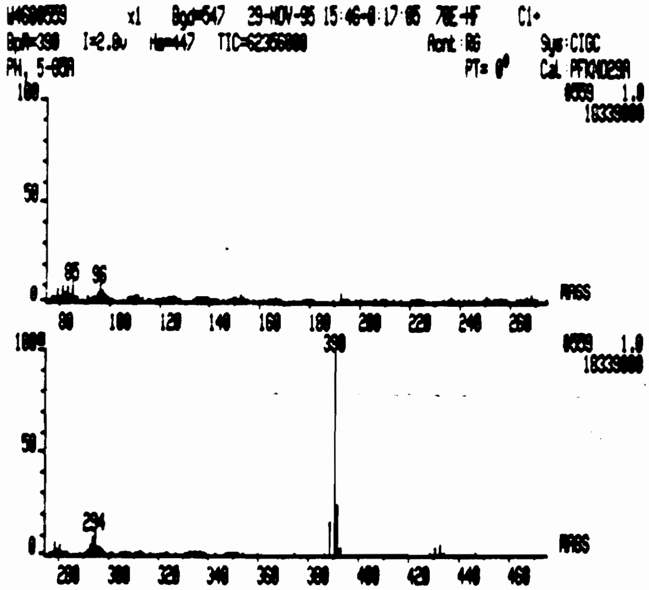

```
W481#6           x1    Bga=5     6-DEC-95  11:15+0:01:05  70EHF       C1+
BpM=88    I=8.0v   Hm=486   TIC=215742000                 Acnt:RG          Sys:DPCI2
PH-5-87, DCI                                                PT= 00    Cal:PFKAU17A
```

