



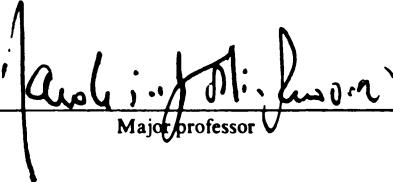
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dissertation entitled
SYNTHESIS OF
PHOSPHOLIPID ANALOGS HAVING NO PHOSPHATE GROUP
AND CHIRAL INTERMEDIATES

presented by

Guangfei Huang

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in CHEMISTRY


Major professor

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SYNTH

**SYNTHESES OF PHOSPHOLIPID ANALOGS HAVING NO PHOSPHATE GROUP
AND CHIRAL INTERMEDIATES**

By

Guangfei Huang

A DISSERTATION

**Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of**

DOCTOR OF PHILOSOPHY

Department of Chemistry

1999

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ABSTRACT

SYNTHESES OF PHOSPHOLIPID ANALOGS HAVING NO PHOSPHATE GROUP AND CHIRAL INTERMEDIATES

By

Guangfei Huang

Phospholipids are the main components of a lipid bilayer in a biological membrane system. They consist of a nonpolar part, usually long chain fatty acids, and a polar part containing a phosphate group. With both exceedingly hydrophilic and hydrophobic regions in the same molecule, phospholipids can self-assemble to form bilayers.

Two phospholipid analogs with the substitution of an amide group for the phosphate were synthesized by utilizing (S)-3-hydroxy- γ -butyrolactone to provide the diacyl substructure. The synthesis of the phosphatidylcholine analog was straightforward and easy to carry out. This cationic analog was shown to be amphiphilic and form lipid bilayers in both the solid state and aqueous solutions by the analyses of X-ray diffraction. Its tight packing order and stability indicate that this analog can be used as a good substitute for phosphatidylcholine as a membrane material. It was also shown to have the ferroelectric properties. The synthesis of the phosphatidylserine analog was accomplished, but the yield was very low. This analog bears a negative charge in the carboxylic group.

A new cationic membrane probe bearing a trimethylammonium function in the head group and pyrenyl groups in the two acyl chains was synthesized. It is a chiral

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phospholipase-C and D insensitive membrane probe and should be more stable than similar ones containing the phosphodiester linkages. It was shown to have the broad excimer emission between 400 and 550 nm. This probe will allow the measurement of membrane dynamics by excimer emission.

Optically active synthons are very valuable intermediates in the synthesis of many chiral complex compounds. (R)-3-Hydroxycarboxylic acids and their derivatives are found as moieties in many biologically important products such as lipid A, and have been utilized as chiral intermediates in the synthesis of many biologically important compounds such as tetrahydrolipstatin. A short and efficient synthesis of (R)-3-hydroxytetradecanoic acid was achieved by using (R)-oxiranyl acetic acid ethyl ester as the source of the chirality from (S)-3-hydroxy- γ -butyrolactone. The method is general and can be used in the preparation of other chiral 3-hydroxy acids.

Dedicated to my parents,
Brother Guanghong, Sister Lanyan,
Wife Jianhua, and Son Zongyi.

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I would also like to thank my parents, Brother Guanghong, Sister Lanyan, and the rest of my relatives. Without their love and support, this dissertation would not be possible. I am indebted to all my teachers and my friends for their help.

Finally, I would like to thank my wife, Jianhua, and my son, Zongyi, for their love, encouragement, sacrifices, support, and the happiness brought to me.

Thank you all

Guangfei Huang

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Figure 2.4:
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Scheme 1.2: S

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Scheme 3.1: S

Scheme 3.2: A

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acid

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acid

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Ac

AIBN

R-Alpine-Bo

BHT

Binap

Bn

Bz

COD

d

dd

δ

DHP

Dibal

DMAP

DMF

DMSO

e. e.

EIMS

Et

HRMS

Hz

LIST OF ABBREVIATIONS

Ac	Acetyl
AIBN	Azo- <i>bis</i> -isobutyronitrile
R-Alpine-Borane®	(R)-(<i>B</i> -isopinocampheyl-9-borabicyclo [3,3,1]-nonane
BHT	2,6-Di- <i>tert</i> -butyl-4-methylphenol
Binap	2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl
Bn	Benzyl
Bz	Benzoyl
COD	1,5-cyclooctadiene
d	Doublet
dd	Double doublet
δ	Chemical shift
DHP	3,4-dihydro-2 <i>H</i> -pyran
Dibal	Diisobutylaluminum hydride
DMAP	<i>N,N</i> -4-Dimethylaminopyridine
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
e. e.	Enantiomeric excess
EIMS	Electron ionization mass spectrometry
Et	Ethyl
HRMS	High resolution mass spectrometry
Hz	Hertz

IR

LDA

LPS

m

Me

mp

MS

NBS

NMR

Ph

PyT

q

s

t

TBAC

TBOC

TEMPO

THF

THL

TMS

Tr

Ts

IR	Infrared
LDA	Lithium diisopropylamide
LPS	Lipopolysaccharide
m	Multiplet
Me	Methyl
mp	Melting point
MS	Mass spectrometry
NBS	<i>N</i> -Bromosuccinimide
NMR	Nuclear Magnetic Resonance Spectroscopy
Ph	Phenyl
Pyr	Pyridine
q	Quartet
s	Singlet
t	Triplet
TBAC	Tetrabutylammonium chloride
TBOC	<i>t</i> -Butyloxycarbonyl
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy, free radical
THF	Tetrahydrofuran
THL	(-)-Tetrahydrolipstatin
TMS	Trimethylsilyl
Tr	Trityl (Triphenylmethyl)
Ts	Toluenesulfonyl

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CHAPTER 1

SYNTHESIS OF PHOSPHOLIPID ANALOGS HAVING NO PHOSPHATE GROUP AS MEMBRANE MATERIALS

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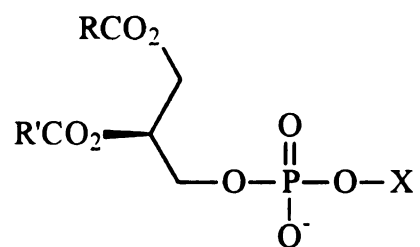
INTRODUCTION

Structures of Phospholipids

Phospholipids are the main components of a lipid bilayer. An enormous variety of their structures have been found in nature, exhibiting great diversity in the structures of both the apolar and the polar moieties of the lipid molecules. Almost all biologically occurring phospholipids are constructed from two combinations of apolar and 'backbone' moieties: a glycerol (or other polyol) moiety substituted with one or two acyl or alkyl chains; or an *N*-acylated sphingoid base (i.e. a ceramide). While glycerol itself is a symmetrical molecule, its C-2 becomes a chiral center when the 1- and 3-positions are not symmetrically substituted. In virtually all natural phospholipids, the polar head group is attached to the 3-position of '*sn*-glycerol', in which the configuration of substituents about C-2 would be designed as R in the (R,S)-system.¹ But in the archaeobacterial phospholipids that are based on a glycerol backbone, the hydrophobic substituents are coupled to the 2- and 3-positions, and the polar head group to the 1-position, of the *sn*-glycerol backbone.

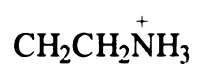
The most common type of phospholipid is phosphoacylglycerol, in which a glycerol moiety is esterified to two carboxylic acids as well as to phosphoric acid, which is linked in turn to a second alcohol² (**Figure 1.1**). Phosphoacylglycerols are classed as phosphatidic acids and phosphatidyl esters. Further classification of the latter depends on the nature of the second alcohol esterified to the phosphoric acid. Some of the most

Figure 1.1: S

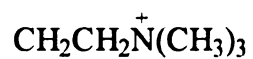


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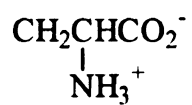
Phosphatidic acid



Phosphatidylethanolamine (cephalin)



Phosphatidylcholine (lecithin)



Phosphatidylserine

Figure 1.1: Structures of some phosphoacylglycerols.

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important phosphatidyl esters are phosphatidylethanolamine (cephalin), phosphatidylcholine (lecithin), and phosphatidylserine. In all these types of phosphatidyl esters there can be wide variation in the nature of the fatty acid moieties. All these compounds have long, nonpolar, hydrophobic tails and a polar, highly hydrophilic head group and thus are markedly amphiphilic. In phosphoacylglycerols the phosphate head group is charged, since it is ionized at neutral pH. Frequently there is also a positively charged amino group contributed by an amino alcohol esterified to the phosphoric acid as in phosphatidylethanolamine, phosphatidylcholine, and phosphatidylserine.

Properties of Phospholipids

Esters and phosphoesters are the most common chemical functional groups in the majority of phospholipids. These chemical features determine the main properties of phospholipids and their stability. Phospholipids can easily undergo hydrolysis in both acidic and alkaline media. Only at pH 7 are phospholipids stable and under these conditions the ester bond hydrolysis does not proceed to any significant degree.³

With both exceedingly hydrophilic and hydrophobic regions in the same molecule, phospholipids are a family of compounds that can be used as building blocks to produce entities that display complexity and diversity on the same scales as proteins composed of amino acids, or polysaccharides from single sugars. The complexity displayed by phospholipids is their ability to form supramolecular structures that self assemble. Phospholipids associate with each other in aqueous media to form micelles or

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lamellar structures and can interact with lipids and other compounds to give mixtures of discrete, immiscible phases. In biological membranes, phospholipids together with other lipids form lipid bilayers with the polar head groups in contact with water, and the apolar tails in the interior. The whole bilayer arrangement is held together through noncovalent interactions such as van der Waals and hydrophobic interactions. The embedded proteins can “float” in the lipid bilayers. The resulting biological membranes not only serve as physical barriers, which cause concentration differences for compounds inside and outside the membranes, but also transport substances across the membranes.^{2, 4}

Uses of Phospholipids

Applications of phospholipids as membrane materials make use of physical, chemical, and biological aspects of the behavior of these molecules.⁴ They are excellent surfactants and emulsifying agents. Such properties have been exploited in the paint, dye, and food industries for many years and all manners of creams and lotions in the cosmetic industry have used natural phospholipids to combine oil and water phases in a stable formulation. Phospholipids also have been used in medical areas such as drug delivery, and lung surfactants.⁵

Synthesis of Phospholipids

Many phospholipids are present not only in low natural abundance but also as a mixture which is difficult to separate. Moreover, even a ‘purified’ lipid preparation that is

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obtained from a biological source may be homogeneous with respect to one structural feature (e.g. the polar portions of the lipid molecules) but highly heterogeneous with respect to another (e.g. their hydrocarbon chains). Thus the chemical synthesis of phospholipids has been of importance due to the need for chemically defined structures for a variety of biochemical and biophysical applications involving membrane-associated processes. There are, however, several problems that arise in chemical syntheses because of the unusual properties of phospholipids. These problems include solubility incompatibilities in reactions involving a long chain hydrocarbon and a polar starting material; slow reactions caused by steric hindrance in long-chain compounds; sensitivities of the phosphate group to both acidic and basic conditions; and the need for extensive chromatographic procedures since, in many instances, phospholipids may be oils or wax-like solids.⁶ Even recrystallization may not remove impurities completely. The syntheses of phospholipids are, therefore, lengthy and not easy, which makes them very expensive. The semi-synthetic approach to phospholipids, i.e. enzymatic cleavage followed by chemical replacement of the cleaved substituent, is generally limited to the preparation of small quantities, and also may not generate chemically defined species because the natural source may be heterogeneous at more than one site. Many phospholipid analogs have been synthesized as molecular probes for physical studies of model and biological membranes. Unnatural stereoisomeric forms of bioactive phospholipids (e.g. 2,3-diacyl-*sn*-glycero-1-phospholipids, which have the S-configuration at C-2) has also attracted interest as analogs for analyzing the role of binding to receptors in the action of certain phospholipids on cellular processes.⁷

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Uses of Phospholipid Analogs

As described earlier, the applications of phospholipids as membrane materials without their biological activities only require that the lipid molecules be amphiphilic and can form liposomes in aqueous solutions. If phospholipid analogs can self-assemble and form liposomes, they should be able to replace phospholipids as membrane materials in the conventional applications of phospholipids in medical and biotechnological areas.⁷

Liposomes are microscopic vesicles composed of one or more phospholipid bilayers separated by an equal number of aqueous interspaces. Highly purified phospholipid analogs can be used to replace phospholipids as the materials for the preparation of liposomes, which are the vehicles of choice in some drug delivery systems.^{7, 8} These vesicles can encapsulate water-soluble drugs in their aqueous space and/or lipid-soluble drugs within the hydrocarbon membrane core and act as “Trojan horses”. Drugs entrapped in liposomes can gain entry into cells by the interaction of liposomes with cells through adsorption, material exchange, and fusion.^{9, 10}

All materials currently used commercially for manufacture of medical devices suffer from the major drawback that they interact strongly with proteins and/or cells when they come into contact with biological fluids and tissues. Biomaterials such as plastics and hydrogel polymers leave much to be desired in terms of their compatibility with biological environments. Safe introduction of these materials into the bloodstream, even for short-term applications, must invariably be accompanied by administration of

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anticoagulants to prevent clotting. Catheterization is the single most common route of hospital acquired infection as a result of the propensity for these materials to bind bacteria and support their growth. Even widely accepted applications such as use of hydrogels in contact lenses suffer from problems of protein deposition.¹¹ Phospholipid analogs can be used to coat the surface of biomaterials, providing a barrier to protein and platelet uptake.

Such phospholipid analogs, in the form of bilayer vesicles, can also be employed to prepare microparticles by chemical means and may facilitate their performance in the areas such as heterogeneous catalysis and photosynthesis.^{12, 13, 14, 15} Microcrystals or precipitates can be formed when concentrated solutions of anions and cations are mixed together. This process can be performed selectively within the internal compartment of a liposome by preparing a liposome suspension with different ions contacting with each other, for example, by means of an ionophore in the membrane that permits influx of the external ion but not efflux of the entrapped species.

Phospholipid analogs can be used in areas where stabilization of particle dispersions is required. These areas include paints and other coating materials. They can be added preferably during preparation of the milling paste, where they can act as lubricants between the solid-solid particle interfaces and reduce the pigment grinding time. All of the particles can be coated with a layer of phospholipid analogs, which can stabilize them upon addition to the solvents, reduce the mixing time and prevent aggregation and settling-out. The particles may be smaller, more homogeneous, and

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finely dispersed. Furthermore, after storage, the pigments may be redispersed more easily. In paints containing mixture of pigments of different size and density, differential settling can be reduced, so the correct shade of paint can be retained even over a long time period. The presence of such phospholipid analogs can also aid in the physical application of paint onto a surface, reducing the effort involved in brushing, and preventing flooding, sagging, or formation of striations in the coating.¹⁶

A special application of the technology described above is the use of phospholipid analogs to coat magnetic particles on recording tape. As a result of advantages already referred to, such as improved dispersion and homogeneity of particles, coating these particles with surfactants can increase the ease with which they can be oriented in the initial coating and hence it is possible to improve the electromagnetic responses.¹⁶

Phospholipid analogs are possibly useful in the study of ferroelectric materials, an application of phospholipids that has not been explored. When chiral lipids are packed in a bilayer membrane, they can exhibit ferroelectric properties. Ferroelectric materials have been utilized in a variety of areas, including portable computers, as a modern liquid crystal display technology.¹⁷ High resolution displays require that the chiral molecules are stable and have high optical purity and good packing order. Phospholipid analogs can meet these requirements. Since they prefer to self-aggregate and form bilayer membranes due to the presence of both polar and nonpolar parts in the same molecules, they can have good packing order and thus have good resolutions in the display. The degree to which such phospholipid analogs tilt can be adjusted simply by changing the size of the polar

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head groups, so the materials with the specifically desired ferroelectric properties can be synthesized.

DESIGNS OF PHOSPHOLIPID ANALOGS HAVING NO PHOSPHATE GROUP

As described earlier, phospholipids have been widely used in biological, biotechnological, medical and industrial areas. Many of these applications require that phospholipid membrane materials be stable and easy to obtain. It is necessary to design phospholipid analogs which have these desired properties. Since the presence of phosphate groups causes phospholipids to be unstable and difficult to synthesize, and many properties such as ferroelectricity do not require its presence, analogs in which the phosphate group is replaced are excellent synthetic targets and valued materials. Two kinds of phospholipid analogs having no phosphate groups were, therefore, designed.

As shown in **Figure 1.2**, each phosphate group of the two analogs is replaced by a peptide bond in which the nitrogen atom is linked to the polar head. The reason for using a peptide bond is that it is polar and the most stable among all common carboxylic acid derivatives. The phosphatidylcholine analog retains the trimethylammonium ethyl group and thus bears a net positive charge. The polar head group has a profound effect on the molecular packing.^{18, 19, 20} It is important to make the size of the polar head group larger than the cross-section of the two acyl chains so the two acyl chains tilt to accommodate the larger polar head group and keep the membrane rigid and tightly packed. Otherwise, the two acyl chains would tilt little. The size of the head group can be controlled through

Figure 1.2:
no phosphate

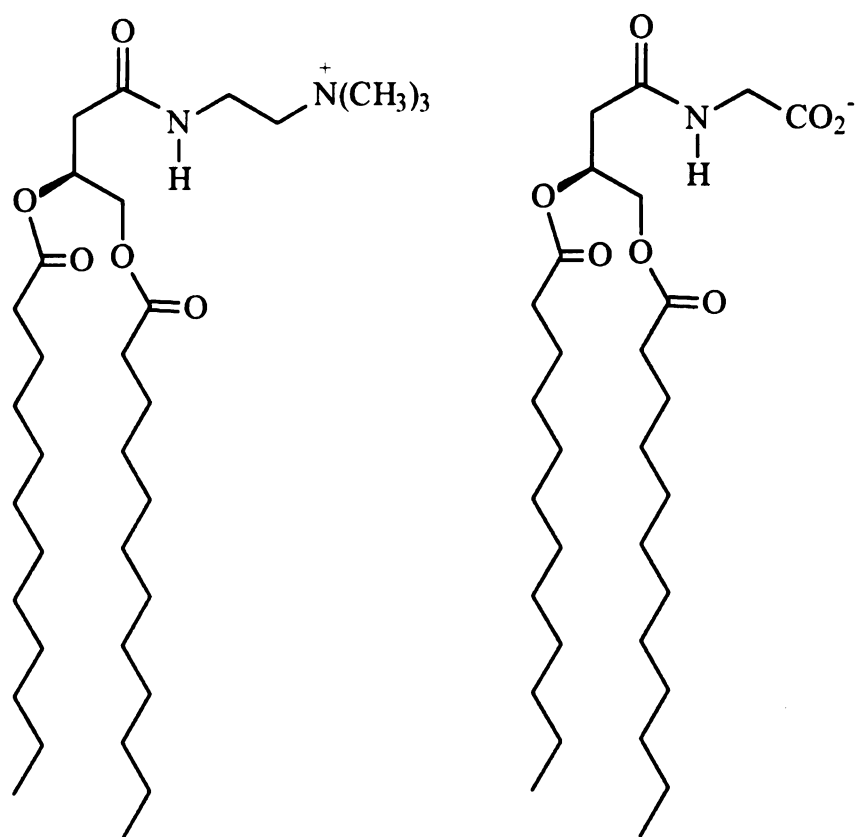


Figure 1.2: Phospholipid (phosphatidylcholine and phosphatidylserine) analogs having no phosphate group as membrane materials.

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changing the chain length of the diamine or the alkyl groups on the quaternary nitrogen atom. In the case of the phosphatidylserine analog, the serine moiety is replaced by glycine, and the phosphate moiety is replaced by an amide group (**Figure 1.2**). The analog has a negative charge on its carboxylic group. Since glycine is easily available and achiral, its introduction will not generate two diastereoisomers. Because glycine is much smaller than serine, the resulting head group is smaller than that of the corresponding phosphatidylserine, which could cause the head group bearing glycine to be parallel to the plane of the bilayer and the acyl chains to tilt little.²¹ This can be overcome when an ω -aminocarboxylic acid with a longer carbon chain is used to replace glycine. The counterions of the two analogs are not important since each counterion can be exchanged easily with another ion in solution. Since both analogs have a nonpolar fatty acid region and a polar charged region, they have the basic properties, such as self-assembly, of lipids. Both phosphatidylcholine and phosphatidylserine have more than one charge in their polar head groups, but each analog has only one charge. Thus it is predictable that the analogs will have less solubility than the corresponding phospholipids in an aqueous solution. They will also be much more stable due to the replacement of the phosphate moiety by an amide group.

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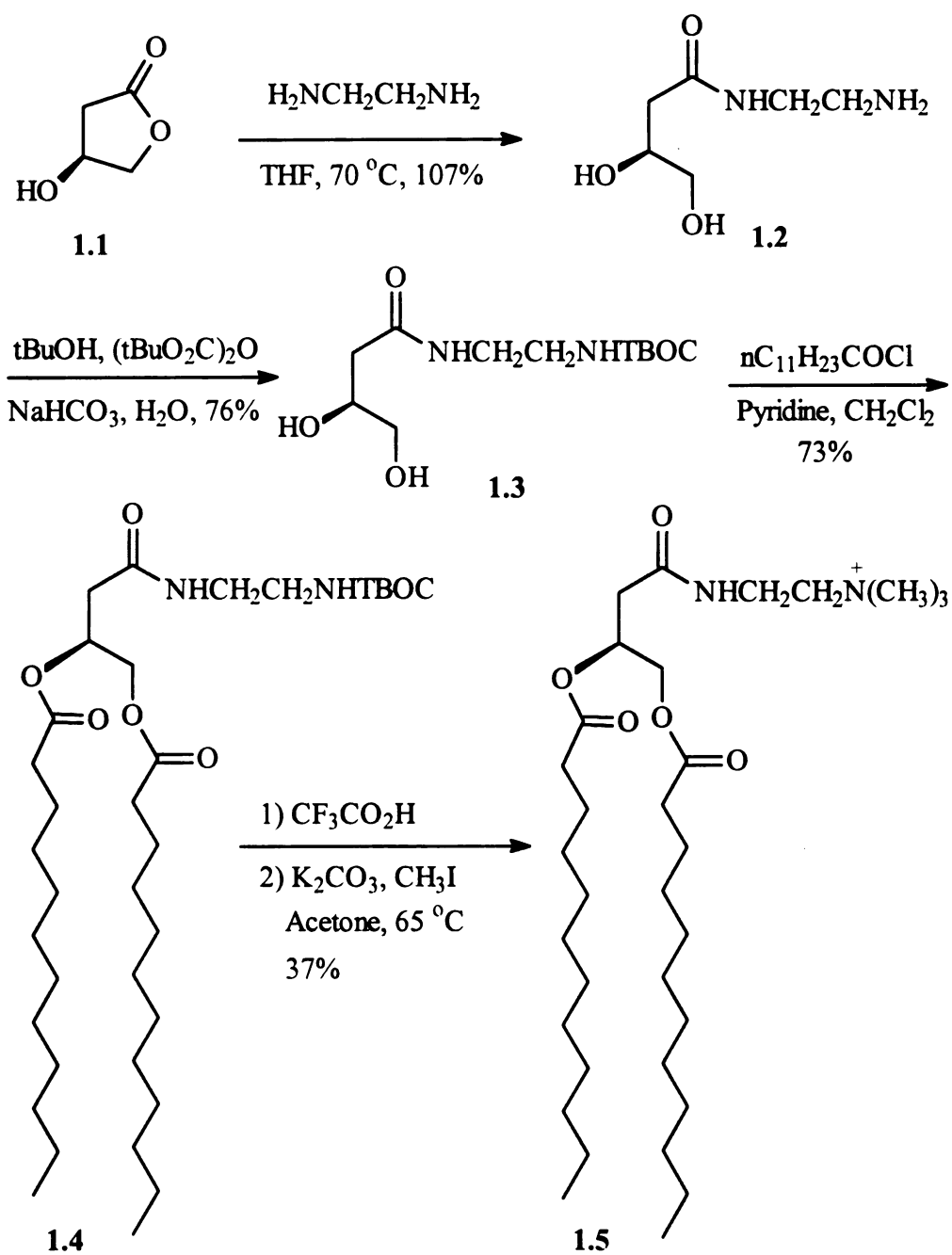
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Scheme 1.1



Scheme 1.1: Synthesis of the phosphatidylcholine analog.

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RESULTS AND DISCUSSION

Synthesis

The chiral replacement of glycerol, (S)-3,4-dihydroxybutyric acid, was obtained as its derivative, (S)-3-hydroxy- γ -butyrolactone, from the degradation of maltose by hydrogen peroxide under basic conditions.²² Of all functional groups present in the phosphatidylcholine analog (1.5), the amide bond is chemically most stable and was installed first. On treatment of optically pure (S)-3-hydroxy- γ -butyrolactone (1.1) with 3 equivalents of ethylene diamine in THF at 70 °C, the lactone ring was opened quantitatively to give the amide derivative (1.2) and it was used for the next step without any purification (**Scheme 1.1**).

Since this amide derivative is highly polar and is almost insoluble in dichloromethane, it was difficult to incorporate the two long chain acyl moieties. Full methylation of the free amino group would also convert it into a charged group, which will make acylation of the two free hydroxyl groups more difficult to accomplish. Hence it is necessary to protect the amino group before the acylation. The TBOC group was used to protect the amino group from acylation and to increase the solubility of the resulting product (1.3) in dichloromethane in the next step.