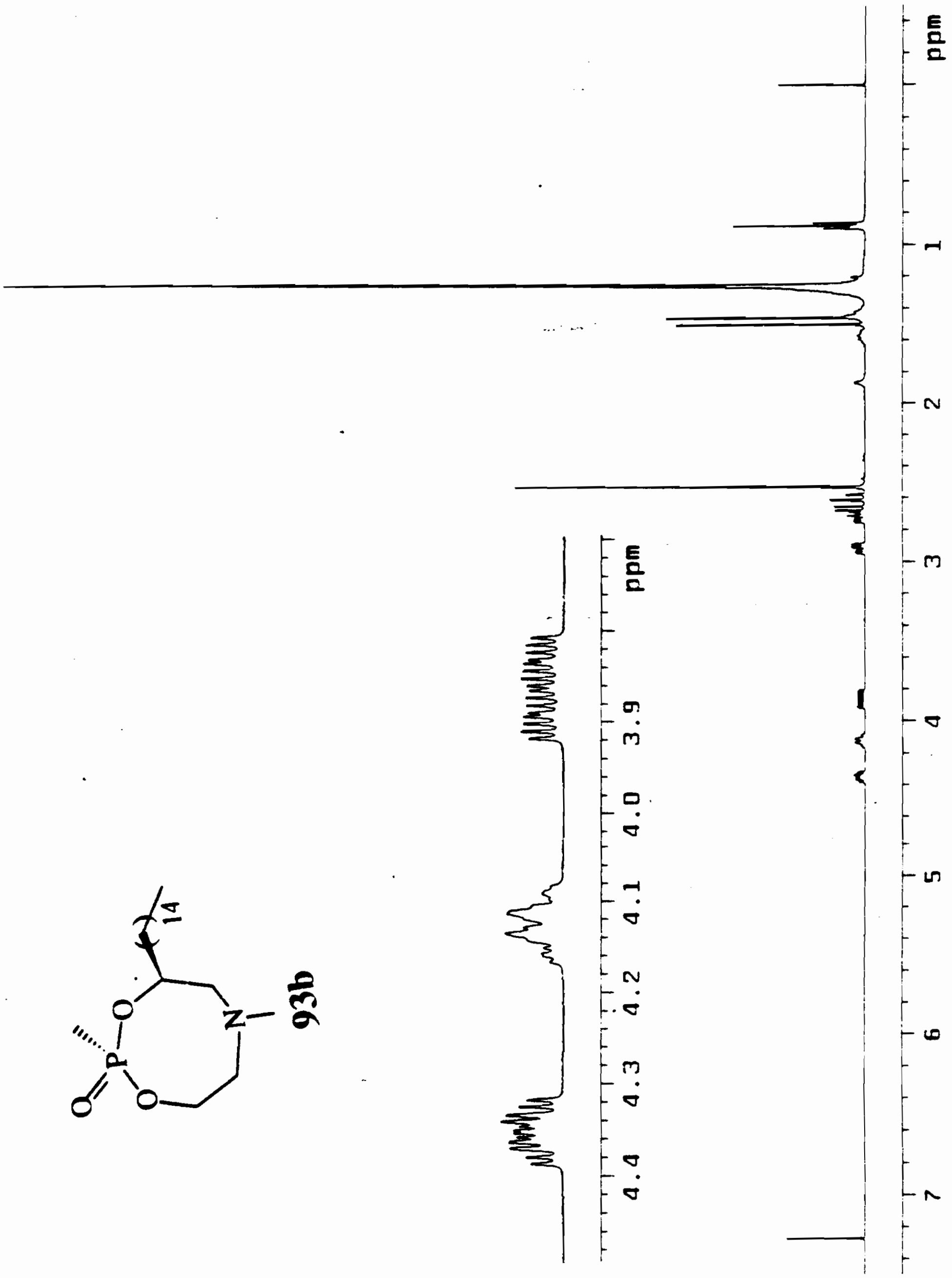


93a

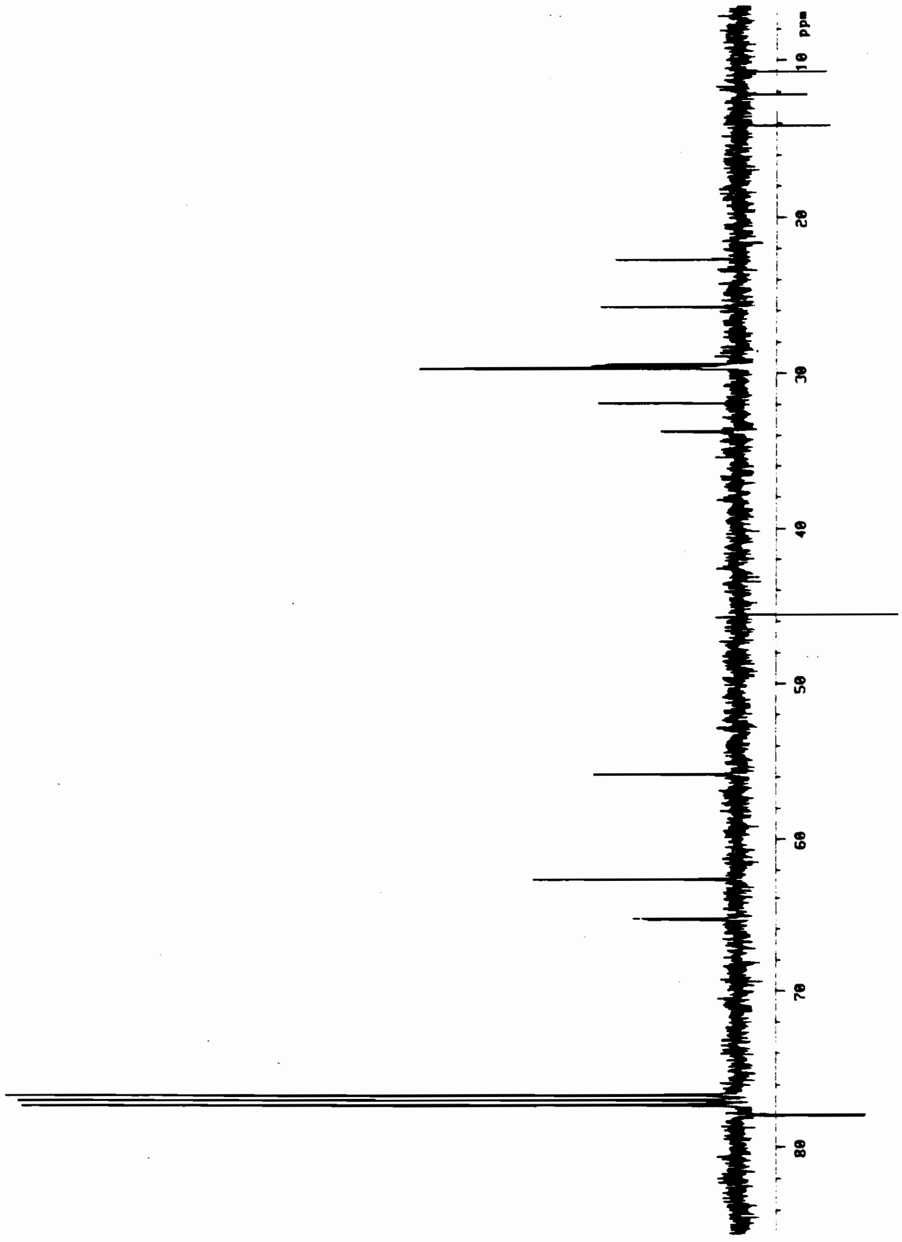

PH 6.49 fraction1
OBSERVE C13
FREQUENCY 100.578 MHz
SPECTRAL WIDTH 25000.0 Hz
ACQUISITION TIME 1.199 sec
RELAXATION DELAY 1.000 sec
PULSE WIDTH 7.3 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 192
DECOUPLE H1
HIGH POWER
DECOUPLER CONTINUOUSLY ON
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
FT SIZE 65536
TOTAL ACQUISITION TIME 7 minutes
Sep 28 95
Virginia Tech STC NMR Facility
6601.42
6593.03
6307.68
5901.79
4238.53
3311.53
3302.37
3207.77
2982.69
2979.64
2969.72
2964.38
2959.80
2949.88
2556.96
2279.24
1417.85
1321.72
1165.31
8000
7000
6000
5000
4000
3000
2000
1000
Hz

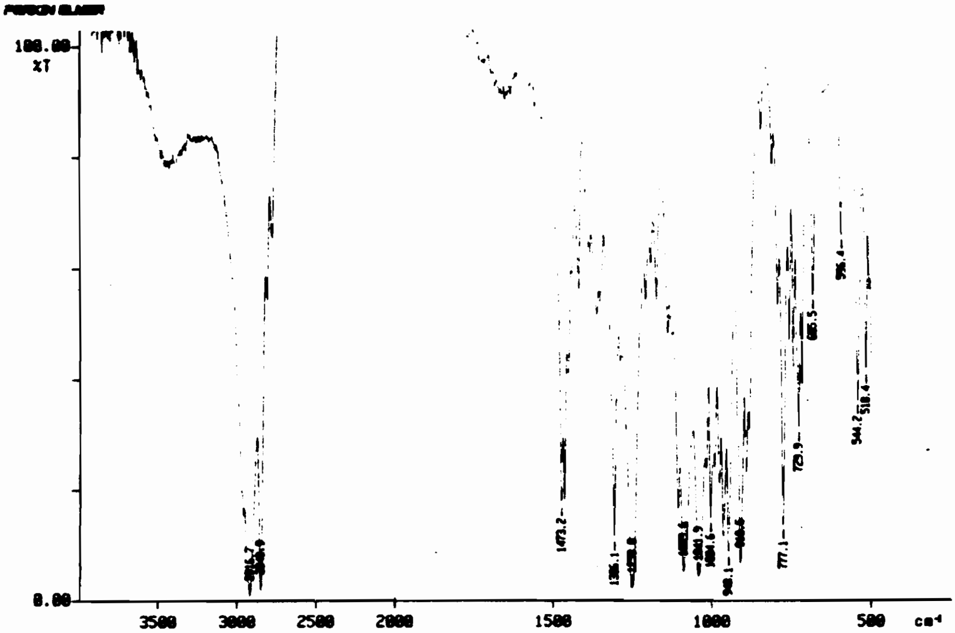

PERKIN ELMER
21.52
%T
-1.50
2915.4
2847.9
1466.1
1311.0
1231.3
1060.1
962.3
908.3
788.0
722.5
545.6
491.1
4000
3500
3000
2500
2000
1500
1000
cm-1
500


W4G00559 x1 Bgd=547 29-NOV-95 15:46+0:17:05 70E+HF CI+
BpM=390 I=2.0v Hm=447 TIC=62366000 Acnt:RG Sys:CIGC
PH, 5-60A PT= 0° Cal:PFKND29A
0559 1.0
18339000
100
50
0
85
96
MASS
80
100
120
140
160
180
200
220
240
260
100
390
0559 1.0
18339000
50
294
0
MASS
280
300
320
340
360
380
400
420
440
460

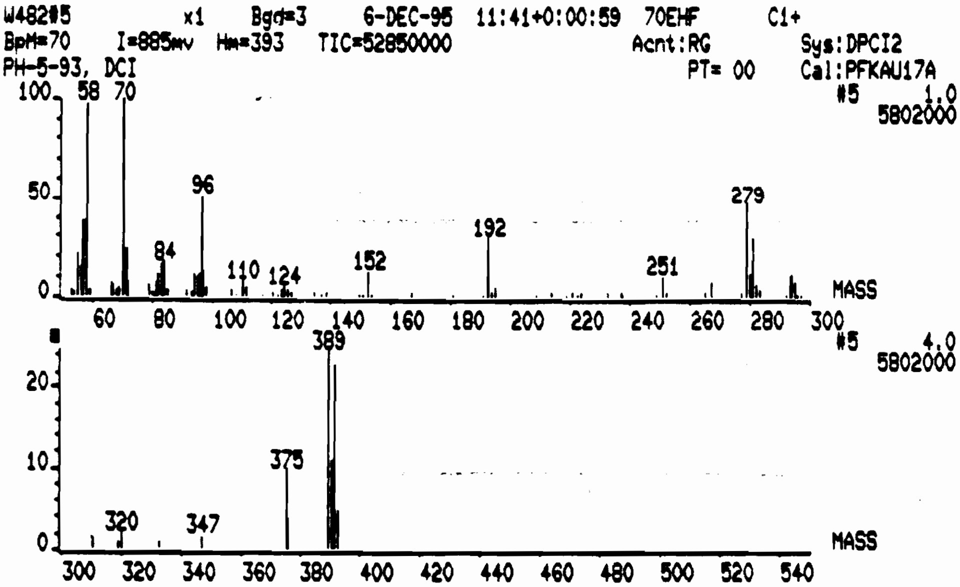


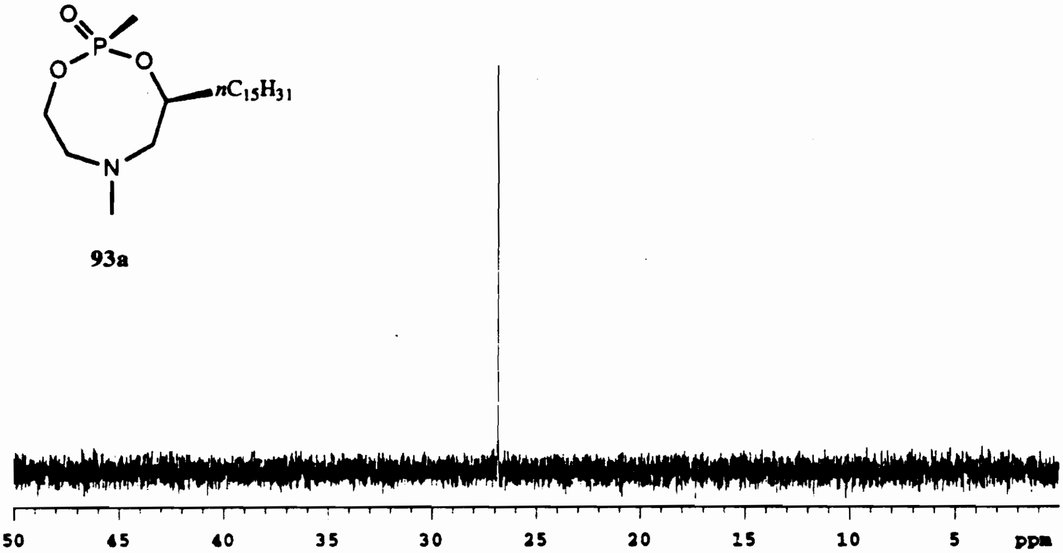
93b


ppm
10
20
30
40
50
60
70
80



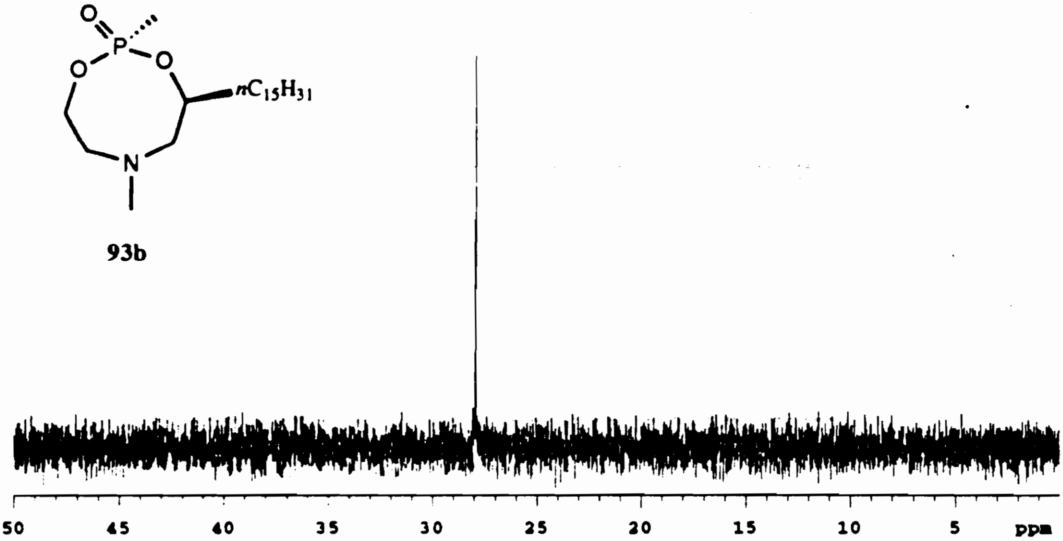
95/10/25 15:46
Y: 4 scans, 4.0cm-1