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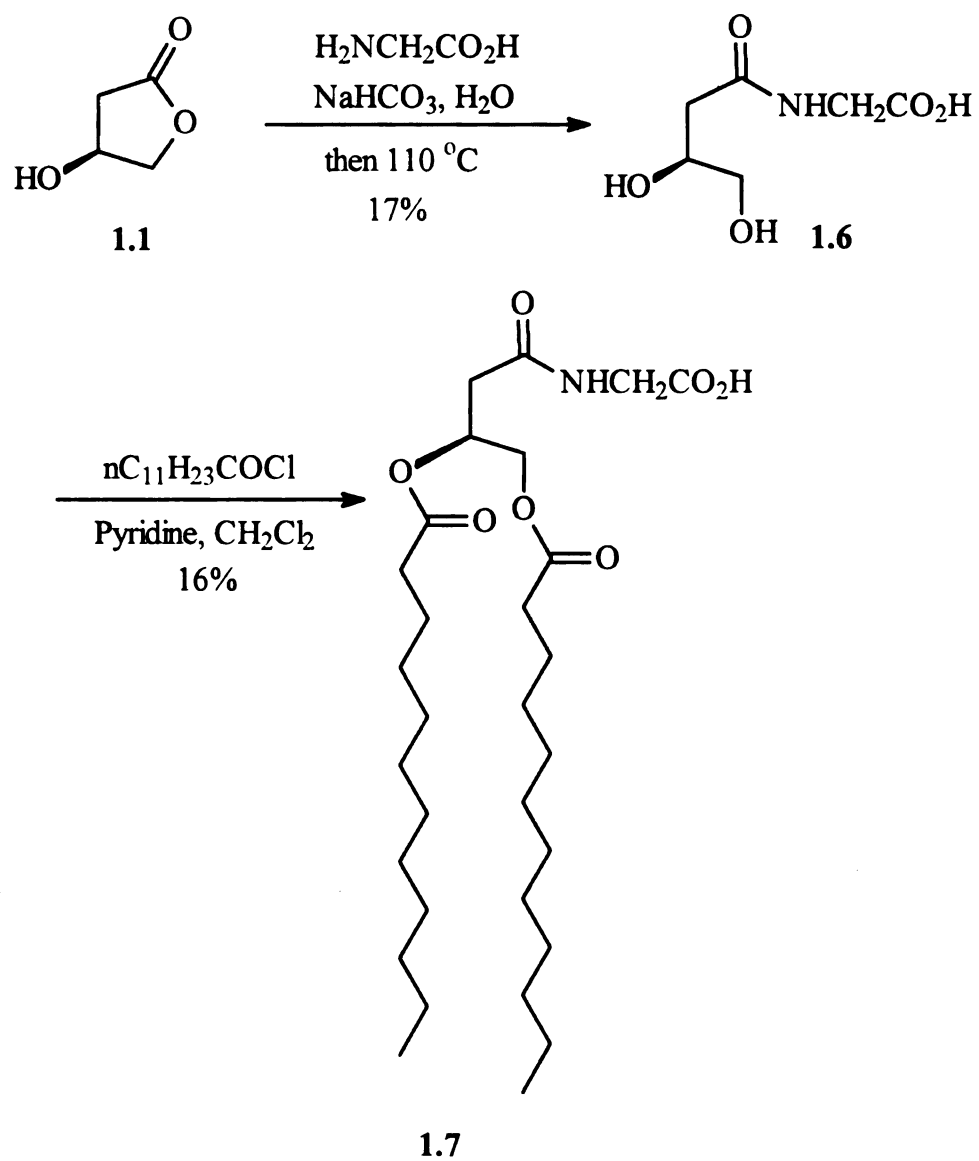
two reactions v

Acylation of the hydroxyl groups with dodecanoyl chloride in the presence of anhydrous pyridine in anhydrous dichloromethane gave the ester derivative (1.4). The removal of the TBOC group was accomplished by treatment of the ester derivative with trifluoroacetic acid for a short time to give the ammonium derivative. This ammonium derivative was methylated with iodomethane in the presence of potassium carbonate in acetone under refluxing conditions and α,β -elimination did not occur. The phosphatidylcholine analog (1.5) was obtained through recrystallization from acetone in 37% yield.

The synthesis of the phosphatidylserine analog (1.7) was completed in two steps (**Scheme 1.2**). Since glycine is a zwitterionic compound, it was treated with sodium bicarbonate to release the amino group. On treatment of the glycine sodium salt with (S)-3-hydroxy- γ -butyrolactone (1.1) in aqueous solution, the solution was heated to remove water and kept at 110 °C overnight to give the desired amide derivative (1.6) in 17% yield after chromatographic separation. Without removal of water and under refluxing conditions at 110 °C no desired product was obtained. This reaction needed optimizing to improve the yield. During the course of the endeavor to form the amide bond, glycine methyl ester was used but resulted in no desired product. The acylation of the two hydroxyl groups was achieved by treatment with *n*-dodecanoyl chloride and anhydrous pyridine in dichloromethane to give the final product (1.7) in 16% yield because of the poor solubility of the amide derivative (1.6) in dichloromethane. Although the synthesis of the phosphatidylserine analog (1.7) was accomplished in two steps, the yields of both two reactions were very low.

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Scheme 1.2:



Scheme 1.2: Synthesis of the phosphatidylserine analog.

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X-ray Diffraction Analysis of the Phosphatidylcholine Analog

X-ray is a principal technique for investigating the physical properties of lipids and their aggregates.^{7, 23, 24} In X-ray diffraction, only regular repeating planes of atoms (lattices) can diffract the radiation. The angle of the reflection (diffracted X-rays) obeys Bragg's law:

$$2d \sin\theta = n\lambda$$

where d is the separation of the repeating planes, θ is the angle of the incidence, n is an integer and λ is the wavelength. Note that the diffraction angle is 2θ . X-ray diffraction can identify the symmetry and long range organization of the bilayer repeat.²⁵

The diffraction pattern of the phosphatidylcholine analog at room temperature is shown in **Figure 1.3**. Seventeen equally spaced reflections from low to wide angle regions ($2\theta = 2.01^\circ, 4.03^\circ, 6.13^\circ, 8.15^\circ, 10.17^\circ, 14.29^\circ, 16.39^\circ, 18.41^\circ, 20.51^\circ, 22.59^\circ, 24.69^\circ, 26.79^\circ, 28.81^\circ, 30.97^\circ, 35.23^\circ, 37.39^\circ$ and 43.89°) are indicative of a lamellar phase with a repeating distance (d) of 43.9 Å. The phosphatidylcholine analog in water (5.0% by weight) has a dramatically changed phase (**Figure 1.4**). Only one discernible lamellar reflection was observed with a repeating distance (47.2 Å). This means that the analog formed predominantly bilayer instead of multibilayer membranes in the aqueous solution. The lamellar repeat distance increased by 3.3 Å due to the hydration of the cationic head groups.

Figure 1.3: X-ray diffraction pattern of the phosphatidylcholine analog. Note that the horizontal axis is diffraction angle and the vertical axis is intensity.

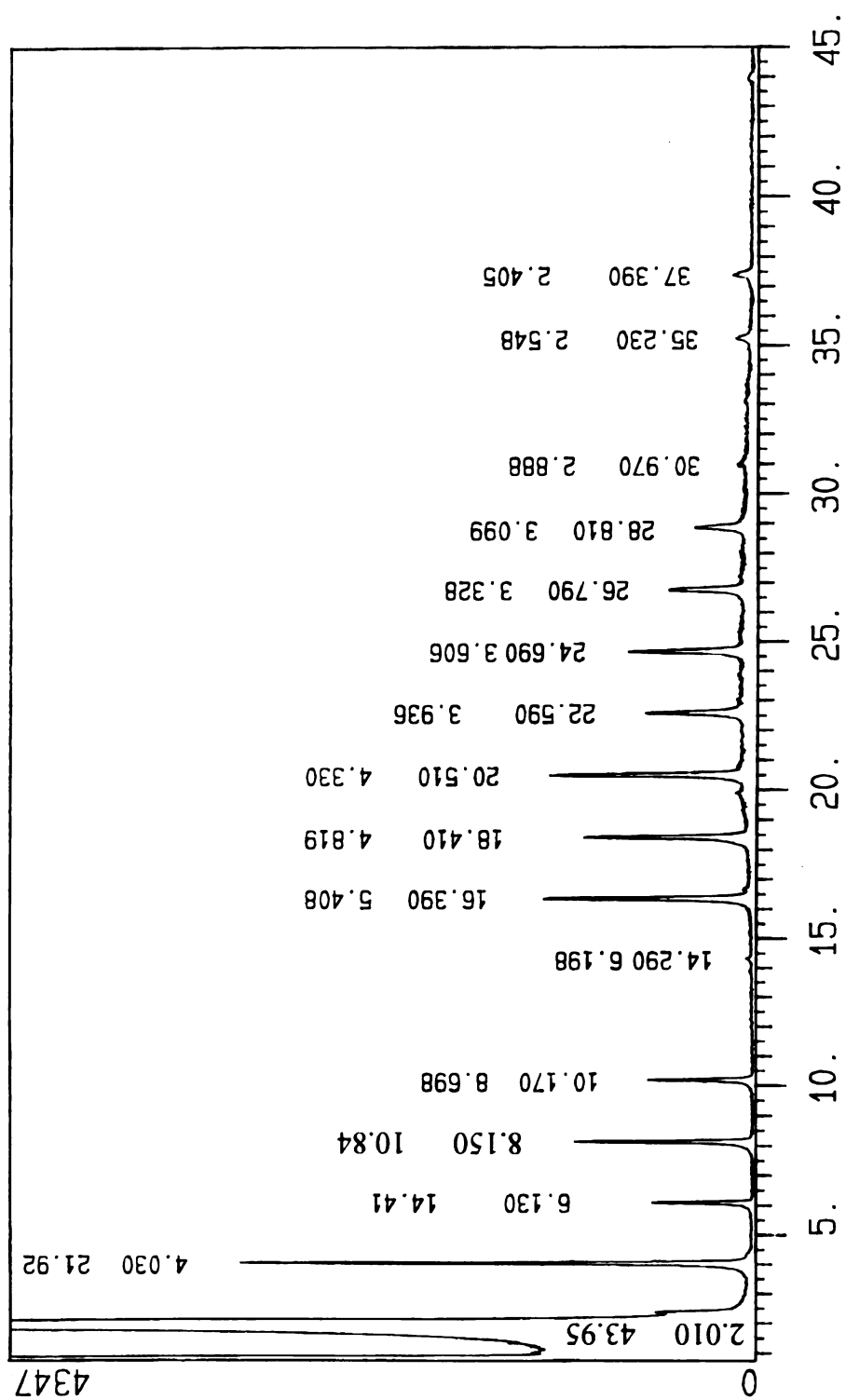


Figure 1.4: X-ray diffraction pattern of the phosphatidylcholine analog in water (5% by weight). Note that the horizontal axis is diffraction angle and the vertical axis is intensity.

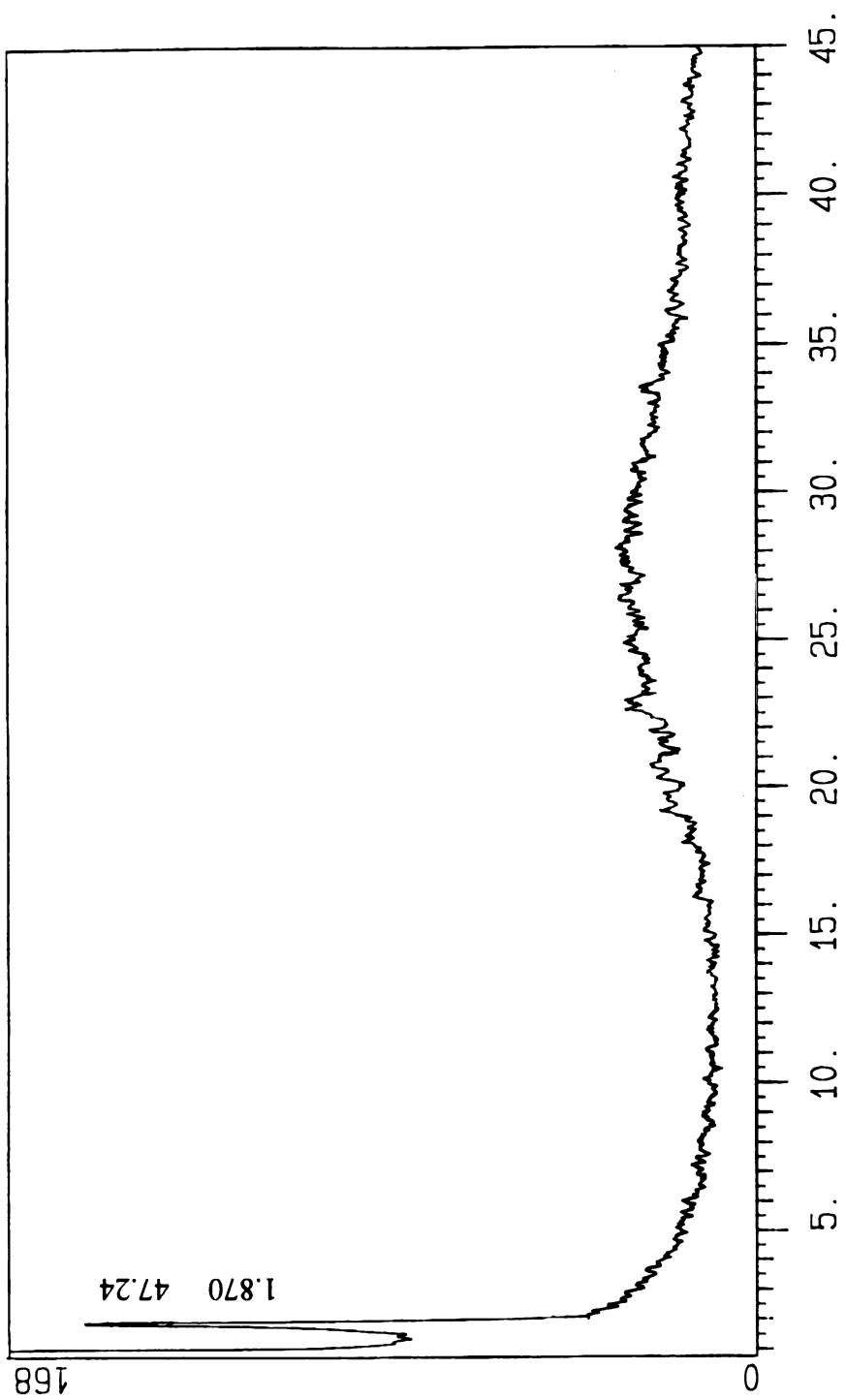


Figure 1.5:
BIOGRAF.

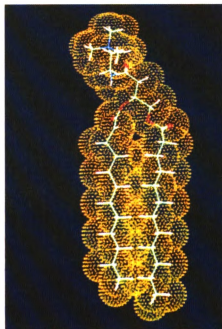


Figure 1.5: The computational structure of the phosphatidylcholine analog by using BIOGRAF.

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The computational structure by using BIOGRAF was shown in **Figure 1.5**. The head-to-tail distance (from the methyl group of the ammonium moiety to the methyl group of the long chain fatty acid moiety which is esterified to the chiral center) of the molecule is 23.0 Å. The calculated distance is between the halves of the repeating distances (22.0 Å and 23.6 Å) given by X-ray. The calculation can support that the phospholipid analog formed bilayers and has a tilted alignment in both the solid state and the aqueous solution.

Characterization of Smectic Phases of the Phosphatidylcholine Analog

Lipid bilayers are liquid crystals. The liquid crystal state is a distinct phase of matter observed between the solid and the liquid states. As one of the three main phases of liquid crystals, the smectic phase is characterized by molecules which not only have positional order but also tend to point in the same direction.¹⁷ Lipid bilayers have smectic phases. If the lipid molecules are achiral, they can align perpendicularly to the bilayer membrane and result in the smectic A phase. If the lipid molecules are chiral, they can tilt and result in the smectic C phase. The smectic C phase can exhibit ferroelectric properties when its constituent molecules are themselves optically active. The smectic C can have a phase transition into the smectic A when the alignment changes from tilted to perpendicular. The phase transition can be caused by factors such as temperature.

Figure 1.6: S

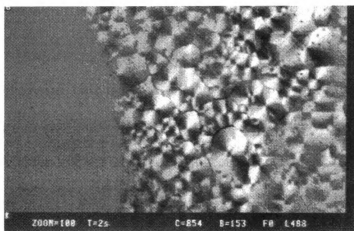


Figure 1.6: Smectic phases of the phosphatidylcholine analog.

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The smectic phase of the phosphatidylcholine analog was characterized by optical microscopy. These conic domains (**Figure 1.6**) were the smectic C phases of the phosphatidylcholine analog bilayers. These results show that the phosphatidylcholine analog form bilayers with good packing in its liquid crystal state and can have the ferroelectric properties. Thus the analog can be used as a material in the area of the liquid crystal display.

CONCLUSIONS

In conclusion, both phosphatidylcholine and phosphatidylserine analogs having no phosphate group were obtained by using (S)-3,4-dihydroxybutyric acid as a substitute for the glycerol phosphate moiety. The synthesis of the phosphatidylcholine analog was straightforward, and easy to carry out compared to that of the corresponding phosphatidylcholine. The analog was recrystallized and thus was easy to purify. The chemistry did not involve any transformation of the chiral center of the backbone and thus the resulting phosphatidylcholine analog should be optically pure. Both the head group and the long fatty acid chains can be easily replaced by other fragments if their sizes need to be varied. The synthesis of the phosphatidylserine analog was very short but gave a very low yield and thus was not efficient. Hence it needs further improvement.

The sheet-like phosphatidylcholine analog is so stable that it can be stored at room temperature without decomposition for more than two years. Due to the absence of

a phosphate group in this analog, its stability makes it useful under conditions in which phospholipids can not be used. It was also shown to form multibilayers by X-ray diffraction. In 5.0% aqueous solution, it formed predominantly bilayer liposomes. This well-packed property in both aqueous solutions and the solid state is of importance to the applications of phospholipids as membrane structural materials. The efficient synthesis and the properties of self-assembly will make this analog a promising excellent substitute for phospholipids in many areas.

EXPERIMENTAL

^1H NMR and ^{13}C NMR spectra were measured on a Varian-300 spectrometer (300 MHz). The chemical shifts are given in δ values with TMS as the internal standard or relative to the CDCl_3 line at 7.24 ppm for ^1H and 77.0 ppm for ^{13}C .

Preparation of 1.2

A solution of (S)-3-hydroxy- γ -butyrolactone (1.0 g, 10 mmol) and ethylene diamine (1.8 g, 30 mmol) in tetrahydrofuran (1 mL) was heated at 70 °C for 12 hours. The reaction mixture was dried on a rotary evaporator to give the product (**1.2**) as syrup. It was used without further purification. The yield was 107%.

IR (KBr): 3357 (bs), 1644 (s), 1562 (s), 1040 (s) cm^{-1} ; ^1H NMR (300 MHz, D_2O): δ 3.90 (1 H, m), 3.43 (1 H, dd, $J = 11.3, 4.5$ Hz), 3.34 (1 H, dd, $J = 11.3, 6.0$ Hz), 3.09 (2

H, t, J = 6.0 Hz), 2.55 (2 H, t, J = 6.0 Hz), 2.29 (1 H, dd, J = 14.5, 4.5 Hz), 2.19 (1 H, dd, J = 14.5, 8.9 Hz); ^{13}C NMR (75 MHz, D_2O): δ 173.9, 68.7, 64.8, 41.6, 39.6, 39.5; HRMS Exact mass: calcd for $\text{C}_6\text{H}_{15}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$, 163.1084. Found 163.1078.

Preparation of 1.3

The diamine derivative (1.2) (6.5 g, 40 mmol) and *tert*-butyl alcohol (30 mL) were added to a two-necked round bottom flask containing sodium carbonate (7.0 g, 66 mmol) freshly dissolved in water (66 mL) and equipped with a reflux condenser and a dropping funnel. The mixture was stirred and di-*tert*-butyl dicarbonate was added dropwise through the dropping funnel. Stirring was continued for 16 hours and then pentane (200 mL) and H_2O (80 mL) were added and the layers separated. The aqueous layer was extracted again with pentane (200 mL) and then twice with ether. It was dried and the product (1.3) was recovered from the salts by extraction of the solid with methanol. The methanol solution was concentrated to yield the product (1.3) as an amorphous solid in a highly pure state. It was used without further purification. The yield was 76%.

IR (CHCl_3): 3351 (bs), 1779 (m), 1694 (s), 1655 (s) cm^{-1} ; ^1H NMR (300 MHz, D_2O): δ 3.88 (1 H, m), 3.41 (1 H, dd, J = 12, 4 Hz), 3.31 (1 H, dd, J = 12, 6 Hz), 3.08 (2 H, t, J = 6 Hz), 3.02 (2 H, t, J = 6 Hz), 2.26 (1 H, dd, J = 15, 6 Hz), 2.16 (1 H, dd, J = 15, 9 Hz), 1.22 (9 H, s); ^{13}C NMR (75 MHz, D_2O): δ 173.45, 167.29, 80.72, 68.68, 64.78,

39.59, 39.09, 38.99, 27.50; HRMS Exact mass: calcd for $C_{11}H_{23}N_2O_5$ $[M+H]^+$, 263.1608, Found 263.1617.

Preparation of 1.4

After the diol (**1.3**) (1.32 g, 5.00 mmol) in tetrahydrofuran was dried under vacuum to remove moisture, anhydrous dichloromethane (200 mL), anhydrous pyridine (2.52 g, 30.0 mmol) and dodecanoyl chloride (6.56 g, 30.0 mmol) were added in sequence and the solution was stirred overnight at room temperature with a drying tube. The reaction mixture was added to a beaker containing ice (50 g), hydrochloric acid (10 mL, 6 M) and sodium chloride (5.0 g) and the solution was stirred for 15 minutes. The organic layer was separated and washed with 5% sodium bicarbonate solution (100 mL) three times, water, brine, dried over anhydrous sodium sulfate, and then dried under reduced pressure. The crude product was purified to give the product (**1.4**) by flash column chromatography on silica gel using a gradient elution from pure chloroform to chloroform/methanol 20:1. The yield was 73%.

^1H NMR (300 MHz, CDCl_3): δ 6.57 (1 H, broad, NH), 6.32 (1 H, broad, NH), 5.39 (1 H, m), 4.33 (1 H, dd, $J = 12.0, 3.0$ Hz), 4.15 (1 H, dd, $J = 12.0, 6.0$ Hz), 3.34 (2 H, m), 3.25 (2 H, m), 2.49 (2 H, dd, $J = 7.5, 1.5$ Hz), 2.29 (4 H, m), 1.45 (9 H, s), 1.25 (36 H, s), 0.86 (6 H, t, $J = 6.0$ Hz); ^{13}C NMR (75 MHz, CDCl_3): δ 177.04, 173.32, 172.90, 169.11, 79.54, 68.46, 64.14, 40.35, 39.77, 37.81, 34.12, 33.93, 31.73, 29.44, 29.31, 29.29, 29.10, 28.96, 28.92, 28.17, 24.73, 24.69, 24.64, 22.50, 13.91.

Preparation of 1.5

Trifluoroacetic acid (2.0 mL) was added to compound **1.4** (2.19 g, 3.50 mmol). The solution was allowed to stand for 15 minutes and then concentrated under reduced pressure. Acetone (20 mL) and potassium carbonate (2.76 g, 20.0 mmol) were added and the resulting mixture was cooled in an ice bath. Iodomethane (20.0 g) was added and the solution was refluxed at 65 °C overnight. After the solution was cooled to room temperature, crystallization occurred. Chloroform was added to dissolve the crystals and the reaction mixture was filtered to remove the inorganic salts. The filtrate was concentrated and the crude product was recrystallized in acetone to give the final product (**1.5**). The yield was 37%.

^1H NMR (300 MHz, CDCl_3): δ 8.08 (1 H, broad, NH), 5.41 (1 H, m), 4.26 (1 H, dd, $J = 11.7, 3.6$ Hz), 4.10 (1 H, dd, $J = 11.7, 6.3$ Hz), 3.79 (4 H, s), 3.41 (9 H, s), 2.64 (1 H, dd, $J = 15.0, 5.7$ Hz), 2.56 (1 H, dd, $J = 15.0, 7.5$ Hz), 2.28 (4 H, m), 1.55 (4 H, s), 1.23 (32 H, s), 0.85 (6 H, t, $J = 6.6$ Hz); ^{13}C NMR (75 MHz, CDCl_3): δ 173.53, 173.22, 170.23, 68.29, 65.54, 65.50, 64.44, 54.60, 37.5, 34.13, 31.88, 29.61, 29.52, 29.49, 29.32, 29.29, 29.13, 29.10, 24.92, 24.86, 22.65, 14.08; HRMS Exact mass: calcd for $\text{C}_{33}\text{H}_{65}\text{N}_2\text{O}_5$ $[\text{M}]^+$, 569.4897. Found 569.4862.

Preparation of 1.6

Sodium bicarbonate (1.68 g, 20.0 mmol) was added to a solution of glycine (1.50 g, 20.0 mmol) in water (10 mL) in a vial and the solution was heated at about 60 °C for a half hour. (S)-3-Hydroxy- γ -butyrolactone (1.02 g, 10.0 mmol) was added and the solution was heated at 110 °C for 12 hours with the reaction vial open to let the reaction occur in the solid state. The pure product (**1.6**) was obtained by flash column chromatography on silica gel using methanol as the eluting solvent. The yield was 17%.

^1H NMR (300 MHz, D_2O): δ 3.93 (1 H, m), 3.61 (2 H, d, $J = 3.0$ Hz), 3.45 (1 H, dd, $J = 12.0, 4.2$ Hz), 3.35 (1 H, dd, $J = 12.0$ Hz, 6.6 Hz), 2.34 (1 H, dd, $J = 14.7, 4.5$ Hz), 2.24 (1 H, dd, $J = 14.7, 5.4$ Hz); ^{13}C NMR (75 MHz, D_2O): δ 171.92, 168.93, 64.34, 60.32, 38.58, 34.97.

Preparation of 1.7

The solution of compound **1.6** (0.33 g, 1.7 mmol) in tetrahydrofuran (20 mL) was dried under reduce pressure to remove the moisture. Anhydrous dichloromethane (2 mL), anhydrous pyridine (0.29 g, 3.7 mmol) and lauroyl chloride (0.80 g, 3.7 mmol) were added in sequence and the solution was stirred overnight with a drying tube. The reaction was quenched by addition to the solution of NaCl (1 g), HCl (6 M, 4 mL) in ice (20 g) and then the mixture was extracted with chloroform. The organic layer was washed with 5% sodium bicarbonate solution, water, brine, dried over anhydrous sodium sulfate,

filtered and dried under reduced pressure to give the crude product. The pure product was obtained by flash column chromatography on silica gel using a gradient elution from chloroform/methanol 20:1 to 3:1. The yield was 16%.

^1H NMR (300 MHz, CDCl_3): δ 6.61 (1 H, t, $J = 6.0$ Hz), 5.41 (1 H, m), 4.34 (1 H, dd, $J = 12.0, 3.6$ Hz), 4.15 (1 H, dd, $J = 12.0, 6.0$ Hz), 4.05 (2 H, d, $J = 6.0$ Hz), 2.61 (2 H, d, $J = 6.0$ Hz), 2.30 (4 H, m), 1.59 (4 H, m), 1.25 (32 H, s), 0.87 (6 H, t, $J = 6.3$ Hz); δ 173.62, 173.21, 172.96, 169.68, 68.41, 64.24, 41.40, 37.62, 34.24, 34.08, 31.90, 29.64, 29.60, 29.50, 29.41, 29.34, 29.28, 29.22, 29.14, 29.07, 29.04, 24.81, 22.62, 14.10. EIMS Exact mass: calcd for $\text{C}_{30}\text{H}_{55}\text{NO}_7$ $[\text{M}+\text{Na}]^+$, 564.4. Found 564.6.

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CHAPTER 2

SYNTHESIS OF A CHIRAL PHOSPHOLIPASE-C AND D INSENSITIVE MEMBRANE PROBE WITH EXCIMER EMISSION PROPERTIES

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INTRODUCTION

In 1972, Singer and Nicholson proposed the fluid mosaic membrane model for the structure of a biological membrane.¹ The membrane is a dynamic structure with lipids moving and with the proteins “floating” in the lipid bilayers. Consequently, the question of how these proteins and the surrounding lipids are diffusing and functioning is of considerable interest and of great importance for the understanding of the functions of biological membranes.