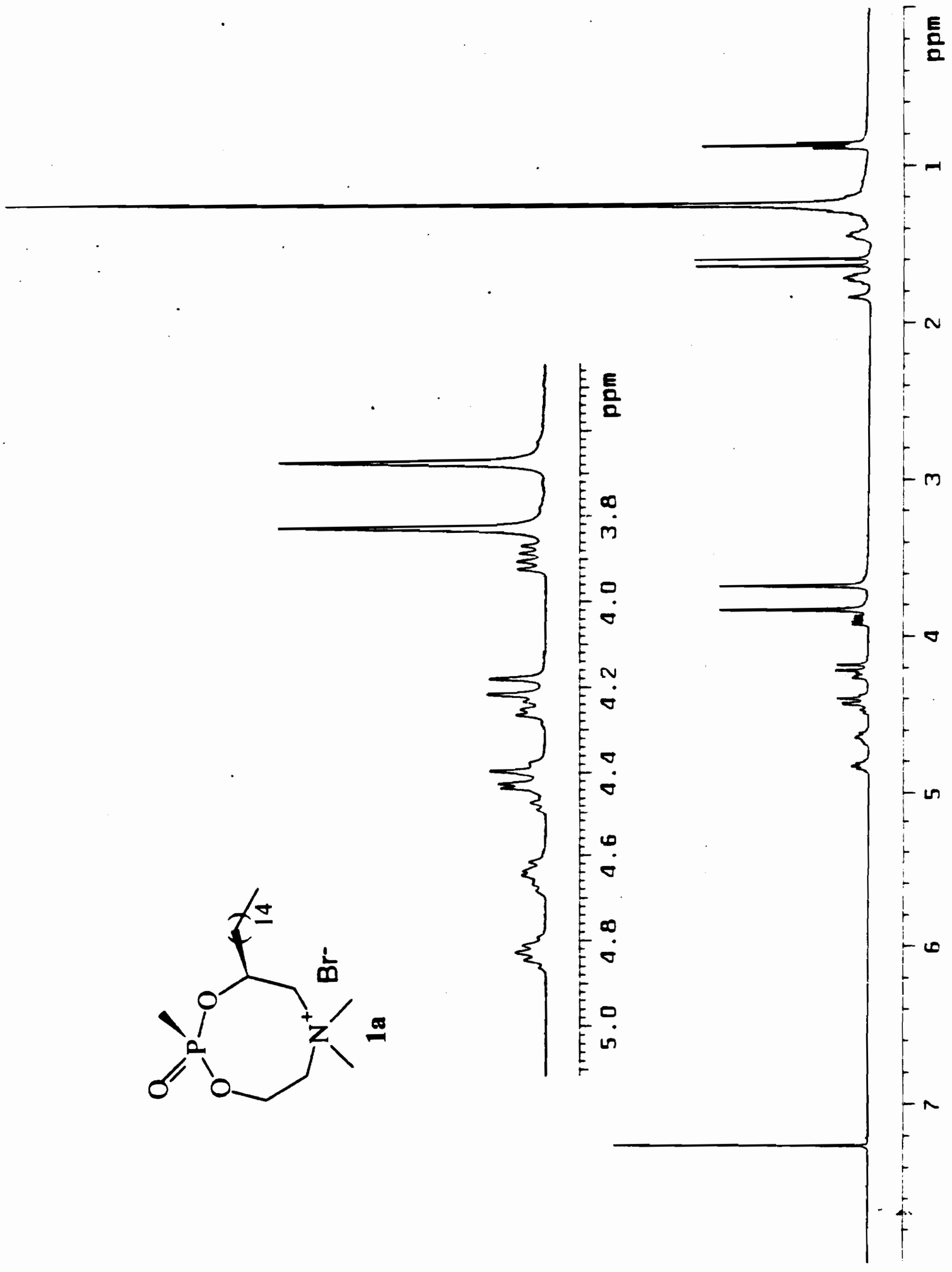
93a

nC15H31
50
45
40
35
30
25
20
15
10
5
ppm

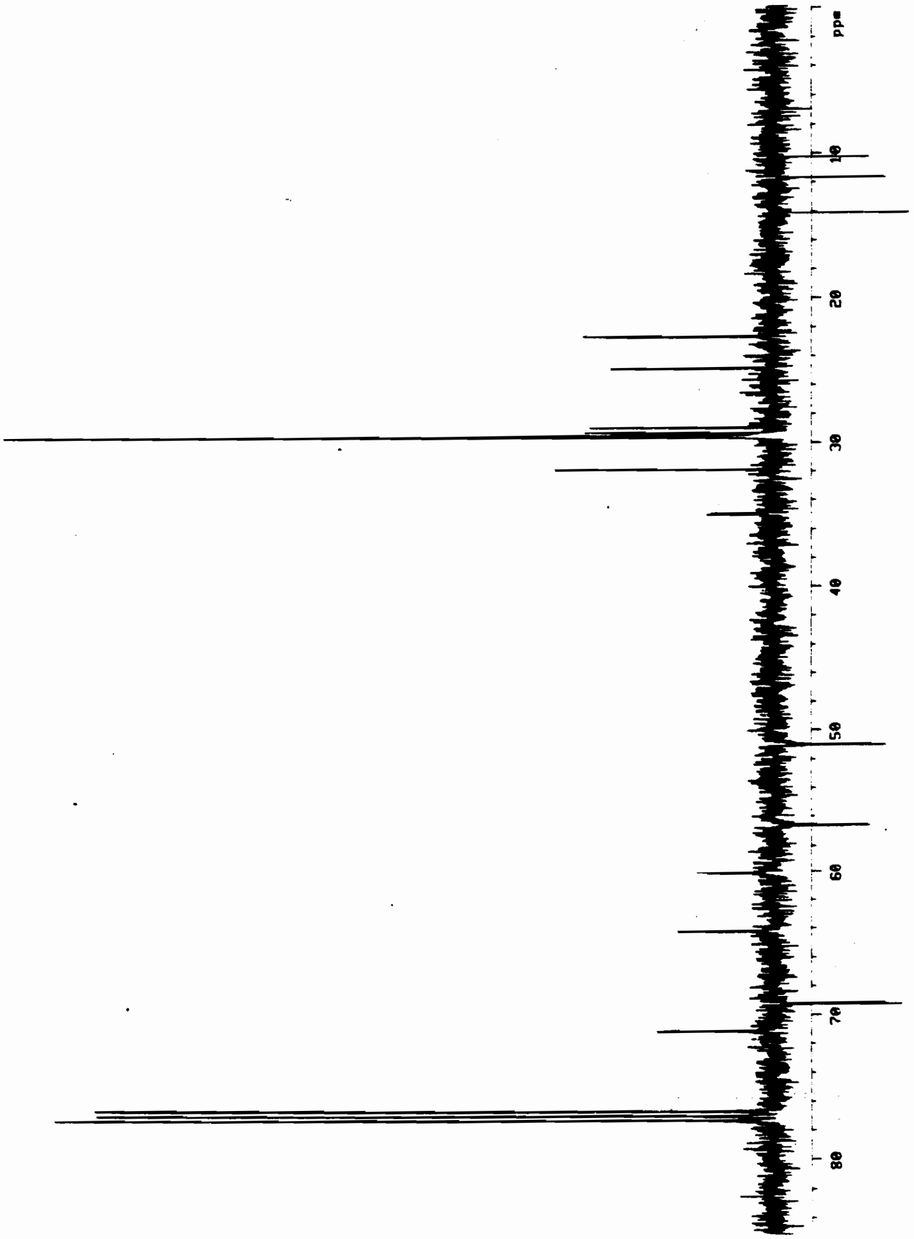


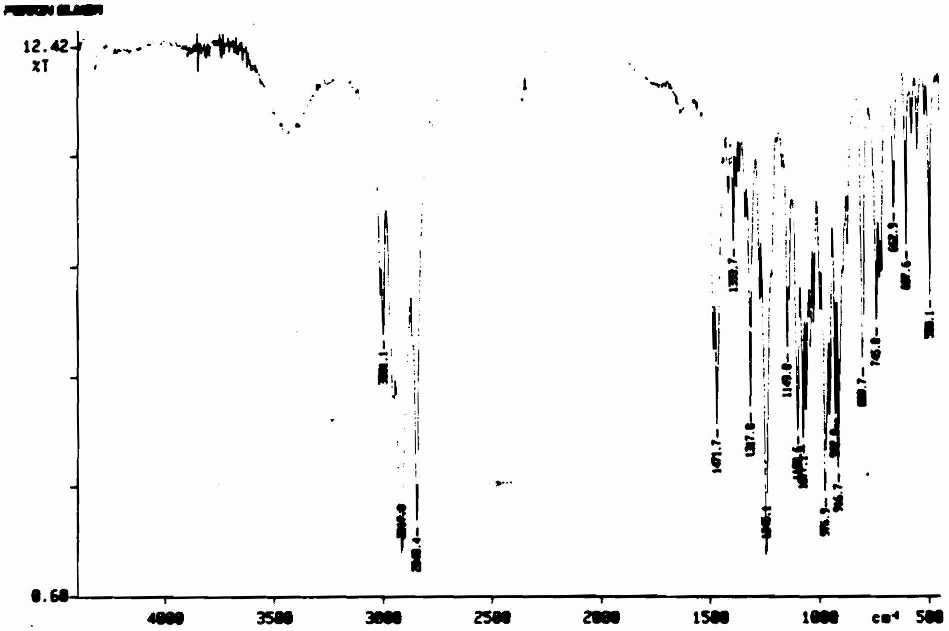
93b

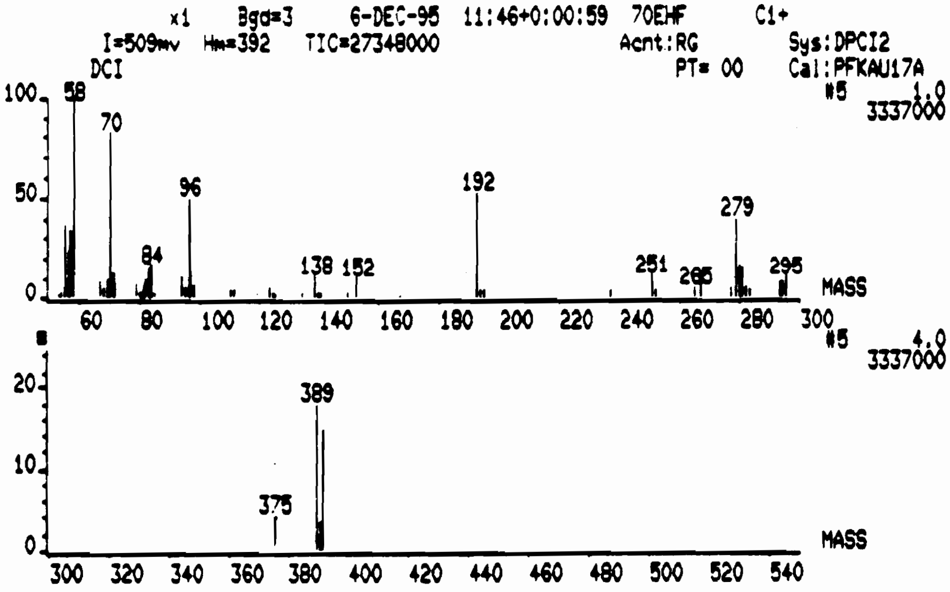
nC15H31
50
45
40
35
30
25
20
15
10
5
ppm