Fluorescence spectroscopy is a tool that is routinely used in the study of membrane organization and dynamics.² Fluorescence emission is observed after an electron that has been brought into an energetically excited state by the absorption of a photon of suitable wavelength spontaneously returns to a lower energy state and thus is deactivated.³ This normally occurs through a $\pi^* \rightarrow \pi$ transition between the states with antiparallel (singlet, $S = 0$) spin orientation. Transitions between the first excited and the ground singlet states give rise to a measurable fluorescence signal; this normally occurs on the time scale of 10^{-9} to 10^{-7} second.

Since most phospholipids do not contain fluorescent groups, many non-lipid fluorescent molecules containing polyunsaturated groups have been used, with varying degrees of success, as probes of membrane fluidity. Examples include parinaric acid (9,11,13,15-octadecatetraenoic acid), 1,6-diphenyl-1,3,5-hexatriene, pyrene and perylene. These probes differ essentially in their shapes, and these differences in shape result in

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differences in their positions and movements within the lipid bilayers. More importantly, these probes are not similar to phospholipids. They do not pack with phospholipids at specific depths in the membrane bilayers, and thus the resulting fluorescence spectroscopy can not accurately reflect the membrane structure and dynamics.

Those probes which bear a reasonable structural similarity to membrane lipids are generally the most useful. A common strategy for using phospholipid analogs as probes is to attach a fluorescing group to the phospholipid.^{4, 5, 6, 7} Such fluorescent labels, as a rule, contain conjugated double bonds, to be excitable even with low energy photons. Many are commercially available from Molecular Probes (Eugene, Oregon.), for example.

In such a configuration that a fluorescing group is attached to each of the acyl chains of the phospholipid, the two fluorescing groups interact to form a dimer complex which emits differently than single fluorescing groups by a phenomenon known as excimer emission.⁸ The process can be described as follows: When two fluorescing groups (one in its excited state and the other in its ground state) are very close, they can interact with each other. The resulting excited state energy level is lower than that of one single fluorescing group, while the resulting ground state energy level is higher than that of one single fluorescing group. It results in a smaller energy gap. Thus the excited dimer emits fluorescence light at a longer wavelength than a fluorescing monomer. This form of emission requires that two fluorescing groups be in close proximity allowing the formation of the excimer (dimer) complex (about 0.35 nm for the separation of pyrene moieties).⁹ Since the wavelength of the excimer emission is longer than that of normal

fluorescence emission, the former can be identified easily. These unique phenomena make excimer emission very useful in the study of biological membranes.

The intensity of the excimer emission is a function of the distance between two fluorescing groups.¹⁰ This relationship can be utilized in the field of membrane study. In the membranous lipid bilayers, lipids are well packed and they interact with each other. If the probe is in a tightly packed domain of a membrane, the separation of the intramolecular fluorescing groups will be small enough for excimer formation within the life time of the excited fluorescing group. The motion of the lipids in a membrane can affect the distance between the fluorescing groups. Thus the resulting spectrum can reflect the lipid lateral motions. The intensity of the excimer emission decreases with a decrease in packing density. The excimer emission is also affected by the microviscosity of the medium. In the case of membrane lipids, this is determined by the lateral mobility of the lipid molecules and their average orientation. In viscous media, the fluorescing groups in the probe are, on average, in the same relative orientation to a greater extent than in less viscous media. If the probe is insensitive to phospholipase-A activity or in absence of such activity, such a probe allows the measurement of the extent of packing of the membrane hydrocarbon chains.¹⁰ If the probe is sensitive to the activity of phospholipase-A, it is possible to follow the hydrolysis of the acyl chains as a result of phospholipase-A activity by monitoring the decrease of the excimer emission.^{11, 12} When an acyl chain bearing a pyrene moiety is cleaved by phospholipase A, the acyl chain can be inhibited from hydrolysis. The phospholipase A bearing the acyl chain can be detected by fluorescence techniques. Thus the probe can be used to detect which enzyme is

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phospholipase A. It is, therefore, necessary to design and synthesize a membrane probe, which is more stable than phospholipids under both chemical and physiological conditions, with excimer emission properties.

The structural difference between a fluorescing group and a fatty acid chain requires that only a very low concentration of the fluorescent probe be present in a membrane system in order not to perturb the lipid bilayer structure to a substantial degree. Thus the quantum yield and the relative position^{13, 14} of the absorption and emission maxima are considered here as the two important factors for the choice of suitable fluorescing groups. Linear type fluorescing moieties such as all trans-9,11,13,15-octadecatetraenoic acid are more similar to saturated fatty acid chains than nonlinear disk-like ones, but they have very low quantum yields.³ Due to these two factors, pyrene moieties were chosen as the fluorescing groups to attach to the two acyl chains in this work. This configuration will not significantly perturb the packing of the lipid layers owing to its low concentration in the membrane system, but the perturbation will be maximal where it probes. One advantage of this probe is that the length of the acyl chain to which a pyrenyl group is attached can be varied so that the resulting probe can be purposely chosen to study the areas at desired depths in the lipid layers.¹⁵

One problem in the design of membrane probes is that those with phosphodiester linkages are susceptible to cleavage by phospholipase-C and D. In the case of phospholipase-D, the phosphodiester linkage distal to the diacyl glycerol moiety is cleaved so that a phosphatidic acid remains. Phospholipase-C activity results in the

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cleavage of the proximal bond and a diacylglycerol is left. The design of a lipid analog that maintains the diacyl structure and the polar aspects of the head group but without the phosphodiester linkage is desirable. The preparation should be simple and the product should be optically pure.

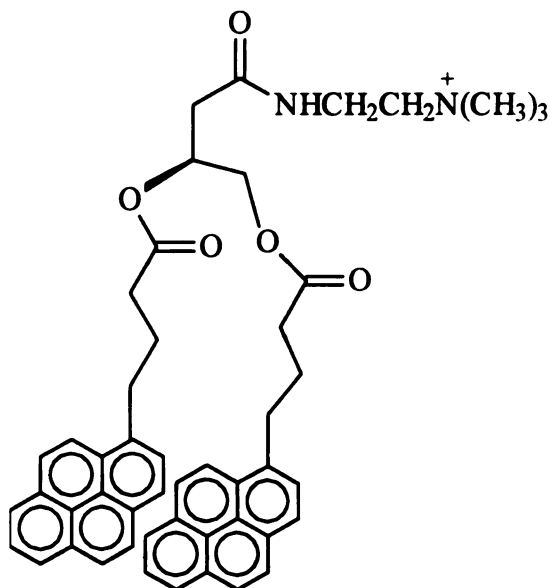


Figure 2.1: A chiral phospholipase-C and D insensitive membrane probe with excimer emission properties.

Here we design a phosphatidylcholine analog (**Figure 2.1**) lacking the phosphodiester group but bearing the trimethylammonium ethyl group and pyrenyl functions in the acyl chains. The molecule has a net positive charge. Phosphatidylcholine in the membrane environment also has a net positive charge since the negative charge on the phosphate group is neutralized by calcium ions at the interface between the hydrocarbon core and the solvation shell. The synthesis is based on (S)-3-hydroxy- γ -butyrolactone as the source of the chiral diol fragment to which the acyl groups are attached. The oxygen atom at the 3-position of glycerol in glycerolipids is replaced by a

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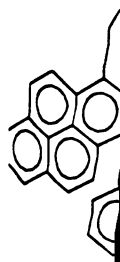
carbon atom in this structure. This probe was designed to be stable to phospholipase-C and D activity but is also very stable to phospholipase-A. It was designed for use in studying membrane dynamics and order^{16, 17, 18} by analyzing excimer emission spectra using fluorescence spectroscopy and for performing studies at the single cell level using fluorescence microscopy.

RESULTS AND DISCUSSION

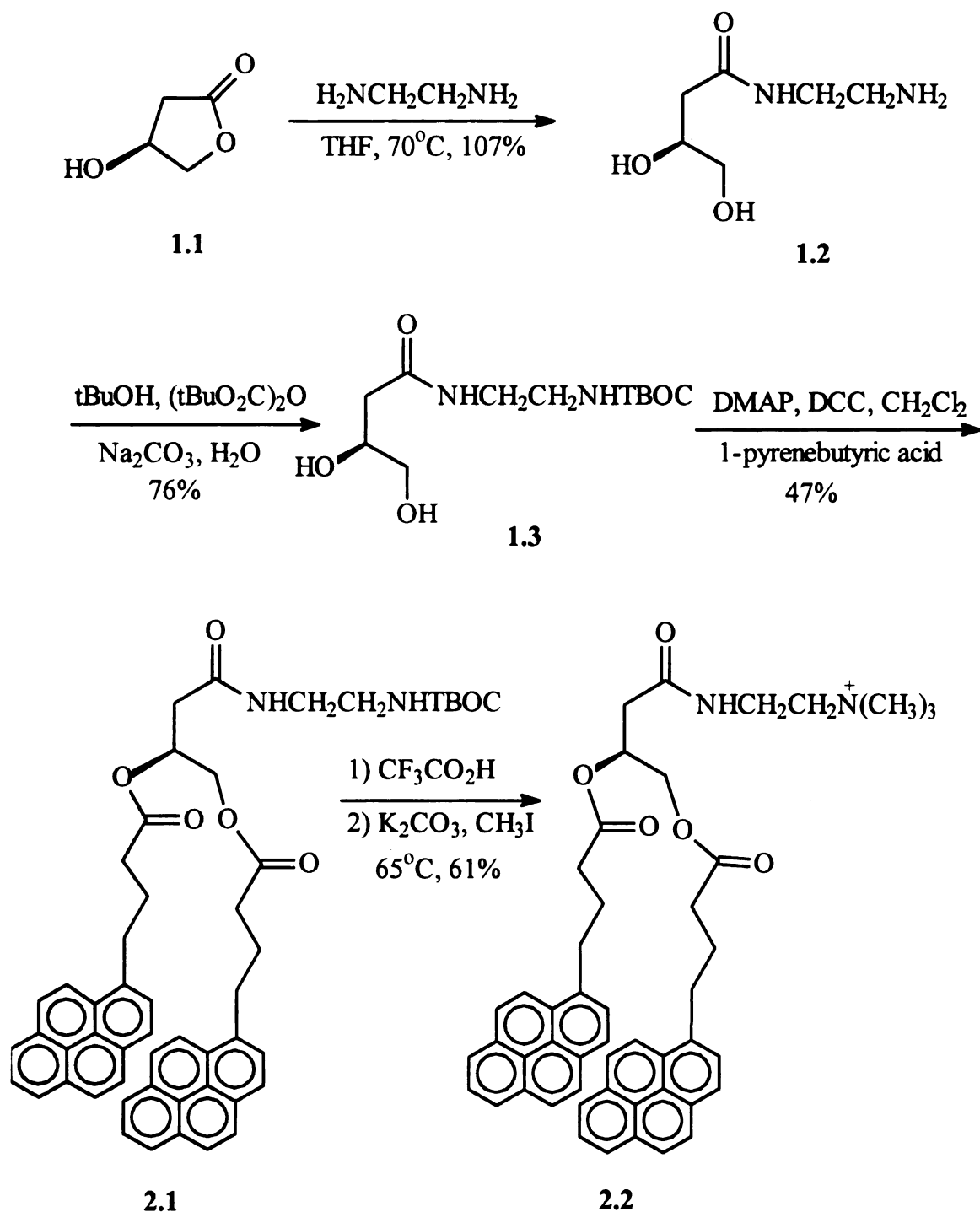
As shown in **Scheme 2.1**, the synthesis of this probe is similar to that of the phosphatidylcholine analog (**1.5**). The chiral head group of the fluorescent probe was obtained from carbons 3-6 of D-glucose by selectively degrading it to (S)-3,4-dihydroxybutyric acid and then cyclizing it to the (S)-3-hydroxy- γ -butyrolactone (**1.1**).¹⁹
²⁰ The lactone was then ring-opened with an excess of ethylenediamine to give the 2-aminoethyl(3,4-dihydroxy)butyramide (**1.2**) and the amino group protected with a *t*-butyloxycarbonyl function.²¹

The acyl groups were then installed by using 1-pyrenebutyric acid, DCC and DMAP in anhydrous dichloromethane in 47% yield. The *t*-butyloxycarbonyl group was removed and the amino group was then completely methylated using iodomethane to give the product (**2.2**) in 61% yield after flash column chromatography.

One important feature of this synthesis is the ease with which the chirality is integrated into the molecule. The starting lactone is obtained >99.8% optically pure (by



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Scheme 2.1: Synthesis of a chiral phospholipase-C and D insensitive membrane probe with excimer emission properties.

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chiral gas liquid chromatography on a cyclodextrin column) and the subsequent transformations cannot disturb the chiral center. The ring opening to form the amide (1.2) is very facile. The chemistry is quite simple and actually represents a general method to access optically pure glycerolipids. The synthesis of optically pure phospholipids and their analogs with a phosphate group is a very laborious affair that usually involves the use of chiral 3-carbon synthons such as glycidols and isopropylidene glycerols. They are expensive, and the installation of the phosphate group requires some effort. The chemistry involves the selective functionalization of the glyceryl moiety, the protection and activation of the phosphate group, the coupling steps, the acylation reactions and some deprotection steps.

Since this probe bears a positive charge in its polar head group, the effect of the positive charge on the fluorophores should be considered. The methyl protons at the amino group of Compound 1.5 have a chemical shift (δ) at 3.41 ppm in CDCl_3 , while those of this probe are at 2.82 ppm. The upfield shift by 0.59 ppm implies the ring current effect of the pyrene. The probe has the conformation in which the three methyl groups are above the pyrene face in CDCl_3 . The pyrenyl ring current has a shielding effect on the methyl groups and drives the protons upfield in the NMR spectrum. The interaction between the aromatic ring and a cation is strong in many organic solvents such as chloroform.²² For the methylated ammonium ion the cation- π interaction is about 9 kcal/mol.²³ In aqueous solution the methylated ammonium ion will be solvated so that the cationic head group will be in the aqueous solution while the bis-pyrenyl moieties will be in the hydrophobic region of a membrane bilayer.

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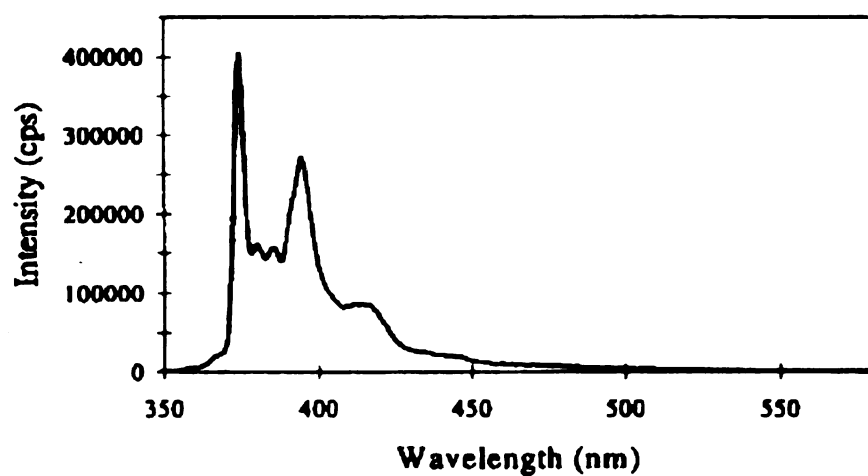


Figure 2.2: Fluorescence emission spectrum of 1-pyrenebutyric acid (*ca.* 10 mM in chloroform) with excitation at 327 nm.

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The length of the linkage chain can be varied to probe different depths in the membrane bilayer. This can be achieved through homologation of the pyrenecarboxylic acid. The other easy method is to attach commercially available pyrenemethanol to ω -halocarboxylic ester from the corresponding lactone through an ether linkage.

The emission spectrum at the absorption maximum of free pyrenebutyric acid in chloroform (327 nm) at room temperature is shown in **Figure 2.2**. The fluorescence emission takes place between 370 and 423 nm. Although the concentration is very high (*ca.* 10 mM), the fluorescence emission is negligible above 423 nm. The excimer emission for the free monomer solution is thus not observed even at such high concentration. This is because the possibility of the formation of an excimer is very low through the interaction between a monomer at its excited state and another one at its ground state.

The probe (2.2) displayed the excimer emission properties that were required. The corresponding spectrum from the probe (*ca.* 10 mM in chloroform) is shown in **Figure 2.3**. The fluorescence emission between 372 and 402 nm belongs to the monomeric pyrenyl moieties. This band is consistent with that of free 1-pyrenebutyric acid. The highly intense, broad spectrum is the excimer emission spectrum. The excimer emission band spreads from 402 to 568 nm with the width of 166 nm. The maximal excimer emission is centered at 469 nm and is 95 nm away from that of the monomer at 372 nm. Even though there is a slight overlap between the excimer and monomer emission bands, the former is very broad and easy to identify. The excimer emission band is very broad

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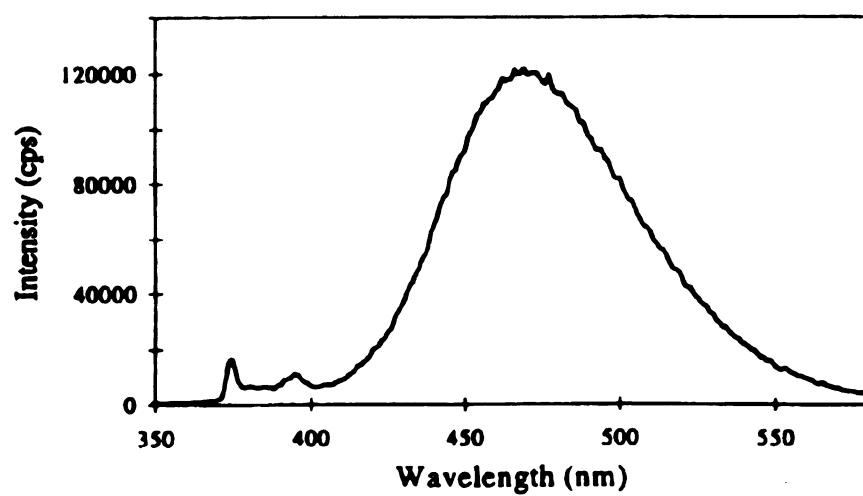


Figure 2.3: Fluorescence emission spectrum of the synthetic probe (2.2) (*ca.* 10 mM in chloroform).

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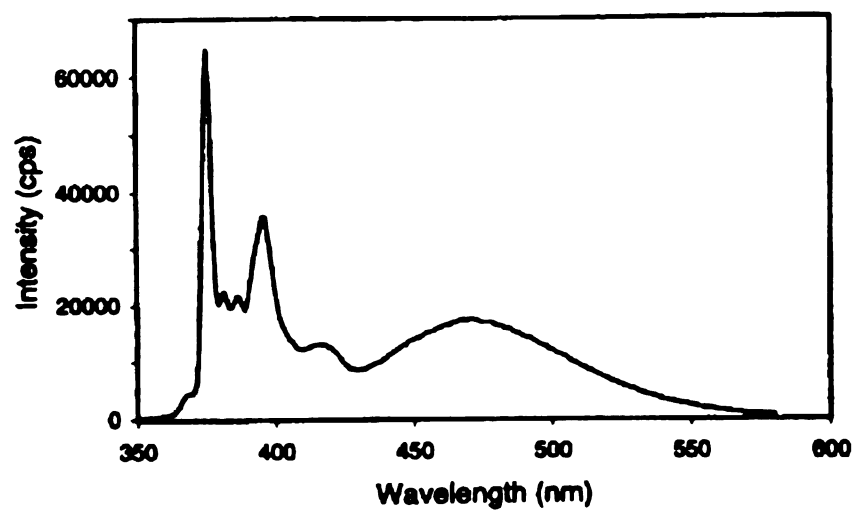


Figure 2.4: Fluorescence emission spectrum of the synthetic probe (2.2) (*ca.* 20 μM) in phosphatidylcholine unilamellar vesicles.

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because many vibronic transitions are possible from the ground vibrational level of the excited state in the excimer to many vibrational levels in the ground state of the monomeric fluorescing group. In this bis-pyrenyl probe, the fluorophores are linked through a flexible chain. In solution, the local concentration of the fluorophores is greatly enhanced, compared with the free monomeric pyrene compound, since the maximal separation of the two fluorophores is limited by the length of the linkage chain between them and they are no longer distributed randomly. The linkage can greatly increase the possibility of the excimer formation. The intramolecular excimer emission, therefore, can be detected at very low concentration (*ca.* 10^{-5} mol L⁻¹). The extent of excimer formation is highly limited by the probability for two fluorophores to reach within the lifetime of the fluorophore at its excited state and the conformation of the two approaching fluorophores. An excimer formation requires that they have a nearly parallel conformation instead of a perpendicular one. Due to the π - π interaction between the two pyrenyl moieties, they prefer to form the parallel sandwich conformation. Both the π - π interaction and the linkage between the fluorophores dramatically enhance the possibility of the excimer formation. The effect is reflected in the excimer emission spectrum.

An excimer emission band was also obtained from the probe in phosphatidylcholine liposomes by Xiaoyang Du in our laboratory (**Figure 2.4**). The excimer emission band is centered at about 470 nm and easy to identify. The probe has less intensity of its excimer emission in phosphatidylcholine liposomes (**Figure 2.4**) than in chloroform (**Figure 2.3**) since the phosphatidylcholine has higher viscosity than and

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the orientations of the intramolecular pyrene moieties in the liposomes are less parallel than in chloroform.

The intensity of the excimer emission decreases as the separation of the pyrenyl moieties increases. Since the separation is affected by the translational and the rotational motions, this phenomenon can give us the information of the probe microenvironments, and thus this probe can allow us to study the dynamic properties of a membrane system. This probe can also be utilized to study the activity of phospholipase A. If the molecule is degraded by a phospholipase A (which removes a pyrenebutyric acid residue), the two pyrenyl rings drift apart and the excimer emission disappears completely. If the probe is not sensitive to the activity of phospholipase A, the cleavage of the phospholipids in the membrane will change the packing order of the lipid membrane. The resulting excimer emission spectrum will reflect this. Treatment of the probe for several hours at pH 7.5 with a phospholipase-A from *Rhizobium trifolii* followed by extraction and thin-layer chromatography analysis indicated that no free pyrenebutyric acid was liberated. Hence the probe was stable to this enzyme.

CONCLUSIONS

In conclusion, a membrane probe with excimer emission properties was synthesized in a few steps by using the 4-carbon synthon, (S)-3-hydroxy- γ -butyrolactone, to provide the chiral substructure. It is a phosphatidylcholine analog with the phosphate group replaced by an amide group and bears a cationic trimethylammonium function in

the head group. Two pyrenyl groups were installed to attach to the terminals of the acyl chains. The chain length can be varied through homologation of the pyrenecarboxylic acid or can be extended through an ether linkage. Thus it can allow the pyrenyl moieties to detect different depths in the membrane system. Due to the replacement of the phosphate group, the probe is insensitive to phospholipases C and D. It is also stable to the activity of phospholipase A. By replacing the phosphate group, the probe is thus much more stable in the systems where the level of phospholipase activity is high.

The fluorescence excimer emission of the probe was demonstrated both in an organic solvent and in phosphatidylcholine unilamellar vesicles. The excimer emission band is centered at about 469 nm. It is in the 400-550 nm region where normal fluorescence emission does not occur, and thus it does not strongly overlap with normal emission. Furthermore, it is very broad and easy to identify in the fluorescence emission spectrum. The intensity of the excimer emission is a function of the separation between the fluorophores, which is sensitive to their microenvironments. This phenomenon will allow the measurement of membrane dynamics by excimer emission.

EXPERIENTAL

^1H NMR and ^{13}C NMR spectra were measured on a Varian-300 spectrometer (300 MHz). The chemical shifts are given in δ values with TMS as the internal standard or relative to the CDCl_3 line at 7.24 ppm for ^1H and 77.0 ppm for ^{13}C .

Preparation of 2.1

Compound **1.3** (0.26 g, 1.0 mmol) was dissolved in THF and the solution was dried under vacuum to remove moisture. Anhydrous dichloromethane (10 mL), 1-pyrenebutyric acid (0.63 g, 2.2 mmol) and DMAP (0.24 g, 2.0 mmol) were added and the solution was stirred. Dicyclohexylcarbodiimide (0.45 g, 2.2 mmol) in anhydrous dichloromethane (10 mL) was added through a dropping funnel. The solution was stirred overnight at room temperature with a drying tube. The reaction mixture was filtered and the precipitate was washed with a small amount of chloroform. The filtrate was concentrated on a rotary evaporator and was then purified by flash column on silica gel starting with pure chloroform and increasing the polarity to chloroform/methanol (1:2) in a stepwise fashion. The yield was 47%.

IR (CHCl₃): 3455 (bs), 3029 (m), 3023 (m), 3017 (m), 3011 (m), 2980 (m), 2942 (m), 2880 (m), 1736 (s), 1705 (s), 1679 (s), 1605 (w), 1588 (w) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 8.21-7.76 (18 H, m, Ar-H), 6.19 (1 H, broad, NH), 5.44 (1 H, m), 4.82 (1 H, broad, NH), 4.38 (1 H, dd, J = 12.3, 3.0 Hz), 4.17 (1 H, dd, J = 12.3, 6.0 Hz), 3.29 (4 H, t, J = 7.4 Hz), 3.23 (2 H, t, J = 5.3 Hz), 3.13 (2 H, t, J = 5.3 Hz), 2.41 (6 H, m), 2.13 (4 H, m), 1.40 (9 H, s); ¹³C NMR (75 MHz, CDCl₃): δ 213.2, 173.0, 172.4, 168.9, 120-123 (many peaks), 79.6, 68.7, 64.3, 40.7, 39.9, 37.6, 33.6, 33.4, 32.5, 32.4, 28.2, 26.5, 26.4; HRMS Exact mass: calcd for C₅₁H₅₀N₂O₇ [M]⁺, 802.3620. Found 802.3647.

Preparation of 2.2

Trifluoroacetic acid (0.5 mL) was added to compound **2.1** (0.38 g). The solution was allowed to stand for 15 minutes and was concentrated on a rotary evaporator. Acetone (3 mL) and potassium carbonate (0.32 g, 5.0 equivalents) were added to the solution which was then cooled in an ice bath. Iodomethane (3.0 g) was added to the solution which was refluxed at 65 °C overnight and cooled to room temperature. Chloroform (5.0 mL) was added and the reaction mixture was filtered to remove inorganic salts. The filtrate was concentrated and compound **2.2** was obtained by flash column chromatography on silica gel using a gradient elution from pure chloroform to chloroform/methanol 1:1. The yield was 61%.

IR (CHCl₃): 2953 (s), 2880 (w), 1734 (s), 1671 (s) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 8.21-7.76 (18 H, m, Ar-H), 5.46 (1 H, m), 4.32 (1 H, dd, J = 12.8, 3.9 Hz), 4.12 (1 H, dd, J = 12.8, 6.0 Hz), 3.43 (2 H, s), 3.40 (2 H, s), 3.10 (4 H, s), 2.82 (9 H, s), 2.51 (2 H, J = 6.0 Hz), 2.33 (4 H, m), 2.00 (4 H, m); ¹³C NMR (75 MHz, CDCl₃): δ 173.3, 173.0, 170.2, 121.6-135.3 (many peaks), 68.3, 64.51, 64.50, 54.2, 54.0, 53.8, 37.3, 33.9, 33.6, 32.3, 32.2, 26.5, 26.4; HRMS Exact mass: calcd for C₄₉H₄₉N₂O₅⁺ [M]⁺, 745.3644. Found 745.3658.

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CHAPTER 3

AN EFFICIENT SYNTHESIS OF (R)-3-HYDROXYCARBOXYLIC ACIDS AND THEIR DERIVATIVES

INTRODUCTION

Chiral 3-Hydroxycarboxylic Acids as Moieties of Natural Products

(R)-3-Hydroxycarboxylic acids and their derivatives are found as moieties in many biologically important natural products, such as lipid A,^{1, 2, 3} secretion products of the leaf-cutting ants,^{4, 5} topstin,⁶ syringostatin,⁷ extracellular glycolipid⁸ and eupassofilin.⁹

(R)-3-Hydroxycarboxylic acids are important moieties of lipid A (**Figure 3.1**), which represents the lipophilic part of the cell surface lipopolysaccharides (LPS, endotoxin) of Gram-negative bacteria and is responsible for most of the biological activities of the LPS, e.g. endotoxicity and antitumor activity.^{1, 2, 3} Lipid A from many different bacterial species shares a common basic structure composed of a β (1'-6) linked D-glucosamine disaccharide moiety.¹⁰ This disaccharide is phosphorylated at hydroxyls 1 and 4' and O, N-polyacylated at positions 2, 2', 3 and 3', usually with (R)-3-hydroxy and/or (R)-3-acyloxy fatty acids. The length of the fatty acid chain varies, and the most common acid is (R)-3-hydroxytetradecanoic acid.

Three (R)-3-hydroxycarboxylic acids [(R)-3-hydroxyhexanoic acid, (R)-3-hydroxyoctanoic acid and (R)-3-hydroxydecanoic acid] are the secretion products of *Atta sexdens* (the leaf-cutting ants from South America), which is a highly developed evolutionary form of the colony-building ants.⁴ These ants demonstrate a uniquely

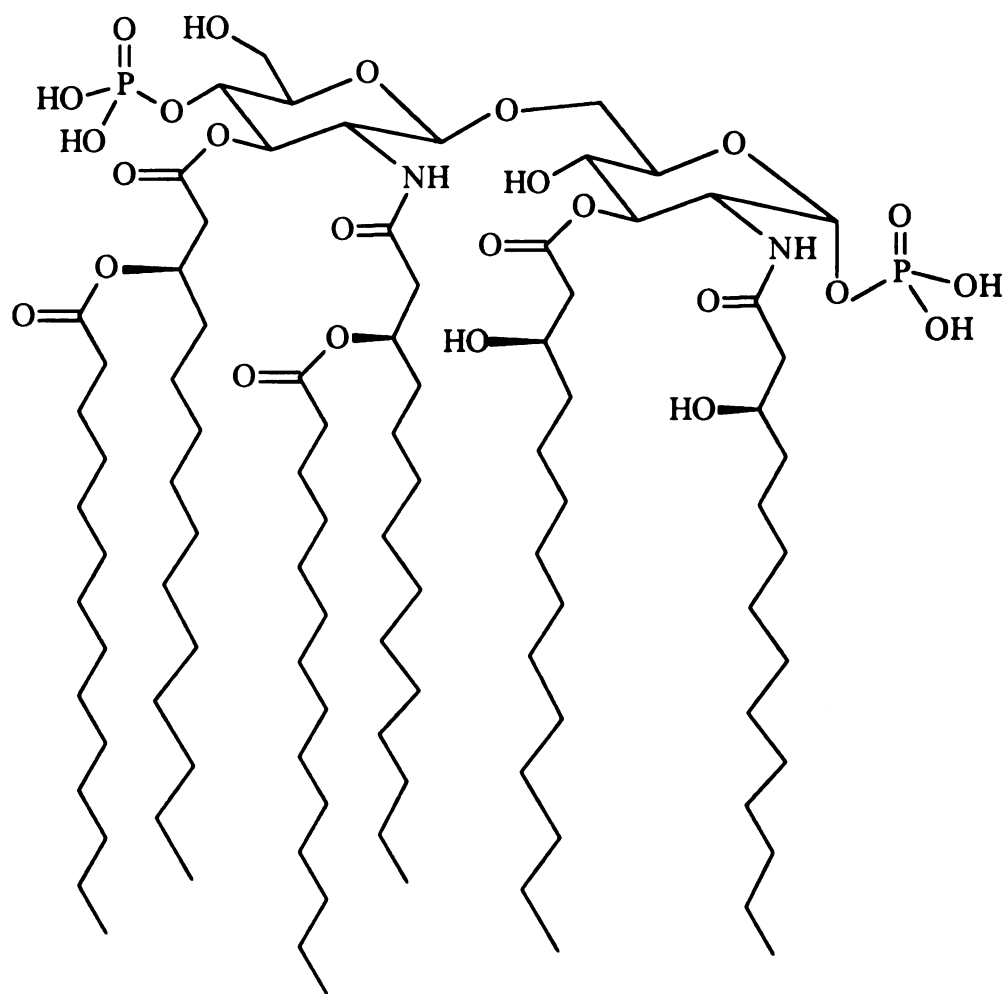


Figure 3.1: A typical structure of lipid A.

effective way of establishing and maintaining their “fungus gardens” as a source of food.⁵ Their behavior has been the subject of many investigations. (-)-(R)-3-Hydroxydecanoic acid found in a secretion of the leaf-cutting ants has been shown to have the strongest germination-inhibiting effect among the (R)-3-hydroxy fatty acids. The harvest ants prevent the gathered grass seed from sprouting and the leaf-cutting ants prevent the intruding spores from sprouting by using Myrmicacin, (R)-3-hydroxydecanoic acid, as a herbicide.

(R)-3-Hydroxyhexadecanoic acid and (R)-3-hydroxy-15-methylhexadecanoic acid are important moieties of topostins, which are the novel inhibitors of mammalian DNA topoisomerase I (topo I) isolated from a culture broth of *Flexibacter topostinus* sp. nov.⁶ Topostin B (**Figure 3.2**), the most active topostin, was a 1:1 mixture of two analogs with molecular weights of 553 and 567. These two substances are lipids containing amino acids. Each of them contains one glycine with the amino group acylated by a fatty acid. The fatty acids are (R)-3-hydroxyhexadecanoic acid in topostin B553 and (R)-3-hydroxy-15-methylhexadecanoic acid in topostin B567 with the 3-hydroxyl groups acylated by another fatty acid. These (R)-3-hydroxycarboxylic acid moieties are shown to play important roles in the activity of topostin B.

Both 3-hydroxydodecanoic acid and 3-hydroxytetradecanoic acid have been found as components of syringostatins, phytotoxins produced by *Pseudomonas syringae* pv. *syringae* which damages crops, fruit trees and various plants world wide.⁷ In the 1980s, lilac blight spread through the central parts of Japan, and *Pseudomonas syringae*

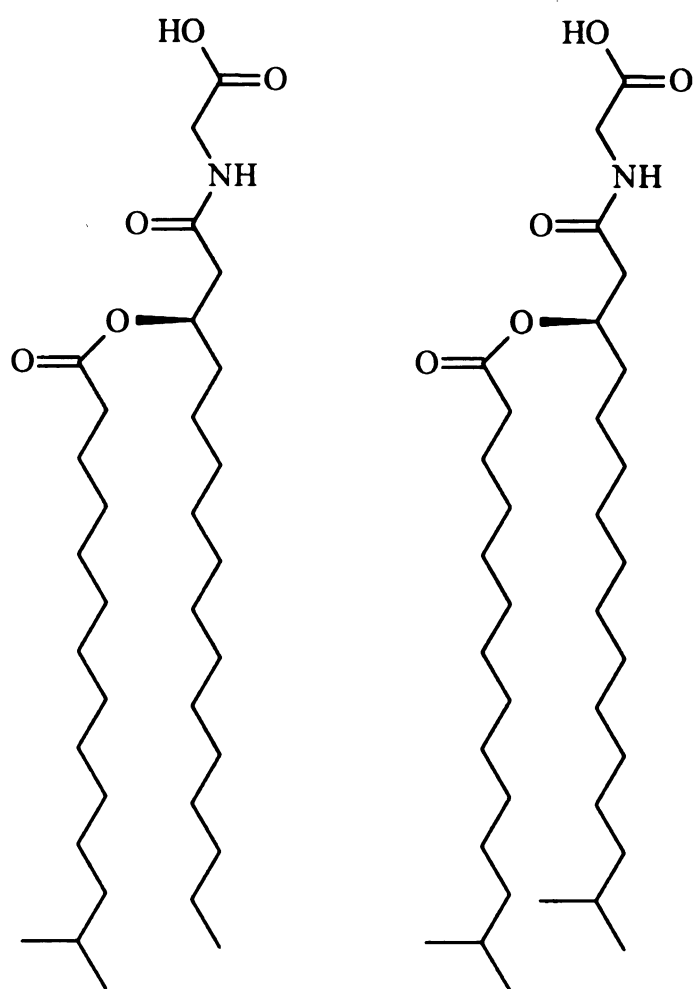


Figure 3.2: The structures of topostins B (B553 and B567) from *Flexibacter topostinus* sp. nov.

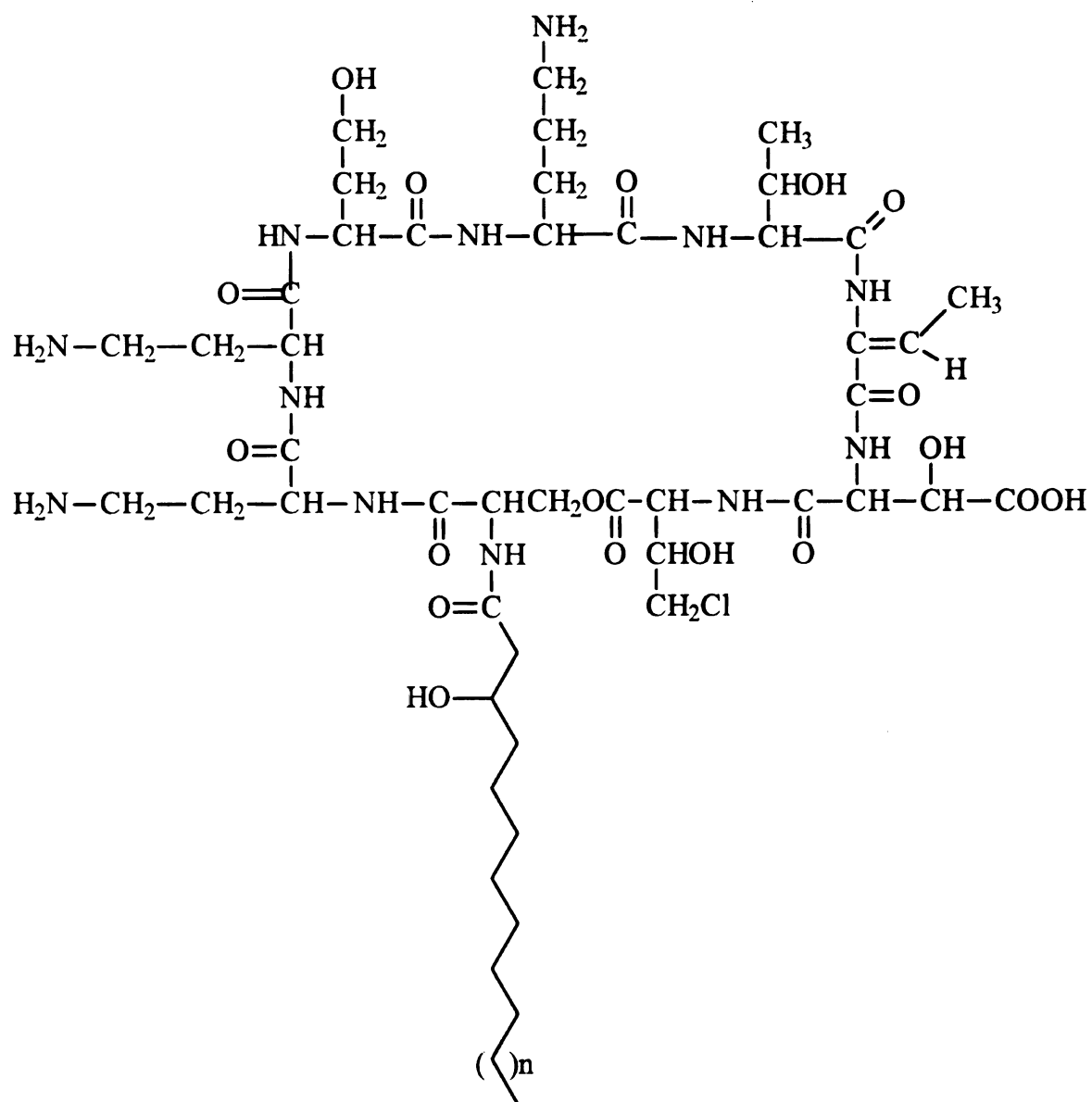


Figure 3.3: The structure of syringostatins A ($n = 1$) and E ($n = 3$). Note that the stereochemistry of the 3-hydroxy fatty acid moieties has not been determined.

pv. syringae was isolated as the causative bacterium of the disease. In 1992, a strain of the bacterium isolated from lilac was found to produce a homologous mixture of phytotoxins. The toxins were termed syringostatins and the structures of the main components, syringostatins A and E, were elucidated by 2D-NMR spectroscopy and mass spectrometry. As shown in **Figure 3.3**, a syringostatin is a cyclic peptide to which 3-hydroxydodecanoic acid or 3-hydroxytetradecanoic acid is attached through an amide bond. But the chirality of the 3-hydroxy fatty acids has not been determined yet.

(R)-3-Hydroxyhexadecanoic acid and (R)-3-hydroxyoctadecanoic acid have been found as constituents of extracellular glycolipids from the red yeast *Rhodotorula*.⁸ These glycolipids consist of a mixture of mannitol and pentitol esters of the 3-hydroxycarboxylic acids. One molecule of the long chain acids is attached to each polyol molecule and most of the remaining hydroxyl groups, including the one on the fatty acid are acetylated.

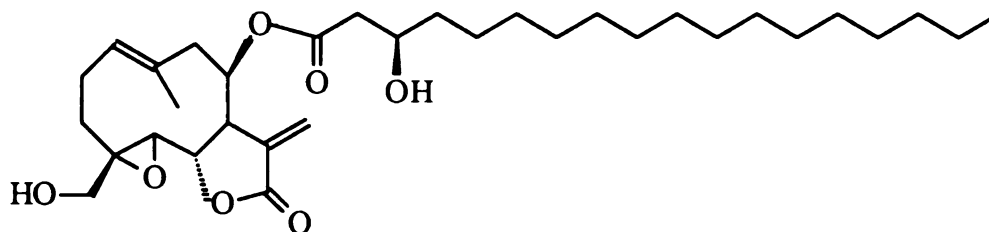


Figure 3.4: The structure of eupassofilin.

(R)-3-Hydroxyoctadecanoic acid is a moiety of eupassoflin, a cytotoxic and antitumor germacranolide, which was isolated from *Eupatorium hyssopifolium* L.⁹ It consists of a sesquiterpene lactone and (R)-3-hydroxyoctadecanoic acid moiety in its lipid ester side chain (**Figure 3.4**).

Chiral 3-Hydroxycarboxylic Acids as Building Blocks in Total Syntheses

Chiral 3-hydroxycarboxylic acids and their derivatives have been used as chiral building blocks in the total syntheses of lipstatin,¹¹ (-)-tetrahydrolipstatin (THL),¹² esterastin, valilactone,¹³ panclicins A-E, and (+)-brefeldin A.¹⁴

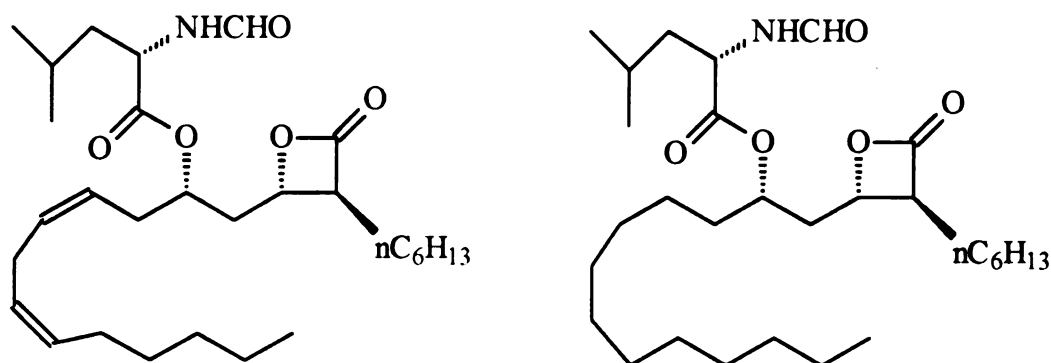


Figure 3.5: The structures of lipstatin and tetrahydrolipstatin.

(R)-3-Hydroxytetradecanoic acid is the chiral starting material for the synthesis of Xenical, a drug for obesity. Obesity is one of the most prevalent clinical conditions

affecting the world. It is estimated that more than 34 million people suffer from it in the USA alone. Scientists at the Hoffman-La Roche Company showed that lipstatin (**Figure 3.5**), a metabolite of *Streptomyces toxytricini*, is a potent and irreversible inhibitor of pancreatic lipase, a key enzyme for the digestion of dietary fat. Its hydrogenated derivative, (-)-tetrahydrolipstatin, has essentially the same inhibitory activity, blocks fat absorption selectively, and has shown in several animal models anti-obesity and anti-hypercholesterolemic activity.¹⁵ In addition to pancreatic lipase, other lipases, such as carboxylester lipase, gastric lipase, and the bile-salt-stimulated lipase of human milk, are also inhibited by THL. In contrast, several bacterial lipases, phospholipase A₂, liver esterase, trypsin, and chymotrypsin are not inhibited by THL. THL is therefore considered to be the first selective irreversible lipase inhibitor.

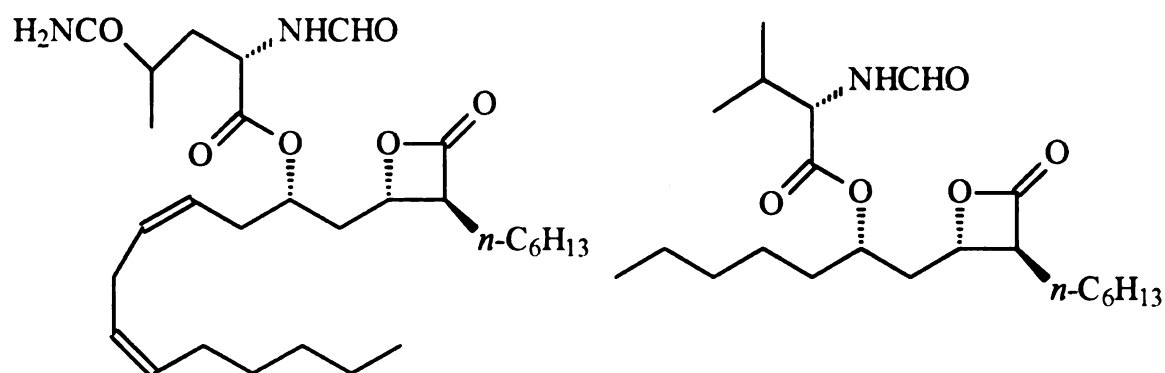


Figure 3.6: The structures of esterastin and valilactone.

A striking feature of lipstatin and THL is the strained and high reactive oxetanone ring (β -lactone) with two alkyl chains, one of which bears an α -N-formylamino acyloxy group. The oxetanone ring is also found in a number of microbial esterase inhibitors

including esterastin (**Figure 3.6**), valilactone, and panclicins A-E. The enhanced reactivity of the oxetanone ring is central to the biological activity of lipstatin and tetrahydrolipstatin.

Clinical studies have shown that a diet supplemented by 50 mg of tetrahydrolipstatin per day leads to sustained weight loss in humans. It has now been developed for the treatment of obesity by the Hoffmann-La Roche Company and is marketed under the tradename Xenical (or Orlistat). Xenical was already approved in European Union, several Latin American countries and the Far East in the summer of 1998. Despite its lower activity, tetrahydrolipstatin has received far more attention in synthetic and biological studies than its parent, lipstatin, because THL is both simpler and more stable and is much easier to synthesize than lipstatin with two isolated *cis* carbon-carbon double bonds in the long chain. To date eight syntheses of THL have been reported, most of which are based on the classic cyclodehydration procedure of Adam or a diastereoselective Lewis acid-promoted [2 + 2] cycloaddition to create the 2-oxetanone ring from appropriate 3-hydroxycarboxylic acid precursors which are converted to the corresponding aldehydes with the 3-hydroxyl group protected (**Figure 3.7**).¹¹ The “incorrect” stereochemistry in the aldehydes is deliberately chosen as a stereocontrolling element in the construction of the oxetanone ring and as a convenient vehicle for introducing the (S)-N-formylleucine at the end of the synthesis under Mitsunobu conditions to accomplish the requisite inversion of configuration.¹² Given the strategic and tactical significance of the aldehydes, it is imperative to secure an efficient and highly enantioselective synthetic route.

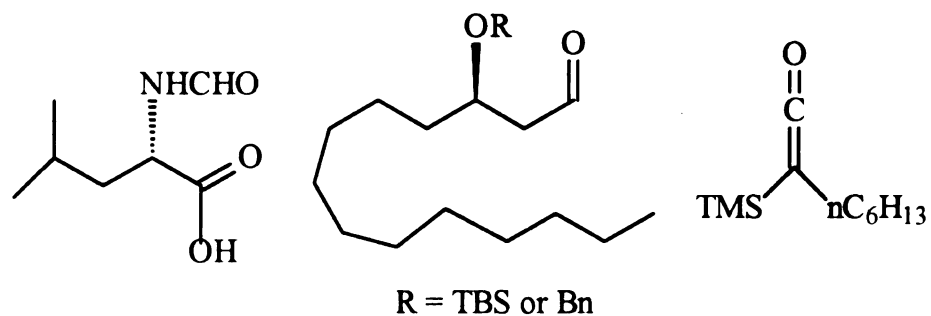


Figure 3.7: A typical retrosynthesis of tetrahydrolipstatin.

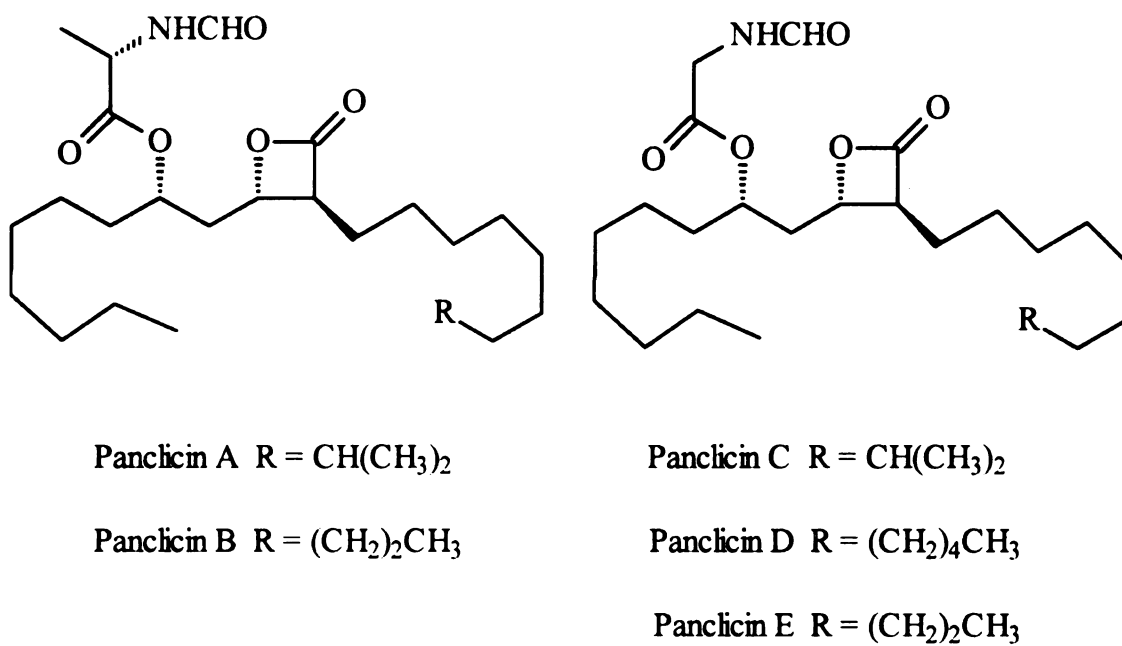


Figure 3.8: The structures of panclicins A-E.

As inhibitors of pancreatic lipase, panclicins C, D, E (**Figure 3.8**) are twice as potent as THL ($IC_{50} = 1.2 \text{ mM}$). SmithKline Beecham Pharmaceuticals claimed that they had finished the synthesis of all five Panclicins based on their syntheses of lipstatin and tetrahydrolipstatin.

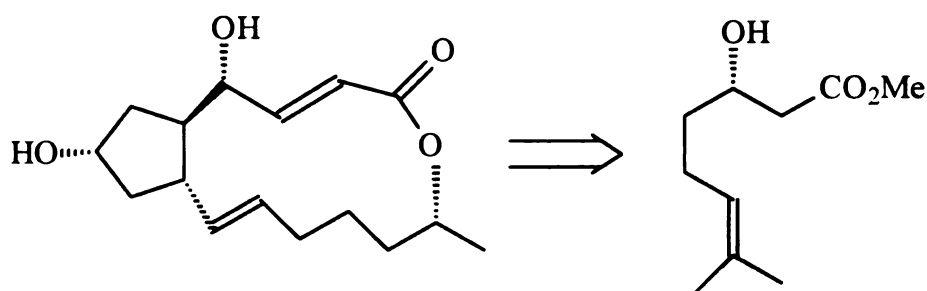
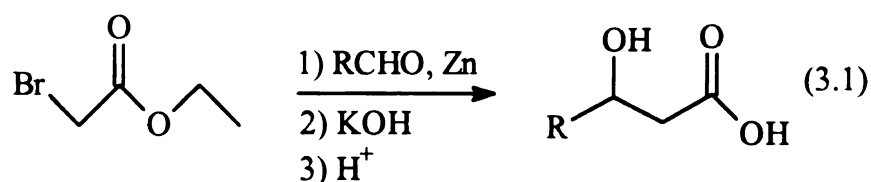


Figure 3.9: (-)-Brefeldin A and its chiral starting material.