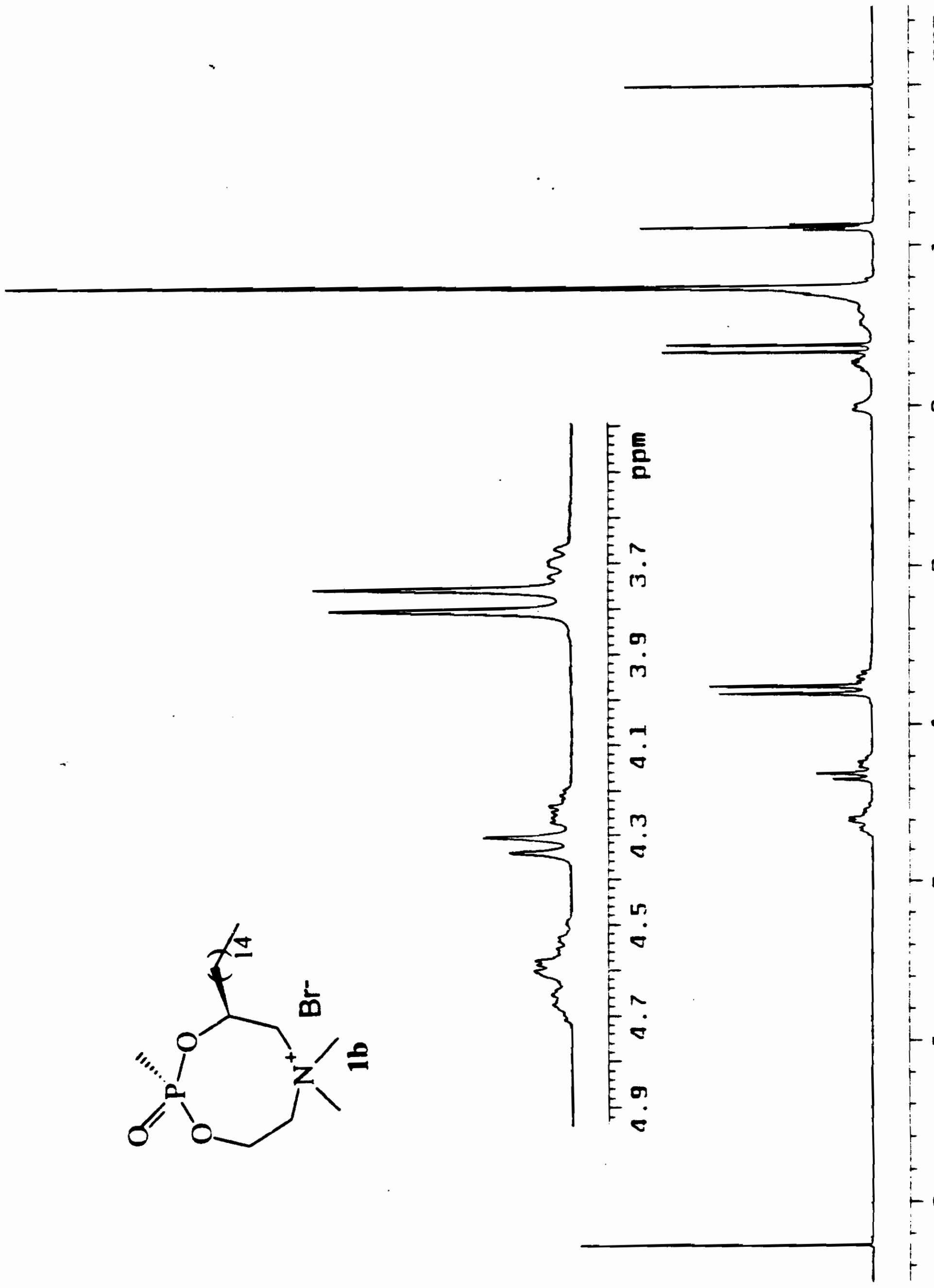


1a

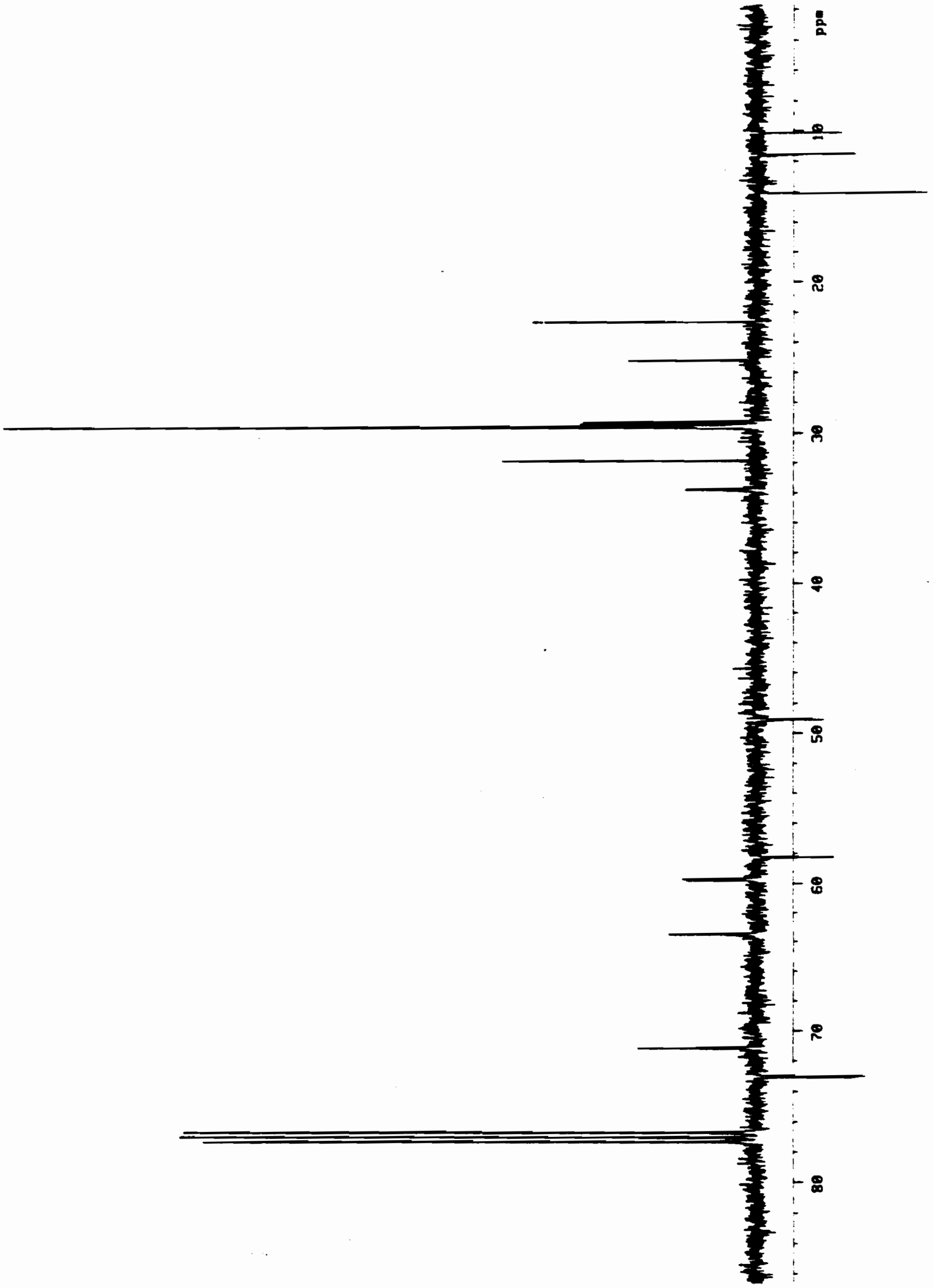

ppm
10
20
30
40
50
60
70
80

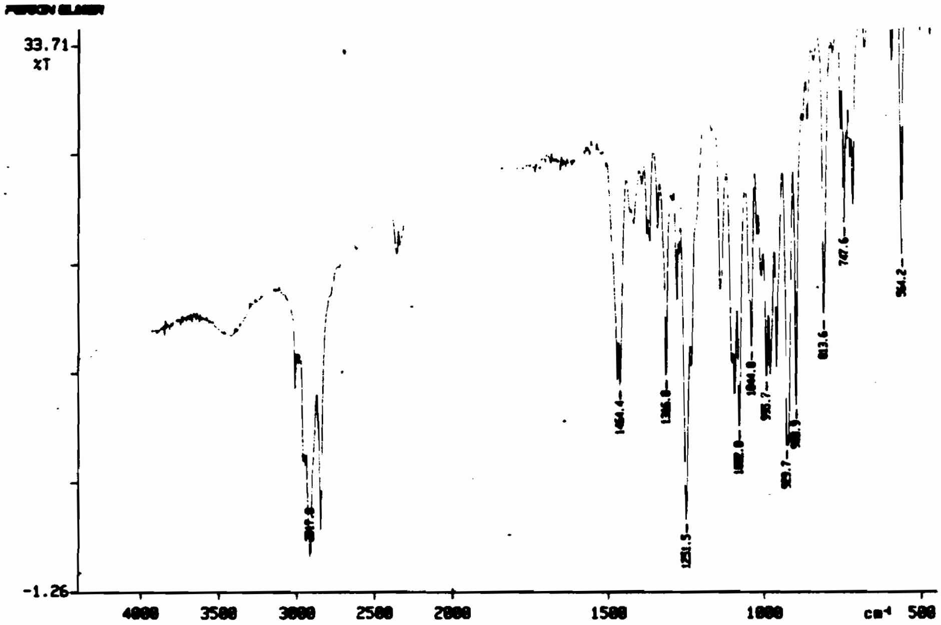

12.42
%T
0.68
4000
3500
3000
2500
2000
1500
1000
cm-1
500
1471.7
1149.0
745.0
602.9

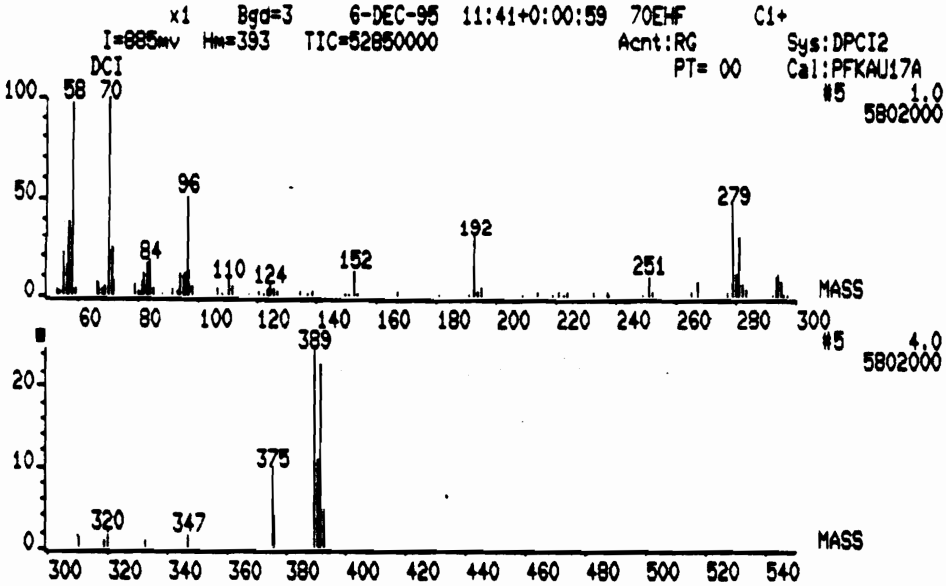

x1 Bgd=3 6-DEC-95 11:46+0:00:59 70EHF CI+
I=509mv Hm=392 TIC=27348000 Acnt:RG Sys:DPCI2
DCI PT= 00 Cal:PFKAU17A
#5 1.0
3337000
100
50
0
58
70
84
96
138
152
192
251
265
279
295
MASS
60 80 100 120 140 160 180 200 220 240 260 280 300
#5 4.0
3337000
20
10
0
375
389
MASS
300 320 340 360 380 400 420 440 460 480 500 520 540