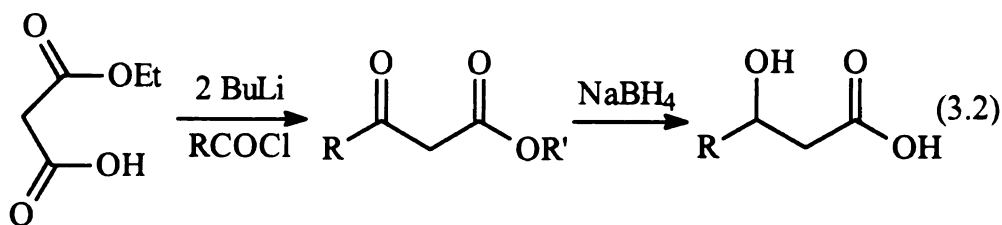
(S)-3-Hydroxy-7-methyl-6-octenoic acid methyl ester was the key starting material for the synthesis of (+)-brefeldin A (**Figure 3.9**),¹⁴ which was isolated from *Penicillium decumbens* and shown to have both antifungal and antiviral activity. Its antiviral activity is due to inhibition by brefeldin A of the intracellular transport of secretory proteins. The finding that brefeldin A specifically blocks the movement of proteins from the *endoplasmic reticulum* to the Golgi apparatus has made it a powerful tool for biochemical investigation. In the years since Corey's initial report,¹⁶ numerous partial¹⁷ and total¹⁸ syntheses have been described. These syntheses utilize the 3-hydroxy acid derivative as a chiral building block. The stereogenic center bearing the hydroxyl group of the five-member ring is from that of the 3-hydroxy acid ester. Today brefeldin A is commercially available from Sigma (Product No. B7651).

Synthetic Approaches to Racemic 3-Hydroxycarboxylic Acids and Their Derivatives

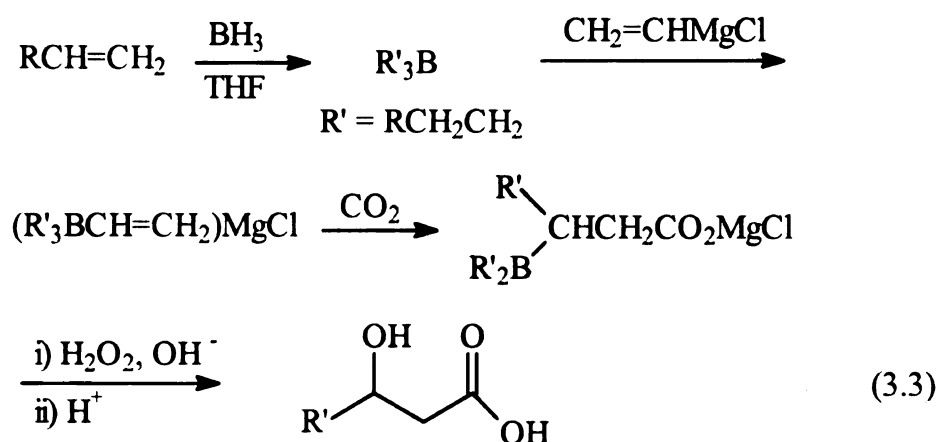
Since 3-hydroxycarboxylic acids have important biological functions, many synthetic methods have been developed to synthesize them and their derivatives, such as the 1,3-diol. Thaler and Geist started with a Zinc Reformatsky reaction (Equation 3.1) as early as in 1939.¹⁹ This method is the most widely used one to synthesize 3-hydroxy fatty acids.



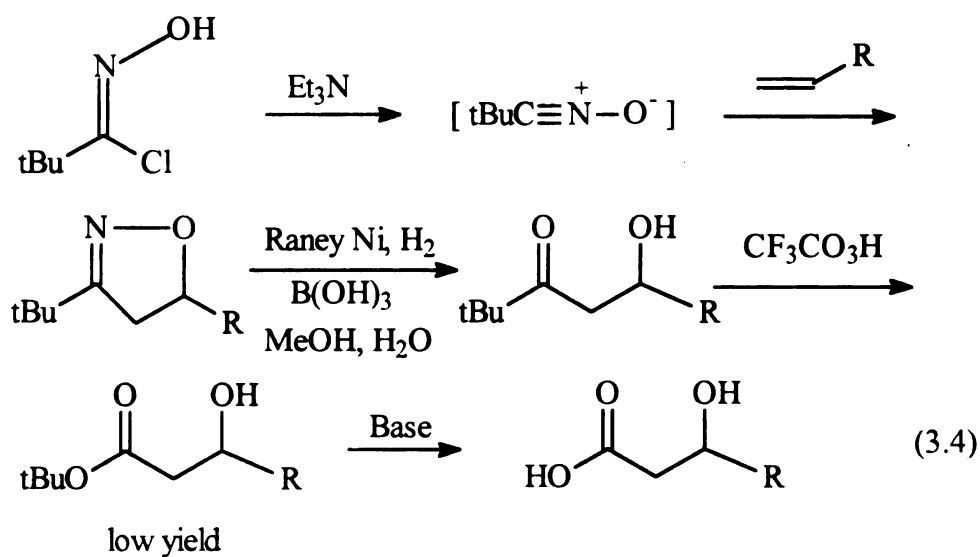
Another method to obtain 3-hydroxy fatty acids is to prepare 3-keto fatty acids or esters followed by reduction of the keto group to the hydroxyl group (Equation 3.2). The first step is done under a harsh condition by using BuLi to generate the anion. Since n-BuLi is not easy to handle, this limits the reaction to a small scale. Several reducing reagents, including sodium borohydride and H₂ / Raney nickel, have been employed to reduce the keto group to the hydroxyl group.^{20, 21}



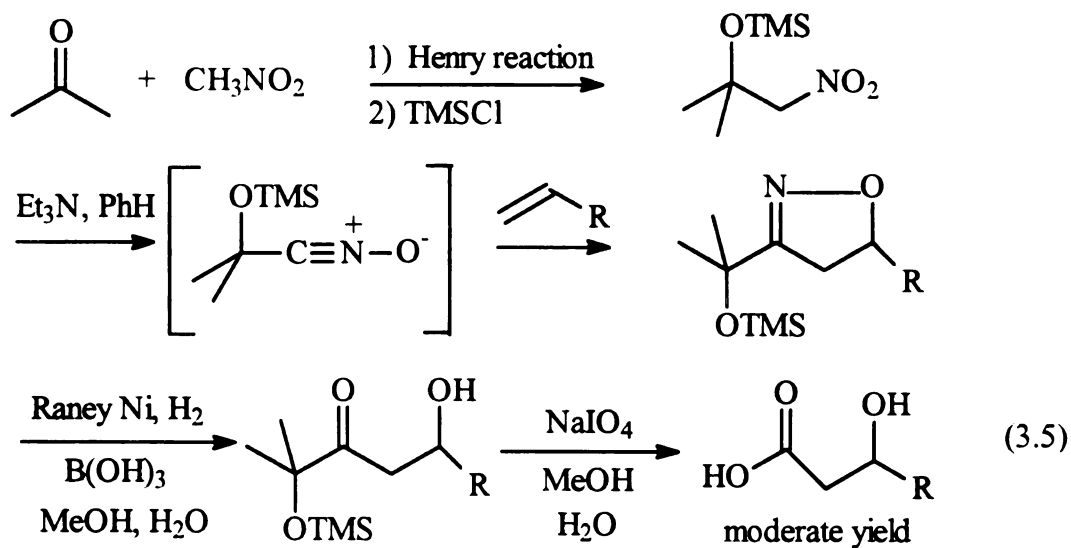
Negishi *et al.* reported a novel method for the preparation of 3-hydroxycarboxylic acids from alkenes via the dialkyl(2-ethoxycarbonylethenyl)borane.²² Deng *et al.* modified Negishi's method and improved the yield.²³ By treating trialkylvinylborate with carbon dioxide, in which any simple alkene may be used, highly regioselective products can be obtained (Equation 3.3). This method thus permits the maximum conversion of a wider range of alkenes into 3-hydroxy fatty acids.



Three separate methods were reported for the formation of 3-hydroxy acid derivatives from readily available substituted Δ^2 -isoxazolines.²⁴ In the first approach, cycloaddition of 2,2-dimethylpropanenitrile oxide with a variety of olefins followed by reductive cleavage produces α' -*tert*-butyl- β -hydroxyketones (Equation 3.4). These were cleaved to β -hydroxy *tert*-butyl esters by Baeyer-Villiger oxidation with peroxytrifluoroacetic acid.

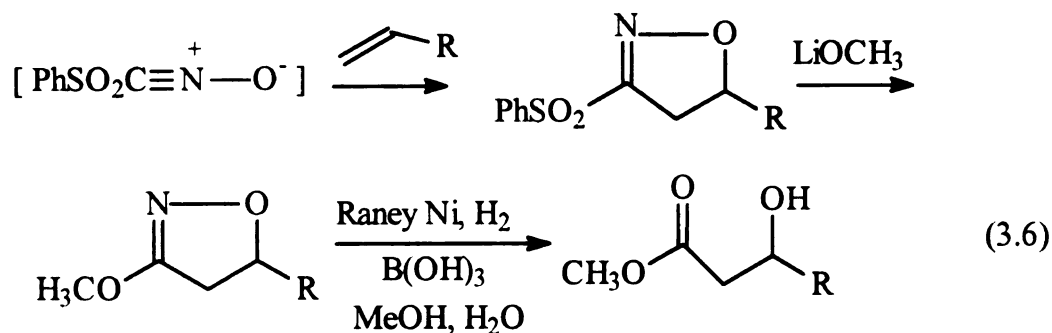


In the second approach, α',β -dihydroxy ketones were generated via cycloaddition of olefins with the nitrile oxide generated from 2-[(trimethylsilyl)oxy]-2-methyl-1-nitropropane followed by reductive ring opening (Equation 3.5). Standard periodic acid cleavage gave β -hydroxy acids.



]

In the third approach, 3-methoxy-substituted Δ^2 -isoxazolines, readily available via benzenesulfonylcarbonitrile oxide-olefin cycloaddition and methoxide displacement, are directly reduced to 3-hydroxy esters in moderate yields (Equation 3.6).

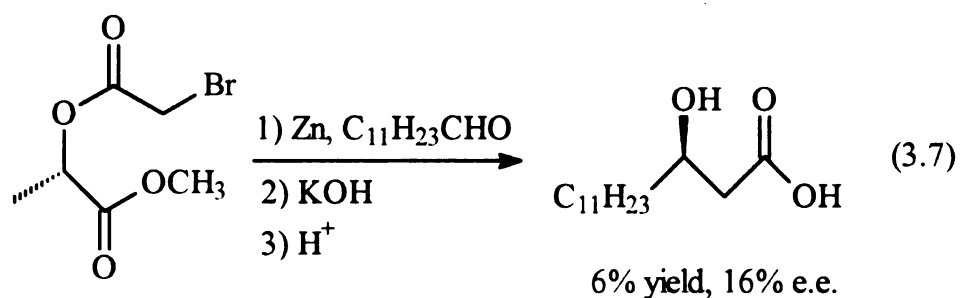


Asymmetric Synthesis of Chiral 3-Hydroxycarboxylic Acids and Their Derivatives

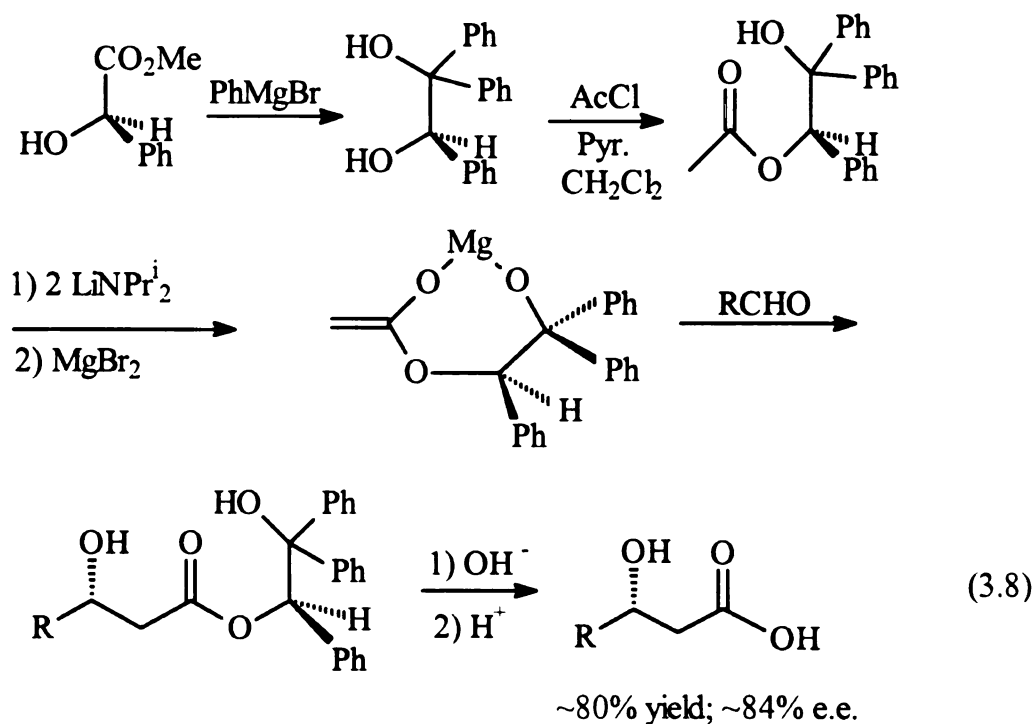
The asymmetric synthesis of 3-hydroxy fatty acids has been extensively investigated due to the importance of their biological activities. Many asymmetric syntheses are based on the methods described earlier, generally speaking, chemically and enzymologically.

Although the Zinc Reformatsky reaction had been applied for the preparation of 3-hydroxyacids, the possibility of the applicability of easily accessible lactic acid derivatives as chiral Reformatsky reagents for the preparation of 3-hydroxyacids was not exploited until 1989 (Equation 3.7). The best result was obtained by using (1-carbomethoxyprop-2-yl)bromoacetate, easily prepared by the reaction of bromoacetyl

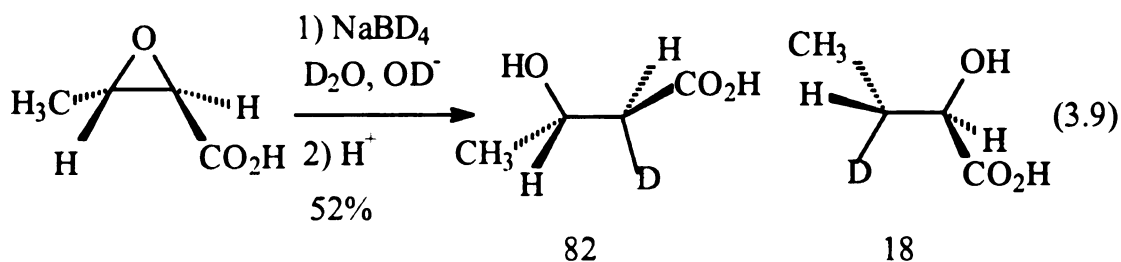
bromide on (S)-methyl lactate. Both yields and ee% were very low for 3-hydroxy fatty acids.²⁵



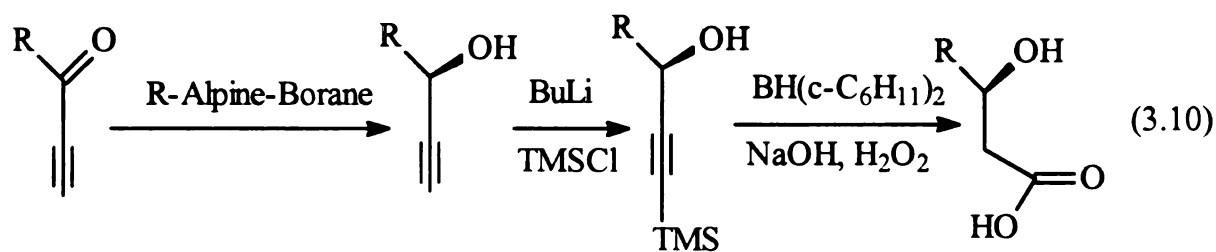
Braun *et al.* described stereoselective aldol reactions, using the dianion enolate, easily available by double deprotonation of (R)-2-acetoxy-1,1,2-triphenylethanol, as a synthetic equivalent of the chiral acetate enolate.²⁶ The chiral auxiliary agent, the diol, was regenerated by the hydrolysis without racemization (Equation 3.8).



Reduction of 2,3-epoxyalkanoic acids with sodium borohydride provides 3-hydroxy acids and 2-hydroxy acids with the former ones being dominant (Equation 3.9). This method is of general utility in stereospecific syntheses of metabolically important 3-hydroxy acid derivatives labeled at C-2 when sodium borodeuteride is used.²⁷

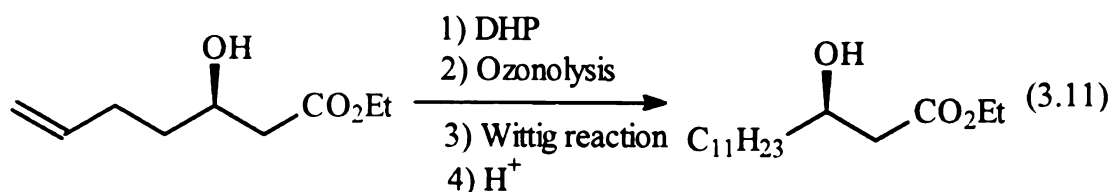


An enantioselective reduction of the ynone (78%, 84% e.e.) using R-Alpine-Borane[®] was reported.²⁸ The resultant alkynol was converted to the 1-trimethylsilyl derivative which underwent smooth hydroboration-oxidation to the 3-hydroxy acid (Equation 3.10).



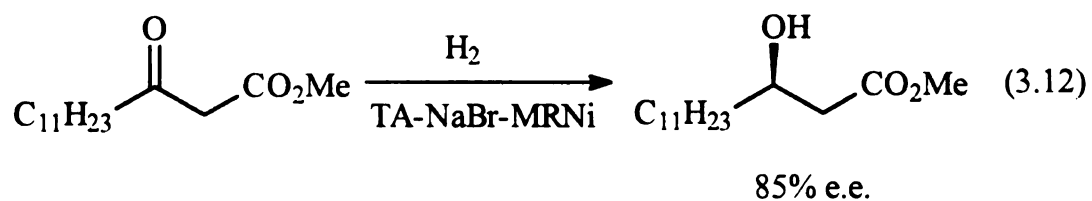
The elongation of a 3-hydroxy acid ester was achieved by Barbier *et al.*²⁹ The known ethyl (R)-3-hydroxy-6-heptenoate was protected as its THP ether. This was followed by ozonolysis to give the aldehyde. The chain was elongated by a Wittig

reaction. This was followed by removal of the THP group and hydrogenation to give (R)-3-hydroxytetradecanoic acid (Equation 3.11).

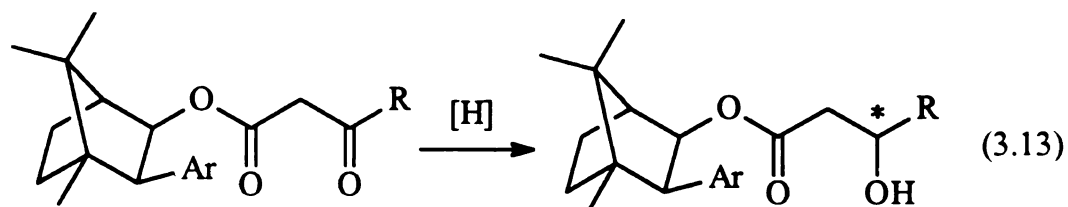


The 3-oxoalkanoic acids and their derivatives, especially their esters, have been asymmetrically reduced by both chemical methods and enzymatical methods.

The enantioface-differentiating hydrogenation of methyl 3-oxotetradecanoate over (R,R)-tartaric acid-NaBr-modified Raney nickel (TA-NaBr-MRNi) under the pressure of hydrogen at 95 kg/cm² at 100°C gave methyl (R)-3-hydroxytetradecanoate in 85% e.e. (Equation 3.12).³⁰ After the saponification of methyl (R)-3-hydroxytetradecanoate, the crude acid was converted to its dicyclohexylammonium salt, which was recrystallized three times from acetonitrile and then treated with acid to give (R)-3-hydroxytetradecanoic acid.

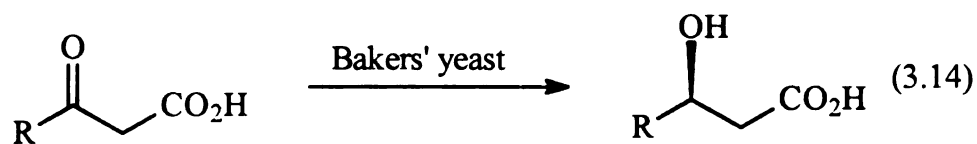


Recently, 3-keto esters were asymmetrically hydrogenated to 3-hydroxy esters with 77-99 % e.e. at atmospheric pressure using chiral ruthenium (II) catalysts prepared by treatment of (COD)Ru(2-methylallyl)₂ in the presence of chiral ligands such as Binap.³¹

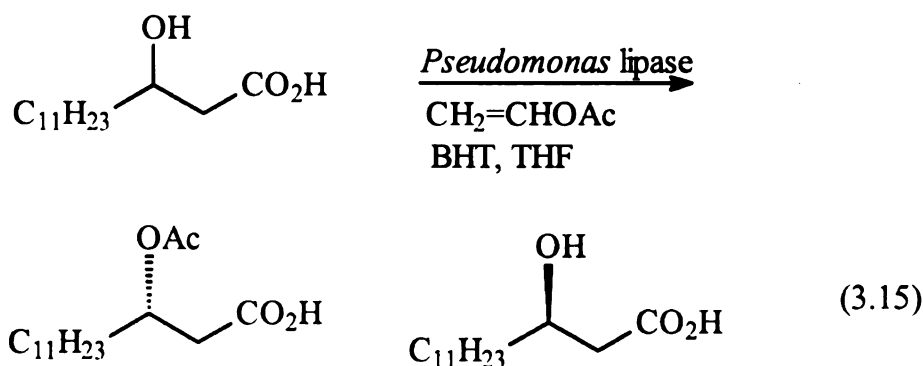


Asymmetric reduction of 3-keto esters using a chiral auxiliary was achieved through differentiating the keto faces (Equation 3.13). Hydride reduction can then proceed either via the transition state in which the carbonyls are syn ($\text{ZnCl}_2 / \text{Zn}(\text{BH}_4)_2$, R / S = 92:8) or via the alternative transition state in which the carbonyls are anti (Dibal-BHT, R / S = 4:96).³²

Asymmetric reduction of aliphatic short- to long-chain 3-keto acids, ranging from 3-oxobutanoic to 3-oxooctadecanoic acids, by use of fermenting bakers' yeast has been extensively investigated by Utaka *et al.* (Equation 3.14).³³ The bakers' yeast works very well for medium-chain substrates with 98% e.e., but short-chain ones such as 3-oxobutanoic acid give only 86% e.e. Inhibition of fermentation for long-chain substrates from 3-oxoundecanoic acid, leading to no reduction, has been observed. For all substrates described above, the yeast gives only from low to moderate chemical yields.



Wong's group chose the lipase-catalyzed kinetic resolution of racemic 3-hydroxytetradecanoic acids (Equation 3.15). The mixture of the racemates was treated with *Pseudomonas* lipase (lipase PS-30, Amano) in a mixture of vinyl acetate and THF in the presence of the polymerization inhibitor, di-*t*-butyl *p*-cresol (BHT) at 60°C. A preferential acetylation of (S)-form was observed. Unacetylated (R)-form was also separated (46.9%, 88% e.e.).³⁴



As described earlier, chiral 3-hydroxycarboxylic acids are not only constituents of many biologically important compounds such as lipid A, but also important chiral building blocks in the syntheses of many important drugs such as tetrahydrolipstatin. A chiral pharmaceutical drug usually requires high optical purity because its enantiomer or diastereoisomer is a different molecule and has unnecessary or even harmful effects.

Since there are four chiral 3-hydroxy fatty acid moieties in a typical lipid A, the synthesis of lipid A requires highly optically pure 3-hydroxy fatty acids. If the 3-hydroxy acid with 93% optical purity is utilized, the resulting lipid A will be only 75% ($0.93^4 \times 100\%$) optically pure and have 16 isomers. This will cause difficulties in separating the 16 isomers. Although many methods for synthesis of 3-hydroxy acids and their derivatives have been reported, the current syntheses of chiral 3-hydroxy acids and their derivatives either give products in low yield, in low optical purity or are limited to small-scale preparation. Because of the importance of the chiral 3-hydroxy acids and their derivatives and the requirement for high optical purity, it is imperative to develop a general method for synthesizing chiral 3-hydroxy acids and their derivatives.

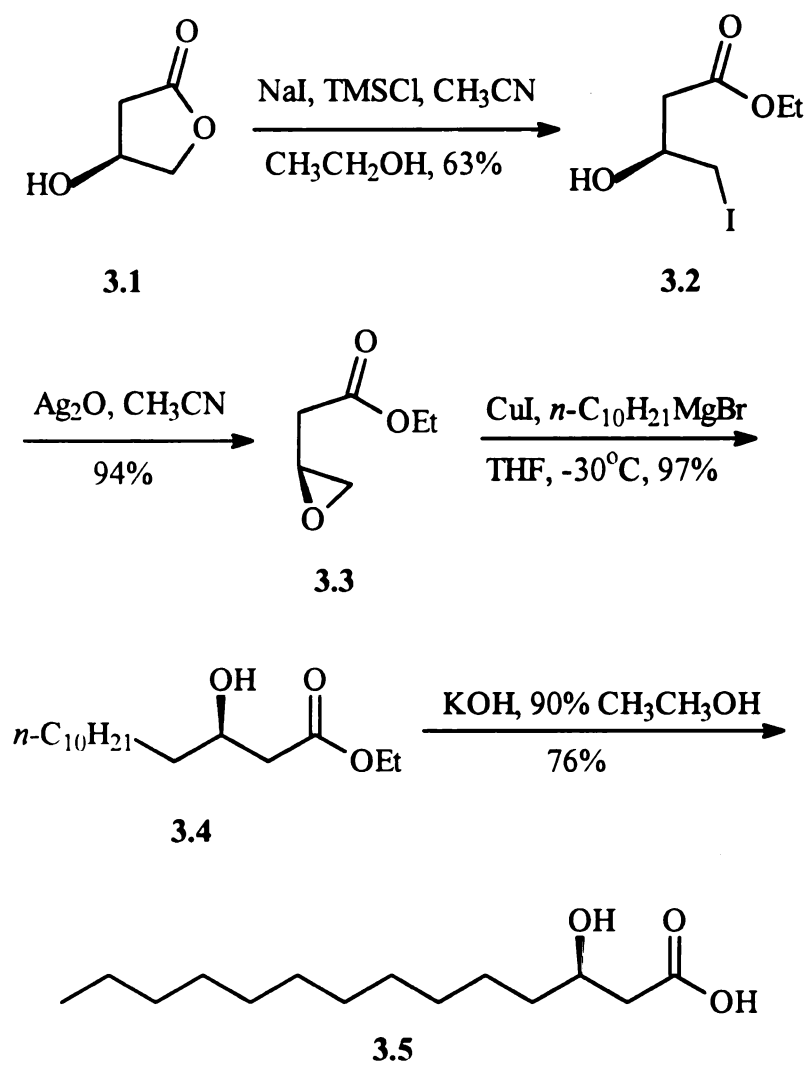
RESULTS AND DISCUSSION

(S)-3-Hydroxy- γ -butyrolactone was obtained from maltose monohydrate by degradation with hydrogen peroxide in basic conditions.^{35, 36} Since the source of the chirality at the β position was from C-5 of the glucose moiety, the resulting lactone has very high optical purity. In addition to this, it is very inexpensive. It was, therefore, chosen as an excellent chiral building block for the synthesis of (R)-3-hydroxycarboxylic acids and their derivatives. Here, the synthesis of (R)-3-hydroxytetradecanoic acid was used as an example because of its importance among this class of compounds.

Strategically the chirality of (R)-3-hydroxytetradecanoic acid is derived from the lactone. Thus, a ten-carbon moiety needs to be connected to the four-carbon unit of (S)-3-

hydroxy- γ -butyrolactone. The long-chain moiety can be introduced as a donor (carbanion) from organometallic reagents, of which the first choice is the commercially available and inexpensive Grignard reagent, *n*-decyl magnesium bromide. The coupling between the donor and the acceptor together with the protection of the chirality without reducing its optical purity are the main elements of the synthesis. Thus, what acceptor to use and how to convert the chiral lactone into the acceptor are very important considerations for a successful coupling. Several chiral acceptors, including epoxides, halides and an aldehyde, were prepared from (R)-3-hydroxy- γ -butyrolactone. Several coupling methods were tried, and the only successful one was that between the chiral epoxybutyric acid ethyl ester and an organocopper reagent.

The successful synthesis is shown in **Scheme 3.1**. It is well known that in the reaction of lactones with trimethylsilyl iodide, the former undergo mild cleavage of the carbon-oxygen bond to provide the corresponding iodoalkylcarboxylic acids after hydrolytic work-up.³⁷ This reaction is catalyzed by hydroiodic acid, and it was shown that in some cases, by operating in the presence of alcohol, it was possible to isolate ω -iodocarboxylic ester. (S)-3-Hydroxy- γ -butyrolactone (**3.1**) (technical purity) in absolute ethanol was added to NaI-TMSCl-CH₃CN³⁸ at room temperature to give the iodohydrin (**3.2**) in 63% yield without any protecting of the free hydroxyl group. Although alcohols are prone to further transformation into iodides with TMSI, the presence of diiodocompounds was not detected. There are several advantages in this reaction. First, a mixture of NaI-TMSCl-CH₃CN was used to generate TMSI *in situ* instead of using the expensive and unstable TMSI directly. Second, the free hydroxyl group was stable



Scheme 3.1: Synthesis of (R)-3-hydroxytetradecanoic acid.

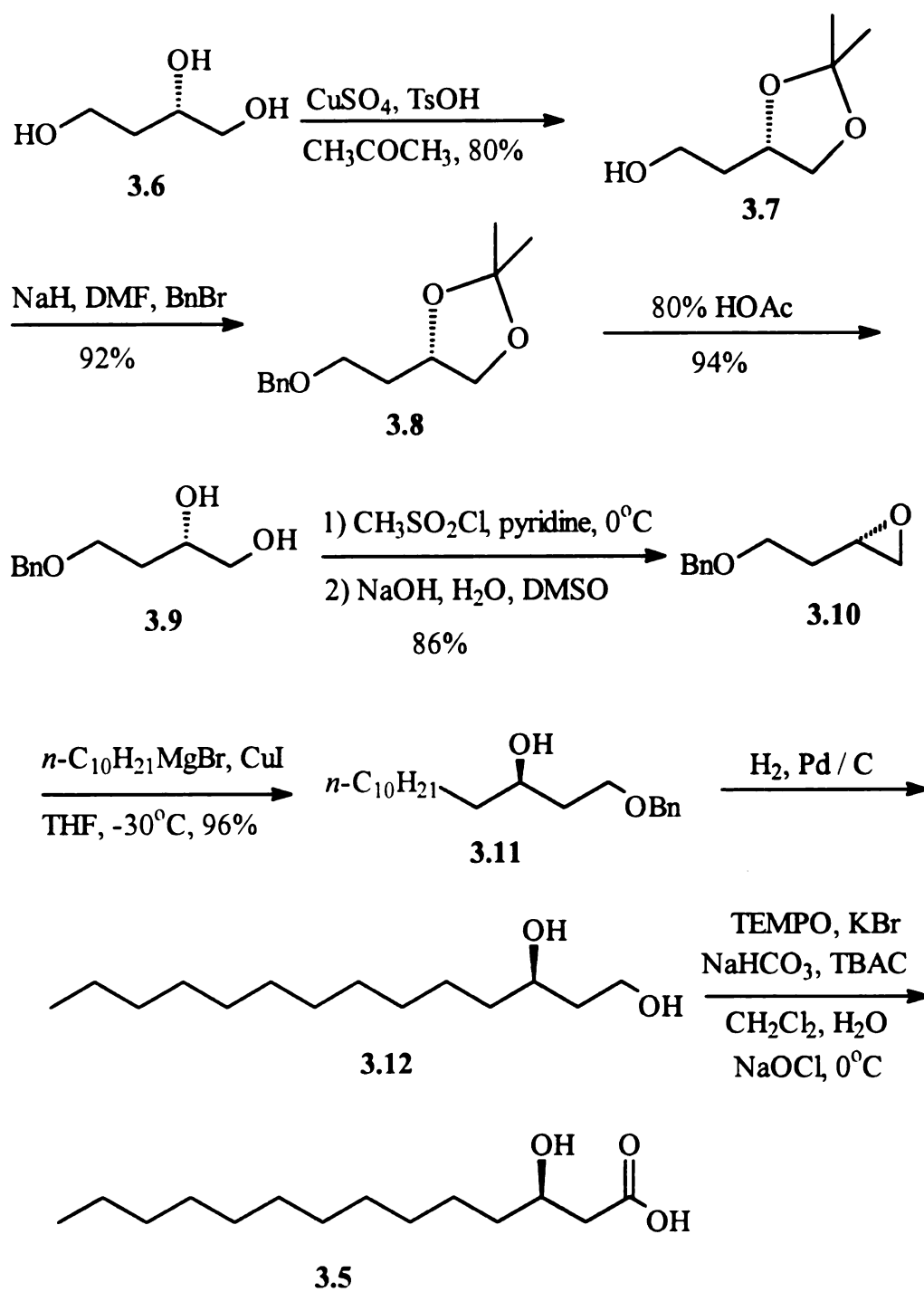
without any protection and did not undergo the transformations that affected the chirality. Third, the carboxylic acid group was protected as an ester with ethanol in the same step. Fourth, the product did not need any purification after the regular work-up procedure. This reaction was therefore efficient, inexpensive and practical.

Typical methods for cyclization of halohydrins into the corresponding epoxides require basic conditions. In the case of the 3-hydroxy-4-iodobutyric acid ester, the cyclization was rather difficult to control because of β -elimination to form an acrylate ester. Thus it was very important to choose the correct base. Silver oxide is a very mildly base and is excellent for carrying out the reaction without the risk of elimination. The iodohydrin (3.2) was cyclized to the desired epoxide ester (3.3) in high yield on treatment with silver oxide in acetonitrile. Since silver oxide is fairly expensive, it is better to add it in small portions to keep a slight amount in excess as indicated by the gray or greenish color of the reaction mixture. In this reaction the chirality at C-3 was preserved under the guise of an epoxide.

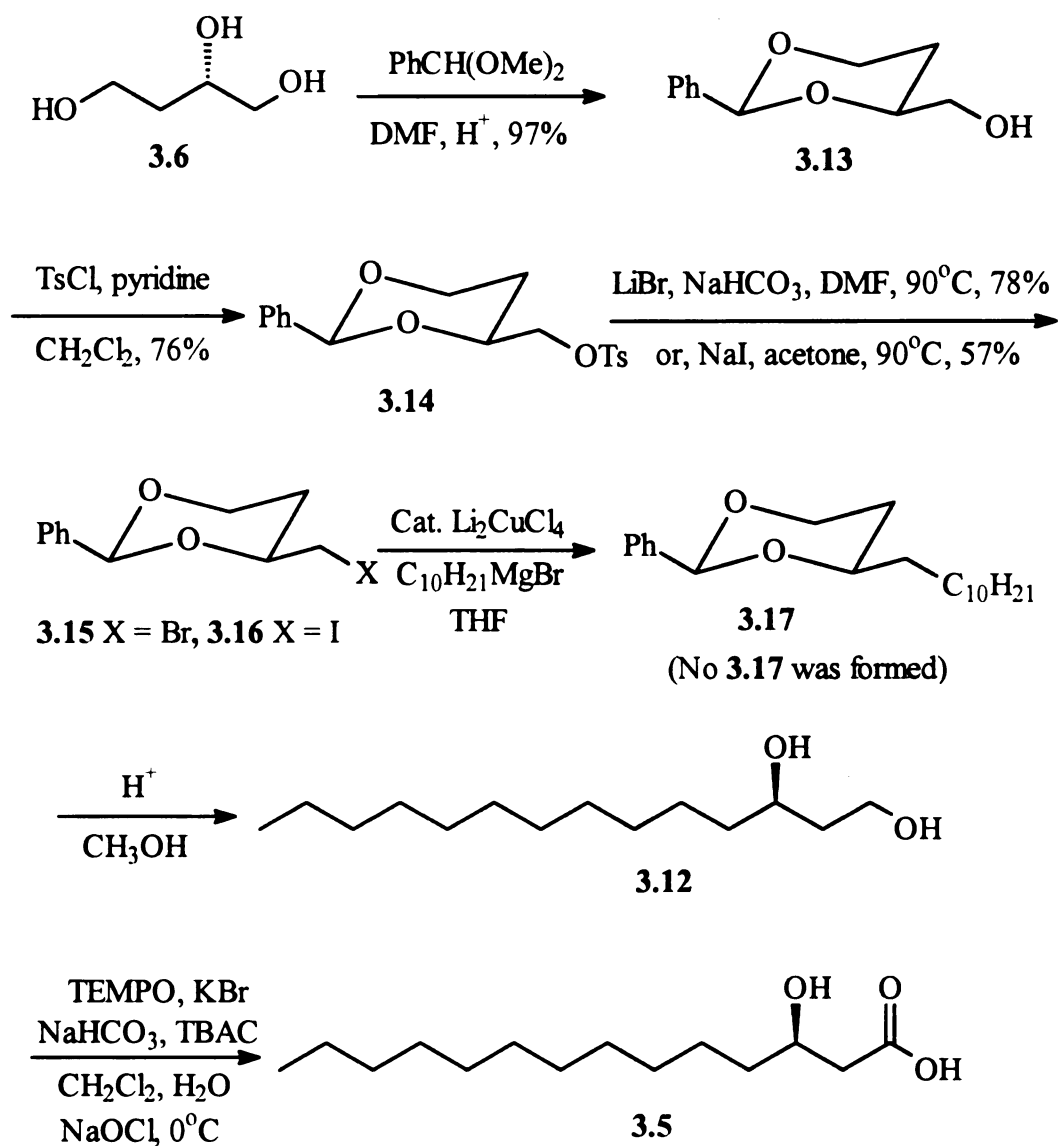
The sensitivity of the epoxide ester to a basic medium requires the use of a mild basic ten-carbon nucleophile in the coupling reaction. The nucleophile should not add to the carboxylic group nor promote the elimination of the epoxide ester. There are two major reactions of organocuprates, and both give products reminiscent of a carbon nucleophile: (1) reaction with halides or epoxides and (2) conjugate addition to the β -position of α , β -unsaturated carbonyls. Hence, *n*-decyl cuprate was chosen as the nucleophile and was generated *in situ* from the Grignard reagent and copper (I) iodide.

Selective ring opening was achieved by adding the epoxide ester to *n*-decyl magnesium bromide and CuI in anhydrous THF at $-30\text{ }^{\circ}\text{C}$. This gave (R)-3-hydroxytetradecanoic acid ethyl ester (**3.4**) in 97% yield. The coupling was exclusive at the C-4 position due to the steric effect. The optical purity of the desired product was 99.3% e.e., which was determined by NMR spectroscopy of the (S)-(-)- α -methoxy- α -(trifluoromethyl)phenylacetyl ester using the integration of the signals for the methine and the methoxyl hydrogens.³⁹ The free fatty acid (**3.5**) was obtained in 76% yield by saponification with potassium hydroxide in 90% ethanol followed by acidification.⁴⁰

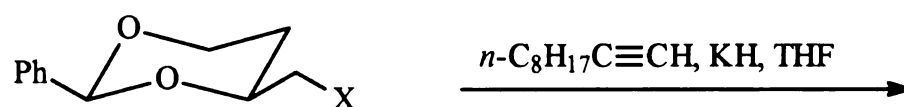
In the course of the synthesis of (R)-3-hydroxytetradecanoic acid, several chiral synthons, including the tosylate (**3.14**), halides (**3.15**, **3.16**), aldehyde (**3.20**) and epoxides (**3.10**), were derived from (S)-1,2,4-butanetriol (Synthon Corporation, Lansing, MI), the reduction product of (S)-3-hydroxy- γ -butyrolactone with sodium borohydride.⁴¹ (S)-1,2,4-Butanetriol (**3.6**) was converted into an isopropylidene derivative (**3.7**) on treatment with anhydrous acetone and anhydrous copper sulfate in acidic conditions (**Scheme 3.2**). The primary hydroxyl group at the C-4 position was protected as a benzyl ether derivative (**3.8**), and then the isopropylidene moiety was removed to release the two vicinal hydroxyl groups (**3.9**). The primary OH group was converted to a mesylate, which was then cyclized under basic conditions to give the epoxide derivative (**3.10**). The epoxide underwent a selective ring opening at the less sterically hindered position with *n*-decyl cuprate to give the (R)-3-hydroxytetradecyl benzyl ether (**3.11**). After debenzylation, the free primary hydroxyl group can be selectively oxidized with TEMPO to give (R)-3-tetradecanoic acid. One advantage of this synthetic route is that this epoxide



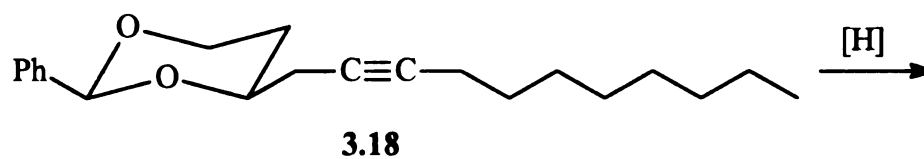
Scheme 3.2: Another synthetic approach to (R)-3-hydroxytetradecanoic acid.



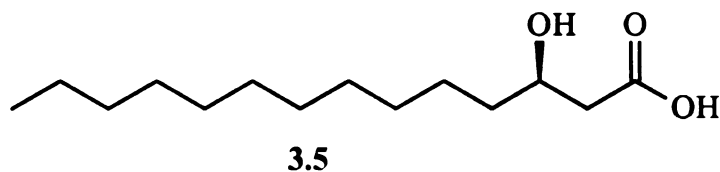
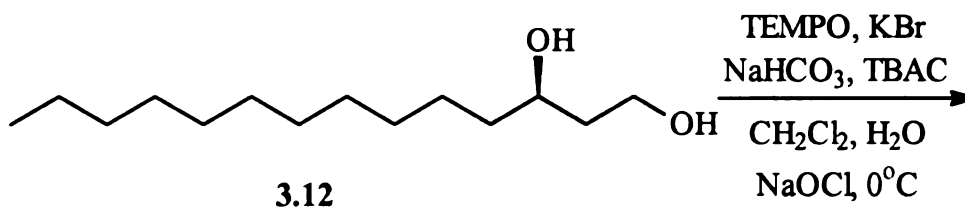
Scheme 3.3: Another synthetic approach to (R)-3-hydroxytetradecanoic acid.



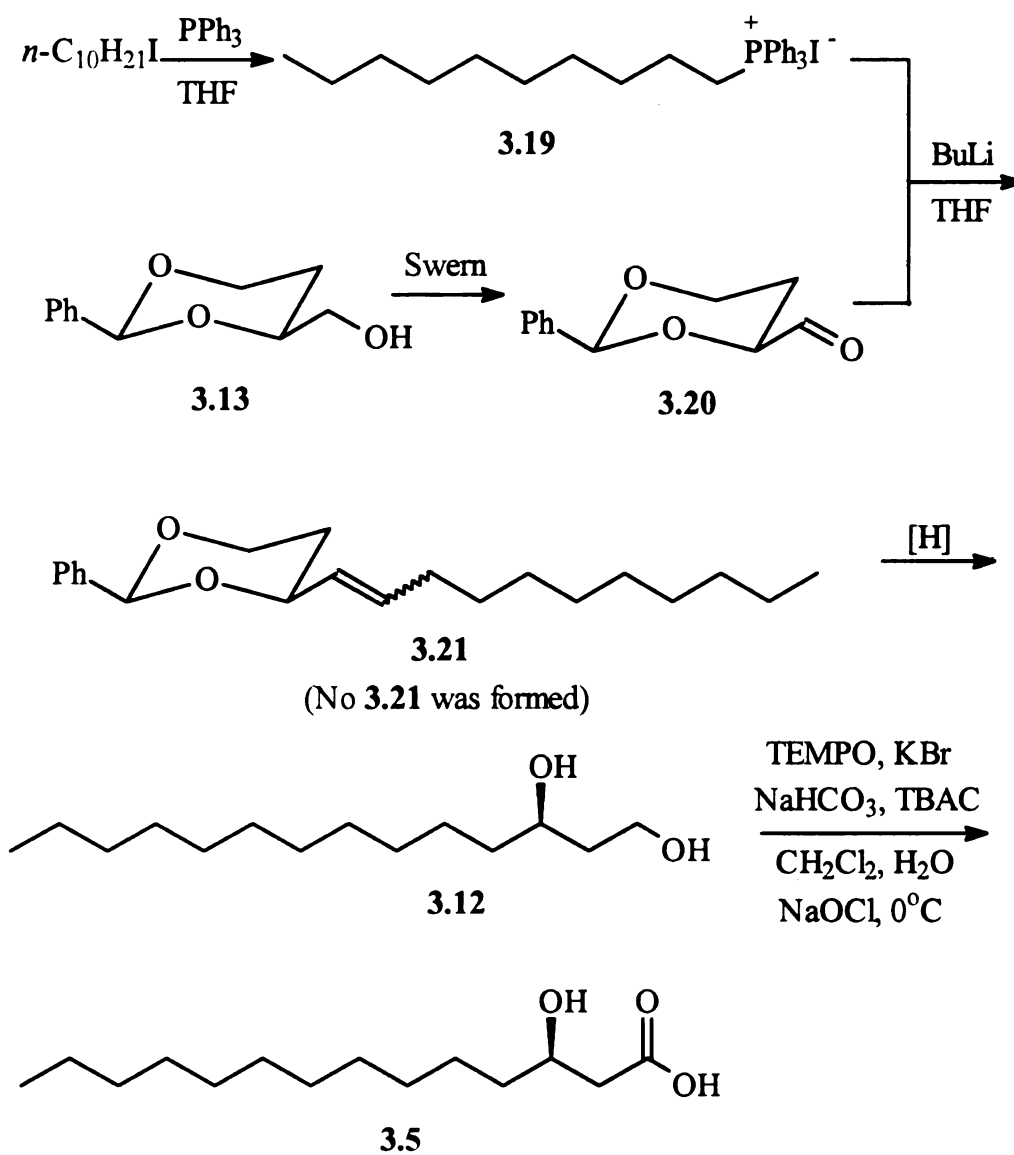
3.15 X = Br, **3.16** X = I



(No **3.18** was formed)



Scheme 3.4: Another proposed synthetic approach to (R)-3-hydroxytetradecanoic acid.



Scheme 3.5: Another proposed synthetic approach to (R)-3-hydroxytetradecanoic acid.

synthon can be prepared without the risk of the elimination reaction that complicates the other route. There is one major disadvantage: the hydroxyl group has to be protected, deprotected, and then converted back into a carboxylic acid group. Several more synthetic steps are therefore involved.

(S)-1,2,4-Butanetriol was also converted into the cyclic benzylidene acetal derivative (3.13), and the free hydroxyl group was transformed into bromide, tosylate or iodide groups (Scheme 3.3). These did not react with any of the carbon nucleophiles tried, including the alkynyl carbanion (Scheme 3.4), Grignard reagent and organic cuprate ($n\text{-C}_{10}\text{H}_{21}\text{MgBr} + \text{cat. Li}_2\text{CuCl}_4$). This may be explained as follows: (1) halides are less reactive to organocuprates than are epoxides, and (2) the cyclic benzylidene moiety sterically hinders the reactivity of the halo groups.

The aldehyde (3.20) formed from the cyclic benzylidene derivative (3.13) by Swern oxidation was reduced back to the cyclic benzylidene derivative (3.13) instead of undergoing a coupling reaction on treatment with *n*-decyl magnesium bromide. The aldehyde was degraded instead of undergoing a Wittig reaction on treatment with the ylide generated from 3.19 (Scheme 3.5).

CONCLUSIONS

An efficient chemical synthesis of (R)-3-hydroxytetradecanoic acid was achieved. Compared with numerous other syntheses, this method is efficient in carbon-counts and

has only four steps from the commercially available (S)-3-hydroxy- γ -butyrolactone. None of four steps involves very dangerous and highly explosive reagents such as *n*-butyl lithium. The starting material is inexpensive and available in bulk. Because the chirality is obtained directly from a natural product (maltose) without transformations at the stereogenic center, the e.e. of (R)-3-hydroxytetradecanoic acid is as high as 99.3% without further chiral separations. This is higher than reported in the literature using other methods. These methods such as asymmetric synthesis and chemoenzymatic resolutions, also gave different enantiomeric excesses when the carbon chain length was varied. This will not be the case for the method described here. This approach can be a general synthesis of chiral 3-hydroxycarboxylic acids and their derivatives by using different Grignard reagents. The chiral 3-hydroxycarboxylic acids can be converted to the corresponding 1,3-diol, 3-hydroxyaldehyde, and the hydroxyl group at the C-3 position can be transformed into a different functional group. (S)-3-Hydroxycarboxylic acids and their derivatives can be obtained from (R)-3-hydroxy- γ -butyrolactone through this method. The method developed here is therefore efficient, simple, practical, and general for the synthesis of both chiral 3-hydroxycarboxylic acids and their derivatives.

EXPERIMENTAL

^1H NMR and ^{13}C NMR spectra were measured on a Varian Inova-300 or Varian-300 spectrometer (300 MHz). The chemical shifts are given in δ values with TMS as the internal standard or relative to the chloroform line at 7.24 ppm for ^1H and 77.0 ppm for ^{13}C .

(S)-3-Hydroxy-4-iodobutanoic acid ethyl ester (3.2)

Trimethylsilylchloride (38.1 mL, 0.300 mol) was slowly added to a stirred suspension of sodium iodide (45.0 g, 0.300 mol) cooled in acetonitrile (300 mL) in a water bath and under dry nitrogen protection. After ten minutes, (S)-3-hydroxy- γ -butyrolactone (**3.1**) (15.3 g, 0.150 mol) in 100 mL absolute ethanol (100 mL) was added dropwise. The reaction mixture was stirred overnight and filtered to remove the salts and then concentrated under reduced pressure. Absolute ethanol (200 mL) was added and removed under reduced pressure. The reaction residue was washed with saturated aqueous sodium thiosulfate solution and then extracted with ethyl acetate for three times. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give the product, (S)-3-hydroxy-4-iodobutanoic acid ethyl ester (**3.2**). The yield was 63%.

^1H NMR (300 MHz, CDCl_3): δ 4.12 (2 H, q, $J = 7.2$ Hz), 3.95 (1 H, m), 3.34 (1 H, broad, -OH), 3.29 (1 H, dd, $J = 10.2, 5.1$ Hz), 3.24 (1 H, dd, $J = 10.2, 5.6$ Hz), 2.63 (1 H, dd, $J = 16.5, 4.2$ Hz), 2.53 (1 H, dd, $J = 16.5, 8.1$ Hz), 1.22 (3 H, t, $J = 7.2$ Hz); ^{13}C NMR (75 MHz, CDCl_3): δ 171.63, 67.35, 60.92, 40.68, 14.03, 12.05.

(S)-3,4-Epoxybutanoic acid ethyl ester (3.3)

Silver oxide (14.4 g, 62.0 mmol) was added to a solution of (S)-3-hydroxy-4-iodobutanoic acid ethyl ester (**3.2**) (16.0 g, 62.0 mmol) in 300 mL. The color of the

reaction mixture turned gray after one hour (if the color turned yellow, more silver oxide was added). The reaction was stirred overnight and then refluxed at 90 °C for two hours. The salts were filtered through Celite and the filtrate was concentrated under reduced pressure to give the product, (S)-3,4-epoxybutanoic acid ethyl ester (**3.3**). The yield was 94%.

^1H NMR (300 MHz, CDCl_3): δ 4.14 (2 H, q, $J = 7.2$ Hz), 3.25 (1 H, m), 2.80 (1 H, dd, $J = 4.8, 3.9$ Hz), 2.53 (1 H, d, $J = 4.8$ Hz), 2.52 (2 H, d, $J = 5.7$ Hz), 1.24 (3 H, t, $J = 7.2$ Hz); ^{13}C NMR (75 MHz, CDCl_3): δ 170.36, 60.84, 47.97, 46.66, 38.02, 14.10.

(R)-3-Hydroxytetradecanoic acid ethyl ester (3.4)

n-Decylmagnesium bromide (40 mL, 1.0 M in ether) was added dropwise to a stirred suspension of CuI (3.81 g, 20.0 mmol) in anhydrous THF (50 mL) at -30°C and under dry nitrogen protection. After a half hour, the epoxide (**3.3**) (2.60 g, 20.0 mmol) in anhydrous THF (10 mL) was added dropwise, and the reaction mixture was stirred for one hour at -30°C , and then was allowed to reach room temperature with continued stirring overnight. The reaction mixture was quenched with saturated NH_4Cl aqueous solution and separated. The aqueous layer was extracted with ether three times. The combined organic layer was washed with saturated NaCl solution, dried over anhydrous Na_2SO_4 , concentrated, and separated to give the product (**3.4**) by flash column chromatography on silica gel using a gradient elution from hexanes/ethyl acetate 8:1 to 1:1. The yield was 97%.