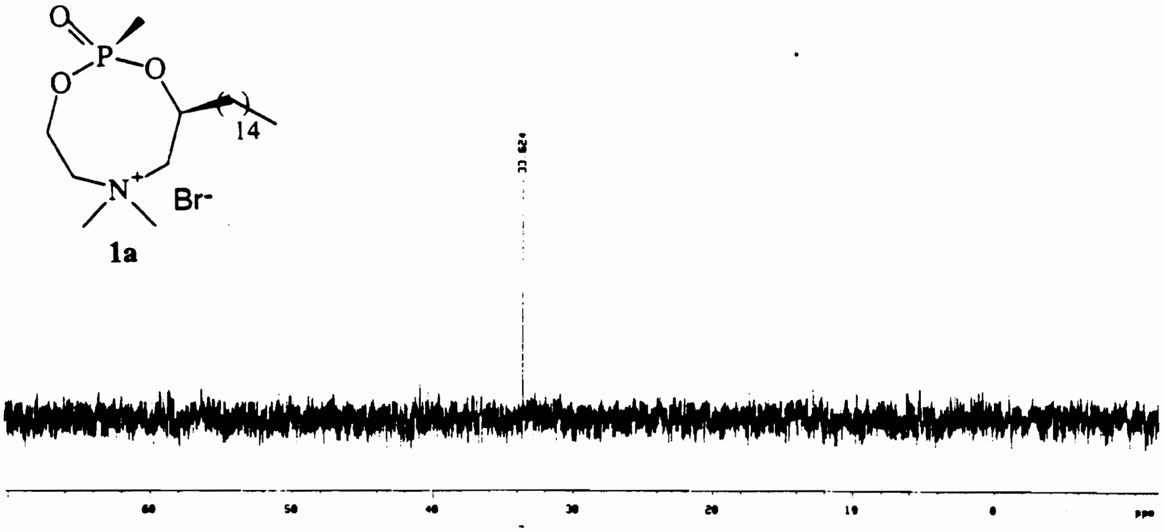


1b

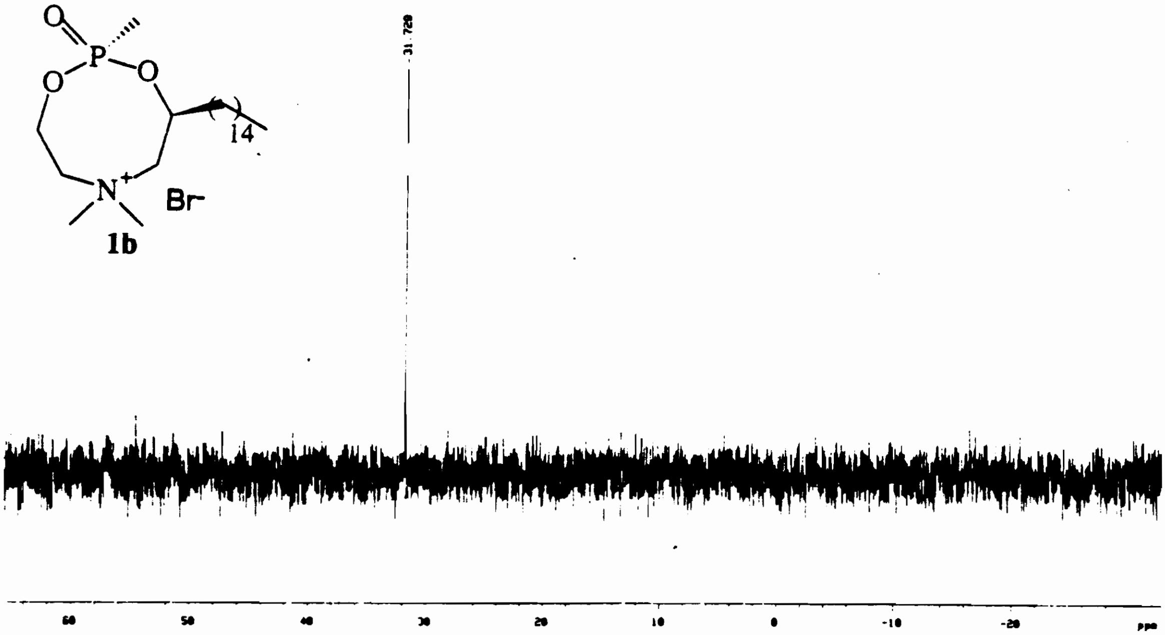

ppm
10
20
30
40
50
60
70
80


33.71
%T
-1.26
4000
3500
3000
2500
2000
1500
1000
cm-1 500
1464.4
1306.0
1251.5
1082.0
1044.0
995.7
929.7
813.6
747.6
564.2


x1 Bgd=3 6-DEC-95 11:41+0:00:59 70EHF C1+
I=885mv Hm=393 TIC=52850000 Acnt:RG Sys:DPCI2
DCI PT= 00 Cal:PFKAU17A
#5 1.0
5802000
100
50
0
58
70
84
96
110
124
152
192
251
279
MASS
60 80 100 120 140 160 180 200 220 240 260 280 300
#5 4.0
5802000
20
10
0
389
375
320
347
MASS
300 320 340 360 380 400 420 440 460 480 500 520 540


O
P
O
O
14
N+
Br-
33.624
60
50
40
30
20
10
0
ppm

1a


O
P
O
O
14
N+
Br-
31.728
60
50
40
30
20
10
0
-10
-20
ppm

1b

VIII. REFERENCES

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IX. VITA

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