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IR (CHCl₃): 3027 (s), 2928 (s), 2857 (s), 1213 (s), 1208 (s) cm⁻¹; [α]_D²⁰ = -11.6° (c = 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 4.15 (2 H, q, J = 7.2 Hz), 3.97 (1 H, m), 2.48 (1 H, dd, J = 16.5, 3.0 Hz), 2.37 (1 H, dd, J = 16.5, 9.0 Hz), 1.40 (2 H, m), 1.23 (21 H, s), 0.86 (3 H, t, J = 6.6 Hz); ¹³C NMR (75 MHz, CDCl₃): 173.15, 68.01, 60.65, 41.24, 36.47, 31.88, 29.60, 29.55, 29.50, 29.32, 25.45, 22.66, 14.14, 14.09; HRMS Exact mass: calcd for C₁₆H₃₃O₃ [M+H]⁺, 273.2430. Found 273.2438.

(R)-3-Hydroxytetradecanoic acid (3.5)

(R)-3-Hydroxytetradecanoic acid ethyl ester (**3.4**) (0.44 g, 1.6 mmol) was added to a solution of 90% ethanol (100 mL) and KOH (4.0 g) at room temperature. The reaction was stirred overnight, and then quenched with 1 N HCl and adjusted to neutral with NaHCO₃. Most of the ethanol was removed under reduced pressure, and the residue was extracted with ethyl acetate three times. The combined organic layer was dried over anhydrous Na₂SO₄, filtered, and dried under reduced pressure to give the crude product. The product (**3.5**) was obtained by flash column chromatography on silica gel using a gradient elution from chloroform/methanol 10:1 to 2:1. The yield was 76%.

¹H NMR (300 MHz, CDCl₃): δ 5.32 (2 H, broad, OH and CO₂H), 4.01 (1 H, m), 2.56 (1 H, dd, J = 16.8, 3.3 Hz), 2.44 (1 H, dd, J = 16.8, 8.7 Hz), 1.57 (2 H, m), 1.23 (18 H, s), 0.86 (3 H, t, J = 6.6 Hz); ¹³C NMR (75 MHz, CDCl₃): δ 177.40, 68.11, 41.10, 36.53, 31.90, 29.65, 29.63, 29.61, 29.57, 29.50, 29.34, 25.46, 22.68, 14.11.

(2S)-1,2-*O*-Isopropylidenebutane-1,2,4-triol (3.7)

Anhydrous CuSO₄ (32.9 g, 0.206 mol) and *p*-toluenesulfonic acid (0.33 g) were added to a stirred solution of the triol (**3.6**) (30.3 g, 0.286 mol) in anhydrous acetone (550 mL) at room temperature. The reaction was stirred for three days before the addition of solid K₂CO₃ (1 g). After two hours, CuSO₄ and the excess base were filtered off and rinsed with a small amount of acetone. The filtrate was concentrated to give the product (**3.7**). The yield is 80%.

¹H NMR (300 MHz, CDCl₃): δ 4.25 (1 H, quintet, J = 6.0 Hz), 4.06 (1 H, dd, J = 8.1, 6.0 Hz), 3.78 (2 H, t, J = 5.7 Hz), 3.57 (1 H, dd, J = 8.1, 7.5 Hz), 2.29 (1 H, s, broad, -OH), 1.79 (2 H, q, J = 5.7 Hz), 1.40 (3 H, s), 1.34 (3 H, s); ¹³C NMR (75 MHz, CDCl₃): δ 109.10, 75.19, 69.43, 60.68, 35.53, 26.88, 25.67.

(2S)-4-*O*-Benzyl-1,2-*O*-isopropylidenebutane-1,2,4-triol (3.8)

A solution of (2S)-1,2-*O*-isopropylidenebutane-1,2,4-triol (33.2g, 0.228 mol) in anhydrous DMF was added to a stirred suspension of sodium hydride (10.9 g, 60% weight in mineral oil, 0.273 mol, 1.2 equivalents, prewashed with hexanes) in anhydrous DMF (500 mL) over a half-hour at room temperature. After an additional half-hour of stirring, benzyl bromide (46.7 g, 0.273 mol, 1.2 equivalents) was added dropwise over a half-hour period as a solution in anhydrous DMF (65 mL). Periodic cooling was

necessary to keep the reaction temperature below 30 °C. The reaction mixture was stirred overnight. Water (32 mL) was added, and the solvent was removed by evaporation under reduced pressure. The residue was dissolved in ethyl acetate, and the resulting solution was washed with water and brine, dried over anhydrous Na₂SO₄, and evaporated to dryness to give the product (**3.8**). The yield was 92%.

¹H NMR (300 MHz, CDCl₃): δ 7.31 (5 H, m), 4.49 (2 H, s), 4.21 (1 H, quintet, J = 6.5 Hz), 4.05 (1 H, dd, J = 8.1, 5.7 Hz), 3.56 (3H, m), 1.88 (2 H, m), 1.39 (3 H, s), 1.34 (3 H, s); ¹³C NMR (75 MHz, CDCl₃): δ 138.25, 128.35, 127.56, 108.50, 73.84, 73.05, 69.62, 67.02, 33.80, 26.90, 25.76.

(2S)-4-*O*-Benzylbutane-1,2,4-triol (3.9**)**

A solution of (2S)-4-*O*-benzyl-1,2-*O*-isopropylidenebutane-1,2,4-triol (**3.8**) (49.6 g, 0.210 mol) in 80% acetic acid (300 mL) was stirred at room temperature for two days and then at 50 °C for one hour. The reaction mixture was concentrated to dryness under reduced pressure to give the product (**3.9**). The yield was 94%.

¹H NMR (300 MHz, CDCl₃): δ 7.30 (5 H, m), 4.49 (2 H, s), 3.87 (1 H, m), 3.72 (1 H, m), 3.64 (2 H, m), 3.45 (1 H, dd, J = 11.3, 6.5 Hz), 3.09 (2 H, s, broad), 1.82 (1 H, m), 1.73 (1 H, m); ¹³C NMR (75 MHz, CDCl₃): δ 137.66, 128.45, 127.80, 127.68, 73.29, 71.27, 68.22, 66.52, 32.73.

(2S)-1,2-Anhydro-4-O-benzylbutane-1,2,4-triol (3.10)

Over 30 minutes methanesulfonyl chloride (16.0 mL, 0.207 mol, 1.05 equivalents) was added to a stirred solution of (2S)-4-O-benzylbutane-1,2,4-triol (**3.9**) (38.6 g, 0.197 mol) in anhydrous pyridine (250 mL) at 0 °C. After an additional 20 minutes, the resulting solution was added dropwise over 15 minutes to a stirred solution of sodium hydroxide (25.0 g, 0.624 mol) in water (250 mL) and DMSO (167 mL) at 0 °C. After an additional 30 minutes of stirring, the solution was poured into ice-water and the mixture extracted twice with ether. The ether phase was washed with water and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was distilled to give the product (**3.10**). The yield was 86%.

¹H NMR (300 MHz, CDCl₃): δ 7.32 (5 H, m), 4.51 (2 H, s), 3.61 (2 H, m), 3.05 (1 H, m), 2.77 (1 H, dd, J = 5.0, 4.1 Hz), 2.51 (1 H, dd, J = 5.0, 2.6 Hz), 1.89 (1 H, m), 1.77 (1 H, m); ¹³C NMR (75 MHz, CDCl₃): δ 138.18, 128.34, 127.55, 73.02, 66.97, 50.03, 47.06, 32.88.

(R)-Benzyl 3-hydroxy-1-tetradecyl ether (3.11)

A solution of *n*-decylmagnesium bromide (90 mL, 1.0 M in ether) was added dropwise to a stirred suspension of CuI (3.42 g, 18.0 mmol) in anhydrous THF (300 mL) at -30 °C. After ten minutes, a solution of epoxide **3.10**, (2S)-1,2-anhydro-4-O-benzylbutane-1,2,4-triol (4.02 g, 22.6 mmol) in anhydrous THF (100 mL) was added

dropwise to the reaction. The reaction mixture was stirred for one hour at $-30\text{ }^{\circ}\text{C}$ and then left to reach room temperature overnight. The reaction was quenched with saturated aqueous NH_4Cl . The aqueous layer was extracted with ether. The combined organic layer was washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified to give the product (**3.11**) by flash column chromatography on silica gel using a gradient elution from hexanes to hexanes/ethyl acetate 1:1. The yield was 96%.

^1H NMR (300 MHz, CDCl_3): δ 7.31 (5 H, m), 4.51 (2 H, s), 3.77 (1 H, m), 3.70 (1 H, dt, $J = 9.3, 5.1$ Hz), 3.62 (1 H, dt, $J = 9.3, 6.0$ Hz), 2.84 (1 H, d, $J = 3.3$ Hz, -OH), 1.71 (2 H, m), 1.41 (2 H, m), 1.23 (18 H, s), 0.86 (3 H, t, $J = 6.9$ Hz); ^{13}C NMR (75 MHz, CDCl_3): δ 138.90, 128.44, 127.73, 127.65, 73.33, 71.58, 69.38, 37.45, 36.35, 31.96, 29.69, 29.63, 29.35, 25.62, 22.69, 14.14.

(2S,4S)-4-Hydroxymethyl-2-phenyl-1,3-dioxane (3.13)

Four drops of concentrated sulfuric acid were added as a catalyst to a stirred solution of (2S)-1,2,4-butanetriol (24.4 g, 0.230 mol) and dimethylbenzylidene acetal (70.0 g, 0.460 mol) in anhydrous DMF (50 mL). The reaction mixture was heated on a steam bath for five minutes and then stirred at room temperature overnight. Triethylamine (1 mL) was added, and the reaction was quenched by adding ice-water, and then extracted twice with toluene. The combined organic layer was dried over

anhydrous sodium sulfate, filtered, and then concentrated under reduced pressure to give the product **(3.13)**, (2S,4S)-4-hydroxymethyl-2-phenyl-1,3-dioxane. The yield was 97%.

^1H NMR (300 MHz, CDCl_3): δ 7.48 (2 H, m), 7.35 (3 H, m), 5.50 (1 H, s), 4.25 (1 H, ddd, $J = 11.4, 5.1, 0.9$ Hz), 3.92 (2 H, m), 3.59 (2 H, m), 2.59 (1 H, s, -OH), 1.84 (1 H, m), 1.38 (1 H, m); ^{13}C NMR (75 MHz, CDCl_3): δ 138.21, 128.82, 128.12, 126.02, 101.08, 77.45, 66.46, 65.37, 26.66.

(2S)-2,4-Benzylidenedioxybutyl tosylate (3.14)

(2S,4S)-4-Hydroxymethyl-2-phenyl-1,3-dioxane **(3.13)** (1.94 g, 10.0 mmol) was added to a gently stirred and cooled mixture of tosyl chloride (1.91 g, 10.0 mmol) and anhydrous pyridine (2 mL) in anhydrous dichloromethane (5 mL) cooled in an ice bath. After stirring for one hour, the ice bath was removed and the reaction mixture was stirred at room temperature overnight. The reaction mixture was poured into ice-water (50 mL) and extracted twice with chloroform. The combined organic layer was washed with saturated sodium bicarbonate solution, water, and brine, and then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give the product **(3.14)**, (2S)-2,4-benzylidenedioxybutyl tosylate. The yield was 76%.

^1H NMR (300 MHz, CDCl_3): δ 7.79 (2 H, m), 7.34 (7 H, m), 5.46 (1 H, s), 4.25 (1 H, m), 4.10 (3 H, m), 3.94 (1 H, m), 2.42 (3 H, s), 1.80 (1 H, m), 1.54 (1 H, m); ^{13}C NMR

(75 MHz, CDCl₃): δ 144.82, 137.84, 132.60, 129.74, 128.83, 128.10, 127.93, 125.96, 100.94, 74.04, 71.47, 66.25, 27.12, 21.58.

(2S,4S)-4-Bromomethyl-2-phenyl-1,3-dioxane (3.15)

Sodium bicarbonate (8.40 g, 100 mmol) and lithium bromide (8.69 g, 100 mmol) were added to a solution of (2S)-2,4-benzylidenedioxybutyl tosylate (**3.14**) (3.48 g, 10.0 mmol) in dry DMF (30 mL). The suspension was stirred overnight at 90 °C. After cooling, the mixture was filtered and the filter cake was washed three times with ether. The combined organic solution was diluted with ether, washed with water and brine, dried over anhydrous sodium sulfate, and concentrated in vacuum to give the raw product. The product (**3.15**), (2S,4S)-4-bromomethyl-2-phenyl-1,3-dioxane, was obtained by flash column chromatography on silica gel using a gradient elution from hexanes/ethyl acetate 5:1 to 1:1. The yield was 78%.

¹H NMR (300 MHz, CDCl₃): δ 7.56-7.40 (5 H, m), 5.53 (1 H, s), 4.28 (1 H, ddd, J = 11.7, 5.1, 1.5 Hz), 4.02 (1 H, ddt, J = 11.7, 6.0, 2.7 Hz), 3.92 (1 H, dt, J = 11.7, 3.0 Hz), 3.50 (1 H, dd, J = 10.5, 6.0 Hz), 3.40 (1 H, dd, J = 10.5, 6.0 Hz), 1.82 (2 H, m); ¹³C NMR (75 MHz, CDCl₃): δ 137.93, 128.58, 127.94, 125.79, 100.80, 75.84, 66.21, 34.29, 29.42.

(2S,4S)-4-Iodomethyl-2-phenyl-1,3-dioxane (3.16)

A suspension of (2S)-2,4-benzylidenedioxybutyl tosylate (**3.14**) (35.6 g, 0.102 mol) and sodium iodide (61.2 g, 0.408 mol, 4 equivalents) in dry acetone was refluxed at 90°C overnight. After cooling, the mixture was filtered and the filter cake was washed with acetone. The filtrate was concentrated under reduced pressure, and the resulting residue was extracted with ether four times. The combined ether solution was washed with sodium thiosulfate aqueous solution, dried over anhydrous sodium sulfate, filtered and dried under reduced pressure to give the crude product. The product (**3.16**), (2S,4S)-4-iodomethyl-2-phenyl-1,3-dioxane, was obtained by flash column chromatography on silica gel using hexanes/ethyl acetate 2:1. The yield was 57%.

^1H NMR (300 MHz, CDCl_3): δ 7.51 (2 H, m), 7.36 (3 H, m), 5.52 (1 H, s), 4.28 (1 H, ddd, $J = 11.4, 4.8, 1.8$ Hz), 3.92 (2 H, m), 3.31 (1 H, dd, $J = 10.2, 6.0$ Hz), 3.23 (1 H, dd, $J = 10.2, 6.0$ Hz), 1.80 (2 H, m); ^{13}C NMR (75 MHz, CDCl_3): δ 138.05, 128.84, 128.22, 125.99, 101.20, 76.43, 66.61, 31.18, 7.96.

(1-Decyl)triphenylphosphonium iodide (3.19)

Iododecane (2.71 g, 10.0 mmol) was added to a solution of triphenylphosphine (3.16 g, 12.0 mmol) in anhydrous THF (10 mL). After stirring at 70 °C overnight, the reaction mixture was concentrated under reduced pressure and recrystallized in ether to give the product (**3.19**). The yield was 90%.

^1H NMR (300 MHz, CDCl_3): δ 7.73 (15 H, m), 3.57 (2 H, m), 1.57 (2 H, m), 1.17 (14 H, s), 0.80 (3 H, t, $J = 8.1$ Hz); ^{13}C NMR (75 MHz, CDCl_3): δ 135.0-128.1 (many peaks), 117.68 (d, $J = 84$ Hz), 31.51, 30.34, 30.12, 29.19, 29.03, 23.17, 22.21, 22.08, 13.87.

(2S)-2,4-Benzylidenedioxybutyl aldehyde (3.20)

Anhydrous DMSO (15.2 mL, 215 mmol) in anhydrous dichloromethane (40 mL) was added through a dropping funnel to a stirred solution of oxalyl chloride (9.40 mL, 107 mmol, 1.1 equivalents) in anhydrous dichloromethane (200 mL) cooled at -50 to -60 $^\circ\text{C}$. The solution was stirred for a further two minutes. (2S,4S)-4-hydroxymethyl-2-phenyl-1,3-dioxane (**3.13**) (18.9 g, 97.6 mmol) in anhydrous dichloromethane (40 mL) was added over five minutes and the stirring was continued for 15 minutes. Triethylamine (70 mL) was added and the solution was stirred for five minutes at -50 to -60 $^\circ\text{C}$, and then the solution mixture was allowed to reach room temperature. Water (400 mL) was added and the layers separated. The organic layer was washed with cold sodium bicarbonate solution, water, and brine, dried over anhydrous sodium sulfate, concentrated to dryness under reduced pressure, and purified to give the product (**3.20**) by flash column chromatography on silica gel using hexanes/ethyl acetate 1:1. The yield was 93%.

¹H NMR (300 MHz, CDCl₃): δ 9.71 (1 H, s), 7.34 (5 H, m), 5.59 (1 H, s), 5.50 (1 H, m), 4.33 (1 H, m), 3.96 (1 H, m), 1.99 (1 H, m), 1.88 (1 H, m); ¹³C NMR (75 MHz, CDCl₃): δ 200.55, 137.58, 128.35, 128.16, 126.05, 101.14, 80.32, 66.56, 25.87.

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CHAPTER 4

SYNTHESIS OF (R)-2-*O*-TRIMETHYLACETYLGLYCERALDEHYDE AS A CHIRAL INTERMEDIATE

INTRODUCTION

Stereoselectivity and Chiral C₃ Intermediates

Stereoselectivity in organic synthesis is of tremendous importance because even moderately complex organic molecules cannot be prepared without reasonable control of stereochemistry. There is an increasing need for organic synthesis to be enantioselective primarily due to the decline in use of racemic products in the pharmaceutical industry. The decline is caused by the fact that a racemic drug in a biological system behaves as a mixture of two different compounds of which only one has the desired properties, while the other is potentially harmful or, at least, undesired.

There are two basic methods for synthesis of chiral compounds. One is to introduce chirality through chiral catalysts or chiral auxiliaries, and the other is to utilize chiral intermediates as building blocks. The former becomes disadvantageous when the molecules are complex and have several functional groups. The latter is thus widely adopted in the total synthesis. As building blocks, these chiral intermediates are usually small molecules. The multifunctional C₃ chiral intermediates are especially useful, and are usually in the form of derivatives of propanol, propanal and propanoic acid.¹

Chiral C₃ intermediates of both propanoic acid and propanol derivatives are well developed, but those of propanal derivatives are not. Thus far, the key role is played by 2,3-*O*-isopropylidene-glyceraldehyde, whose two enantiomers^{2, 3} are readily accessible

and which finds highly diverse uses⁴ due to the free aldehyde and the protected diol functions. It is, however, not stable and undergoes racemization on storage. Storage as a frozen solution in benzene and redistillation prior to use are recommended.⁵ Its instability and redistillation are the big disadvantages of this important chiral synthon. Redistillation prior to use is an inconvenience and also promotes self-aldol condensation. In this chiral synthon, both of the hydroxyl groups are protected by the isopropylidene group and are not differentiated in the subsequent steps.

Design of 2-*O*-Trimethylacetylglyceraldehyde

In principle, glyceraldehyde derivatives with one hydroxyl group free and the other one protected could offer new applications. The more strongly the hydroxyl groups are differentiated, the more easily regio- and stereoselectivities can be manipulated in the subsequent steps. A new chiral synthon, 2-*O*-trimethylacetylglyceraldehyde, is therefore designed to have the properties mentioned above. In order to differentiate the hydroxyl groups, the secondary one is protected with a pivaloyl group (trimethylacetyl) as its ester. The reason for keeping the 3-OH group free is that 3-hydroxyaldehyde can form a cyclic acetal dimer so that the aldehyde group becomes more stable to oxidation, and the chirality at the C-2 position can also be more stable to racemization than those of 2,3-*O*-isopropylideneglyceraldehyde. Here a pivaloyl group is selected to protect the α -hydroxyl group. Unlike many hydroxyl-protecting groups such as trimethylsilyl and acetyl, the pivaloyl group does not migrate between hydroxyl groups. It can be cleaved by basic hydrolysis with aqueous methanolic tetraethylammonium hydroxide at room

temperature or by aqueous methylamine.⁶ Due to its stability, the pivaloyl group has been widely used in both carbohydrate and nucleoside areas. The benzyl group does not migrate either and can be removed by hydrogenolysis, and thus can also be a choice for protecting the α -hydroxyl group.⁷ One of the major uses of the glyceraldehyde derivative, as a chiral intermediate, is to form a carbon-carbon double bond, but the hydrogenolysis of the benzyl group can hydrogenate the carbon-carbon double bond. The pivaloyl group is therefore selected to protect the α -hydroxyl group of the C₃ chiral intermediate.

Applications of (R)-2-*O*-Trimethylacetylglucaldehyde as a Chiral Intermediate

This glyceraldehyde derivative can be utilized to form a carbon-carbon double bond through the Horner-Wadsworth-Emmons reaction under a mild condition by using potassium carbonate as a base to deprotonate the Horner phosphonate. After the functional transformations, it can result in the (S)-5-hydroxy-2-penten-4-olide⁸ or (S)-4-hydroxy-2-penten-5-olide derivatives⁷ with the hydroxyl group protected. These lactones are valuable intermediates⁹ in the synthesis of the optical active compounds such as alkaloids,^{10, 11} carbohydrates,¹² lignans,¹³ nucleosides,^{14, 15} and terpenes.¹⁶

This glyceraldehyde derivative may also be applied in the asymmetric carbon-carbon bond formation by enzymes. (R)-And (S)-oxynitrilases from almonds can catalyze the asymmetric addition of HCN to aldehydes to yield (R)- and (S)-cyanohydrins, respectively.¹⁷ These cyanohydrins are useful precursors for α -hydroxycarboxylic acids, α -hydroxy aldehydes, and vicinal amino alcohols. The aldolases, including pyruvate,

phosphoenolpyruvate and dihydroxyacetone phosphate-dependent lyases, can elongate the carbon backbone by three carbons.¹⁸ The resulting products are useful for the synthesis of complex carbohydrates.

Carbohydrate Precursors for (R)-2-*O*-Trimethylacetylaldehyde

The stereogenic center of this chiral synthon can be either introduced through chiral auxiliaries or from a chiral starting material. The latter strategy is easy to plan and carry out if a useful chiral starting material can be found. Although thousands of optically active natural compounds are known, it is not easy to select an available compound that would not require many steps to convert it into the desired structure.

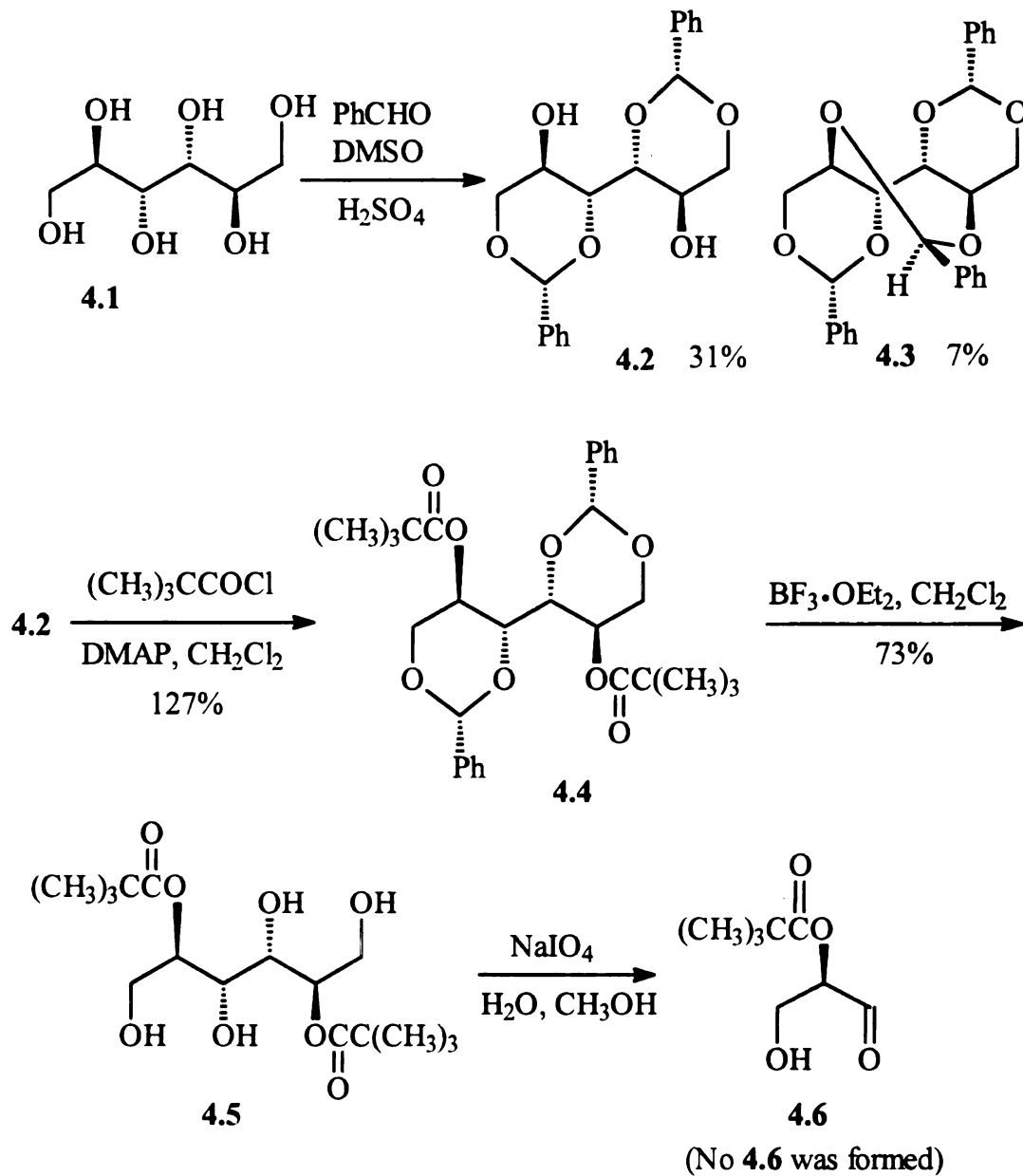
Carbohydrates have been extensively used as starting materials in the synthesis of chiral intermediates since they have very high optical purity and some of them, including D-glucose, D-fructose, D-gluconic acid, D-glucitol (sorbitol), lactose, sucrose, and D-mannitol, are extremely inexpensive (less than \$8 per kilogram in bulk). The prices of these materials are so low that synthesis from them can be carried out on any scale without difficulty, and that even low yield in the initial step is acceptable. Almost all carbons of carbohydrates, such as D-glucose and D-mannitol, are functionalized. This polyfunctionality can be an advantage that gives carbohydrates great potential and versatility. The carbohydrates undoubtedly have their greatest usefulness as chiral starting materials for (R)-2-*O*-trimethylacetylaldehyde containing carbon chains with contiguous functional groups.

RESULTS AND DISCUSSION

In the course of the synthesis of (R)-2-*O*-trimethylacetylglyceraldehyde, carbohydrates such as D-glucose and D-mannitol were utilized as the source of the chirality. Several methods have been tried, but in all of them there are some difficulties to be overcome.

From D-Mannitol

D-Mannitol, an inexpensive hexitol obtained from a variety of natural sources, is obviously the best starting material for synthesis of (R)-2-*O*-trimethylacetylglyceraldehyde due to its C_2 symmetry axis between C-3 and C-4. The cleavage of the diol between C-3 and C-4 will generate two identical chiral synthons so that D-mannitol is ideal for the synthesis of this kind of C_3 chiral synthons in the terms of efficient carbon counts and fewer synthetic steps. It is readily converted into a number of chiral derivatives which are convenient for the subsequent synthesis.¹⁹ Its derivatization depends primarily on the possibilities for selective acetalization of pairs of -OH groups. Thus, by control of the conditions of acetalization it is possible to form 1,3:4,6-di-*O*-benzylidene-D-mannitol with the two free hydroxyl groups at C-2 and C-5, which can be acylated to introduce pivaloyl groups as the protecting groups (**Scheme 4.1**). The selective removal of the two benzylidene groups can free the hydroxyl groups at C-1, C-3, C-4 and C-6, followed by cleavage of the vicinal diol to give (R)-2-*O*-trimethylacetylglyceraldehyde.



Scheme 4.1: A synthetic approach to (R)-2-O-trimethylacetyl-glyceraldehyde from D-mannitol.

Acid-catalyzed condensation of benzaldehyde and D-mannitol in dimethyl sulfoxide at room temperature overnight by slightly modifying Sinclair's method²⁰ gave 1,3:4,6-di-*O*-benzylidene-D-mannitol (**4.2**). The modified method used methanol-water as the cosolvent from which the crude product was directly recrystallized to give the desired product. It not only gave an improved yield of 31% (12% given by Sinclair's method) but also was simpler than Sinclair's method. Although the yield was not yet high, it was acceptable due to the inexpensive D-mannitol, benzaldehyde and other reagents, and the possibility that one desired intermediate could result in two identical final products. The reaction between benzaldehyde and D-mannitol also led to 1,3:2,5:4,6-tri-*O*-benzylidene-D-mannitol (**4.3**) as the by-product. Both the dicyclic and the tricyclic acetals are the equilibrium products.²¹ Solvents had a strong effect on the equilibrium. Since the last ring to be formed is a seven-membered benzylidene ring, a reaction in a homogeneous medium might favor the formation of the dicyclic acetal with two six-membered rings, while the tricyclic acetal formation could be favored in a heterogeneous solution. Thus DMSO was chosen as the solvent, since it can dissolve both the reagents and the desired product. It is also very convenient to remove DMSO in the aqueous layer during the work-up.

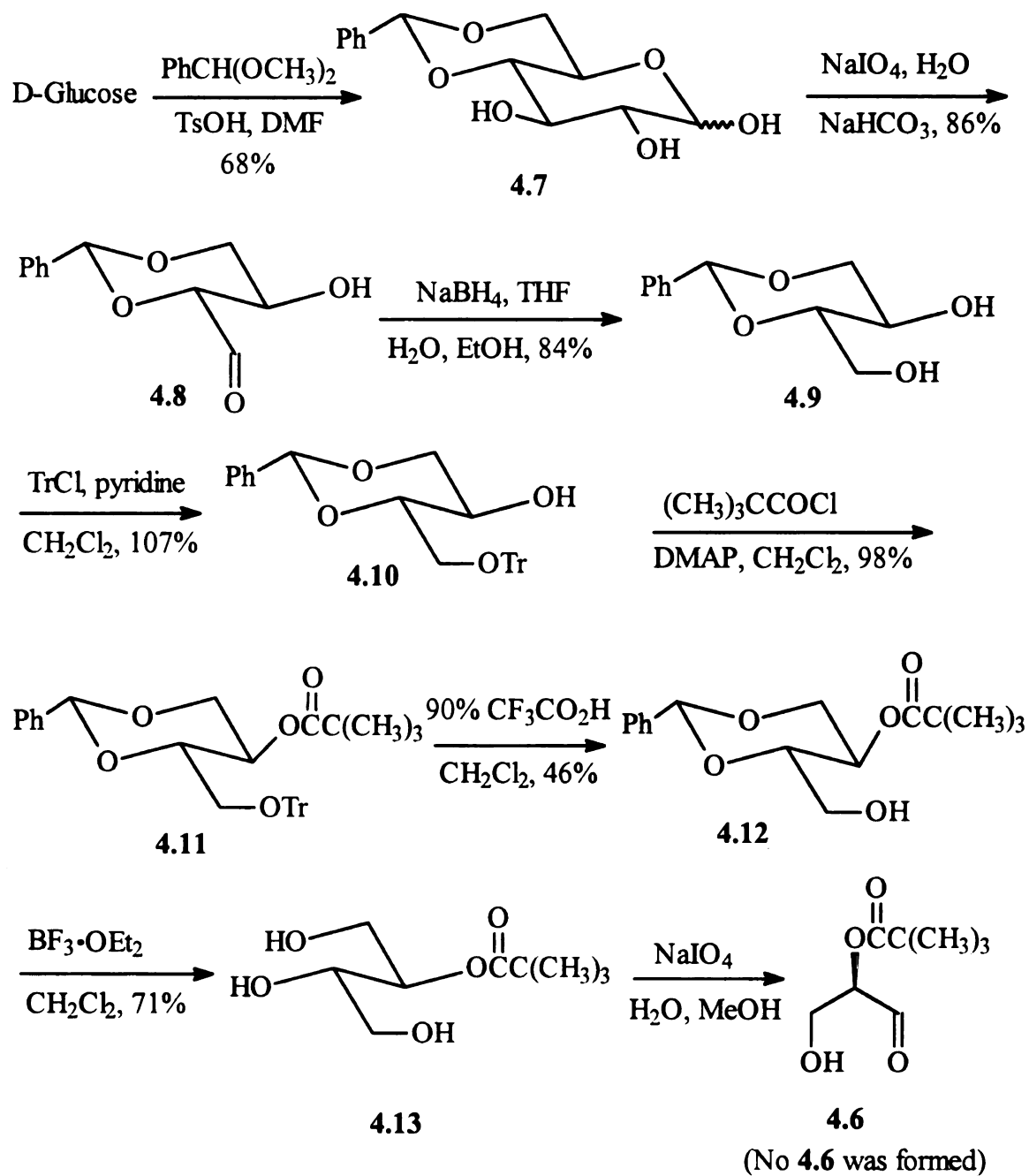
The acylations of 1,3:4,6-di-*O*-benzylidene-D-mannitol (**4.2**) with trimethylacetyl chloride and DMAP as the base in anhydrous dichloromethane gave the desired diester derivative (**4.4**) quantitatively, but it resulted in a poor yield when anhydrous pyridine was used as the base in the reaction.

The chemoselective removal of the benzylidene protecting groups in the presence of the trimethylacetyl groups is an important step. The typically acidic conditions, such as in trifluoroacetic acid or 70% acetic acid aqueous solutions, resulted in poorly selective removals. Boron trifluoride diethyl etherate selectively cleaved the benzylidene groups at room temperature to free the hydroxyl groups at C-1, C-3, C-4 and C-6 to give 2,5-di-*O*-trimethylacetyl-D-mannitol (4.5). The above two steps had a high yield of 93%.

The cleavage of 2,5-di-*O*-trimethylacetyl-D-mannitol (4.5) to the corresponding glyceraldehyde has not yet been achieved by using sodium periodate or periodic acid. The reaction has not resulted in any detectable desired product (4.6) in many conditions including the different solvent systems, phase transfer catalysts, varied acidity and temperature. This high resistance to glycol cleavage can be attributed to the structure of 2,5-di-*O*-trimethylacetyl-D-mannitol (4.5). The three hydroxyl groups at the 1, 3, and 4-positions can form a tridentate complex with a periodate ion.²² This tridentate complex is very stable compared with the transient five-membered cyclic complex, an intermediate required for the oxidative cleavage of glycol.²³

From D-Glucose

D-Glucose and its reduction product, D-glucitol, have been widely used as starting materials for chiral building blocks.¹⁹ D-Glucitol is rarely used as a chiral source for C₃ building blocks because it does not contain a C₂ symmetry axis and is not as convenient as D-mannitol to selectively protect the hydroxyl groups. Although D-glucose



Scheme 4.2: A synthetic approach to (R)-2-O-trimethylacetylglyceraldehyde from D-glucose.

is not as good as D-mannitol to be a source for C₃ multifunctional chiral intermediates, it is much better than D-glucitol. The predominant conformation of D-glucose can be viewed as a six-membered ring pyranose with all its substituents equatorial except for the anomeric position of its α -form and the chirality at C-5 well self-protected by the pyranose ring. This form of D-glucose can be conveniently manipulated as a six-membered cyclic derivative, much the same way as a cyclohexane derivative, with allowance made for the stereoelectronic consequences of replacing a ring carbon by an oxygen atom. The chirality at the C-5 position can be released at the subsequent synthesis and thus utilized as the stereogenic center of (R)-2-*O*-trimethylacetylglyceraldehyde.

As shown in **Scheme 4.2**, the strategy was to carry out periodate cleavage on the protected glucose to give a tetrose derivative and release the hydroxyl group at the C-5 position. After introduction of trimethylacetyl group, the backbone could be degraded to a C₃ unit.

The only primary hydroxyl group of D-glucopyranose is least hindered and can be protected selectively together with the secondary hydroxyl group at the C-4 position as a 4,6-*O*-benzylidene acetal derivative (4.7) of the trans-decalin type structure. Hence benzaldehyde dimethyl acetal was used to react with two equivalents of D-glucose in DMF, using *p*-toluenesulfonic acid as a catalyst, to generate 4,6-*O*-benzylidene-D-glucopyranose (4.7) in 68% yield. The pyranose product was a 40:60 mixture of its α and β anomers and needed no further separating, since the chiralities at the C-5 positions were same and the anomeric carbons (C-1) and their neighboring carbons (C-2) would be

degraded in the following step. Due to its inexpensiveness, an excessive amount of D-glucose was used to improve the conversion of benzaldehyde dimethyl acetal into the desired product and to decrease the formation of any dibenzylidene byproducts.

4,6-*O*-Benzylidene-D-glucopyranose (4.7) was cleaved by sodium periodate in the presence of sodium bicarbonate in aqueous solution to give the tetrose derivative (4.8) in 86% yield compared with in 72% yield in a bi-phasic mixture of dichloromethane and water (2:1).²⁴ Besides a short reaction time (30 minutes), the two-phase reaction mixture was suggested in the literature to minimize the formation of the hemiacetal dimer complex. My result showed that the two-phase reaction mixture had little effect on the formation of the dimer in a short reaction time. Actually, this tetrose derivative can be completely converted into the dimer from the white crystalline solid to the yellowish syrup above 60 °C. It is necessary to control the reaction and the work-up at low temperature if the dimer is not the desired product. Since a large amount of heat can be released during the oxidation, sodium periodate should be added in small portions and the reaction solution should be fully stirred and cooled below room temperature. The organic solvent utilized to extract the tetrose derivative should have a low boiling point and be easily removed under reduced pressure. Thus dichloromethane is a good choice as the solvent. Since both the monomer and the dimer can be reduced to the erythritol derivatives, temperature control is not strictly necessary in this step. After periodate oxidation, the pyranose ring was cleaved and opened, and thus the hydroxyl group at the C-5 position was released and its chirality remained intact.

Prior to the introduction of the trimethylacetyl group, the aldehyde group of the tetrose derivative needed protection. Here the aldehyde group was reduced to the primary hydroxyl group with sodium borohydride in the solvent system of ethanol and water. The resulting product, (2R,3S)-1,3-*O*-benzylideneerythritol (4.9), had two free hydroxyl groups, one primary at the C-4 position (C-3 of D-glucose) and one secondary at the C-2 position (C-5 of D-glucose). The primary hydroxyl group was selectively protected with a bulky trityl group to form an ether linkage (4.10). The protection was achieved by treatment of the erythritol derivative with trityl chloride in the presence of pyridine in dichloromethane at 40 °C in a quantitative yield, and the secondary hydroxyl group remained.

The protection of the primary hydroxyl group was followed by the introduction of a trimethylacetyl group to the secondary hydroxyl group. Trimethylacetyl chloride was reacted with (2R,3S)-1,3-*O*-benzylidene-4-*O*-triphenylmethylethritol (4.10) in the presence of DMAP in dichloromethane to give the desired product (4.11) with the complete retention of chirality at the C-2 position.

The resulting erythritol derivative (4.11) has the cyclic benzylidene, trityl ether and ester moieties. The former two protecting groups are more sensitive to acids and can be selectively cleaved in the presence of the ester moiety. When it was treated with boron trifluoride diethyl etherate in anhydrous dichloromethane, it yielded predominantly (2R,3S)-3-*O*-benzoyl-2-*O*-trimethylacetylerythritol with a small amount of the desired product, (2R,3S)-2-*O*-trimethylacetylerythritol (4.13). The benzoyl derivative was the

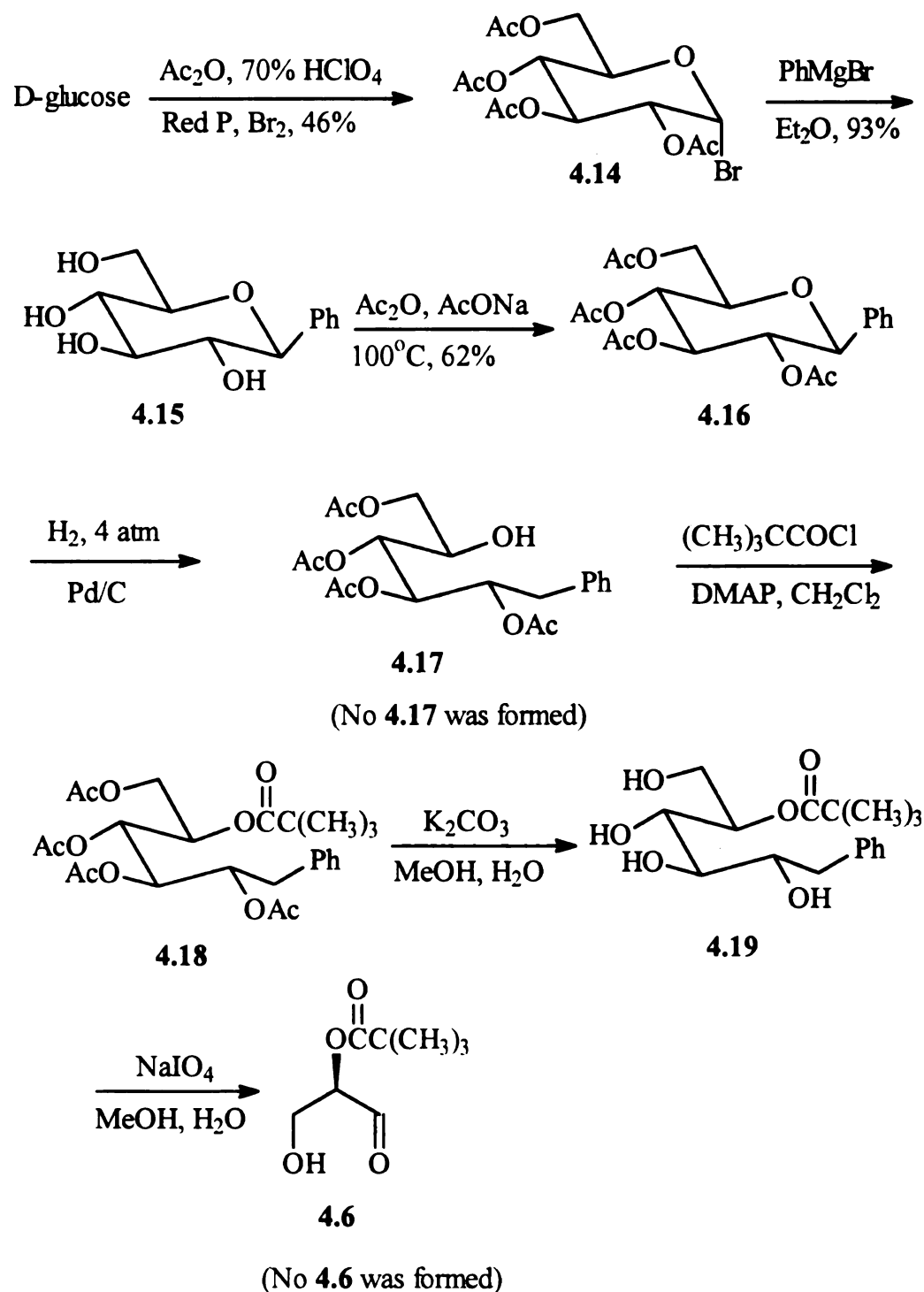
oxidized product from the cyclic benzylidene and the reduced product, triphenylmethane, was also obtained. The oxidation-reduction reaction can be explained as follows. The trityl ether was cleaved by the Lewis acid and generated a stable trityl cation. The trityl cation, as a hydride-abstracting species, obtained a hydrogen atom from the cyclic benzylidene and was reduced to triphenylmethane. After losing a hydride, the cyclic benzylidene was converted into the cationic cyclic species with the charge delocalized between the phenyl ring and the benzylidene oxygen atoms. Due to the steric effect, the incoming nucleophile (H_2O) selectively attacked the methylene carbon of the ring, which was cleaved to give the erythritol derivative with the benzoyl group at the C-3 position. To explore the application of trityl cation as a hydride-abstracting species, methyl triphenylmethyl ether was prepared from triphenylmethyl chloride and methanol. On treatment with methyl trityl ether and boron trifluoride diethyl etherate in dichloromethane, benzaldehyde dimethyl acetal was oxidized to benzoate and triphenylmethane was obtained.

To avoid the oxidation-reduction reaction, the two protecting groups were cleaved in two steps. The trityl group was cleaved to free the primary hydroxyl group with 90% trifluoroacetic acid in dichloromethane in a short time without the cleavage of the benzylidene group. Here the trityl cation was trapped by water and did not function as a hydride-abstracting species. The remaining benzylidene group was removed by boron trifluoride diethyl etherate to give (2R,3S)-2-*O*-trimethylacetylerythritol (**4.11**).

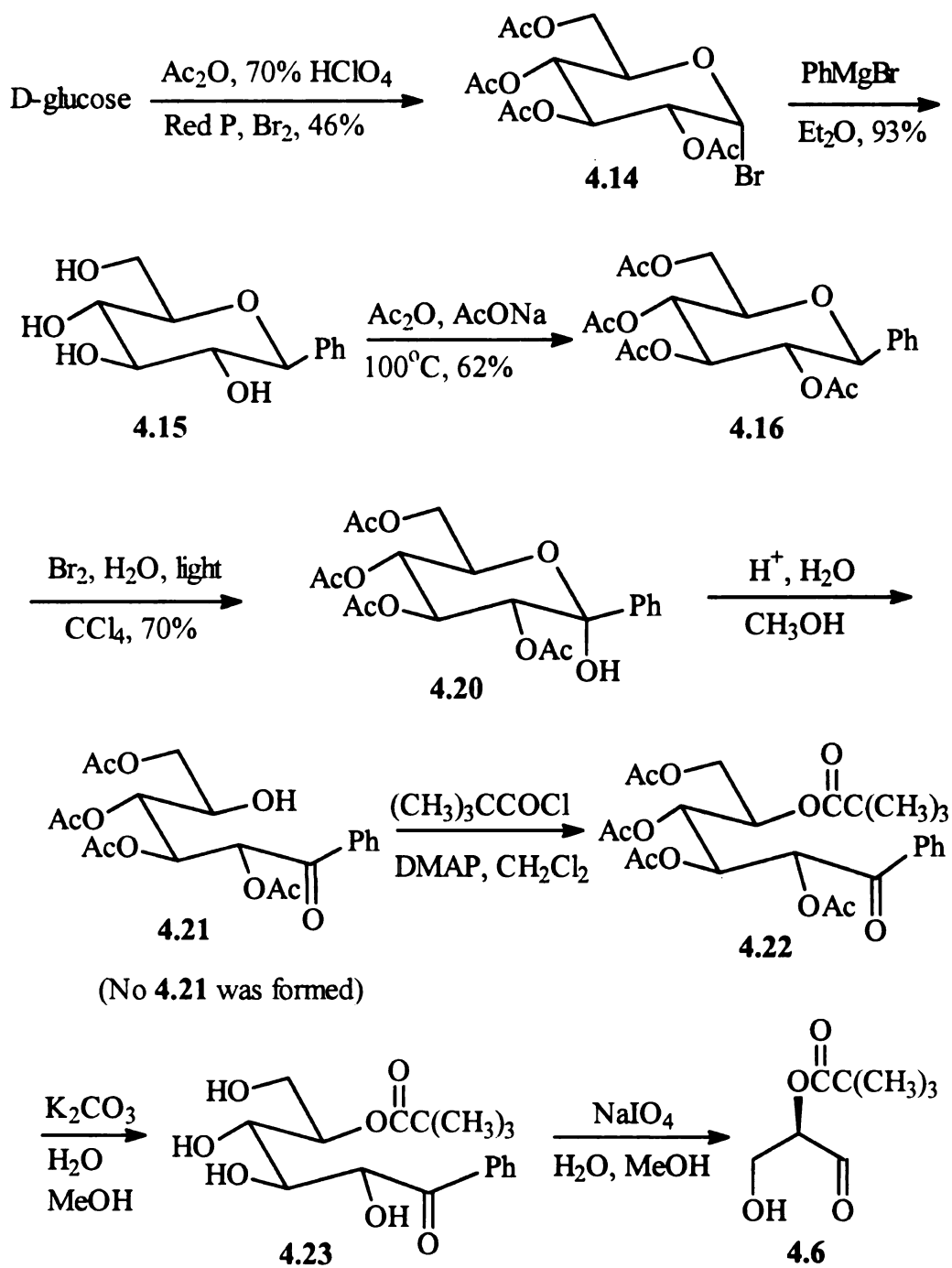
Even though (2R,3S)-2-*O*-trimethylacetylerythritol (**4.11**) has three free hydroxyl groups, it does not dissolve in water. The cleavage of this erythritol derivative to the final product has not yet been achieved on treatment with sodium periodate or periodic acid in homogeneous or heterogeneous solutions, while it was completely decomposed on treatment with lead tetraacetate. This is also due to the formation of the tridentate complex between the 1,2,4-triol and the periodate ion.

As discussed earlier, the chirality of C-5 is self-protected by the D-glucopyranose ring. This advantage is still utilized and the general strategies (**Schemes 4.3** and **4.4**) are similar to the previous one.

As shown in **Scheme 4.3**, D-glucose was converted into tetra-*O*-acetyl- α -glucopyranosyl bromide (**4.14**), which reacted with phenyl magnesium bromide to give D-glucosylbenzene (**4.15**). Before releasing the hydroxyl group at the C-5 position, all four of the free hydroxyl groups were acetylated. The resulting tetra-*O*-acetyl-D-glucosylbenzene (**4.16**) had a secondary benzyl ether. Originally this had been thought to cleave easily, but later it turned out to be very difficult. Many methods for cleavage of a typical benzyl group failed to cleave the bond between C-1 and O-5. These included hydrogenolysis with $\text{H}_2/\text{Pd-C}$, transfer hydrogenation with cyclohexadiene/ Pd-C , reductions with $\text{Et}_3\text{SiH}/\text{BF}_3\cdot\text{OEt}_2$, $\text{Et}_3\text{SiH}/\text{CF}_3\text{COOH}$, $\text{Et}_3\text{SiH}/\text{AlCl}_3$, oxidation with NBS/AIBN, cleavages with FeCl_3 , $\text{Me}_3\text{SiCl}/\text{NaI}/\text{MeCN}$, and several other methods.



Scheme 4.3: Another synthetic approach to (R)-2-O-trimethylacetyl-glyceraldehyde from D-glucose.



Scheme 4.4: Another synthetic approach to (R)-2-O-trimethylacetyl-glyceraldehyde from D-glucose.

On treatment with bromine and under irradiation, tetra-*O*-acetyl-D-glucosylbenzene was converted into tetra-*O*-acetyl-1-*C*-phenyl- α -glucopyranose (**4.20**) with the ring remaining (**Scheme 4.4**). This glucopyranose derivative prefers the cyclic form to the acyclic one and its reduction with $\text{Et}_3\text{SiH}/\text{BF}_3\cdot\text{OEt}_2$ resulted in the glucosylbenzene derivative (**4.16**) with the ring intact. To carry out the strategies shown in **Schemes 4.3** and **4.4**, much more endeavor is needed to overcome the difficulty in opening the benzyl glycoside.

CONCLUSIONS

Both D-mannitol and D-glucose have been utilized as the starting materials for the synthesis of (R)-2-*O*-trimethylacetylglyceraldehyde. D-Mannitol is shown as an excellent precursor for the synthesis due to its C_2 symmetry axis and short synthetic route. The synthetic routes from D-glucose are much longer and involve several steps of protections/deprotections of the hydroxyl groups due to its lack of a C_2 symmetry axis. Several synthetic approaches have been tried, but have not been accomplished. The vicinal diol cleavages of both (2R,5R)-2,5-di-*O*-trimethylacetyl-D-mannitol and (2R,3S)-2-*O*-trimethylacetylerythritol have not been successful with sodium periodate or periodic acid in many different conditions that have been tried. The ring-openings of both the glucosylbenzene and the 1-*C*-phenylglucose derivatives are difficult to accomplish. These two synthetic approaches still involve the oxidative cleavages of the compounds bearing a moiety of 1,2,4-triol. Further endeavor is needed to overcome these difficulties.

EXPERIMENTAL

^1H NMR and ^{13}C NMR spectra were measured on a Varian Inova-300 or Varian-300 spectrometer (300 MHz). The chemical shifts are given in δ values with TMS as the internal standard or relative to the chloroform line at 7.24 ppm for ^1H and 77.0 ppm for ^{13}C .

1,3:4,6-Di-*O*-Benzylidene-D-mannitol (4.2)

After D-mannitol (4.1) (20 g) was dissolved in a mixture of benzaldehyde (40 mL) and anhydrous dimethyl sulfoxide (200 mL), concentrated sulfuric acid (10 mL) was added dropwise (requiring *ca.* 10 minutes) during which time the mixture warmed. After being kept overnight at room temperature, the mixture was poured into an ice-water slush. Oil separated out on the bottom and slowly became solid as the ice melted. This solid was separated by filtration and rinsed with several small portions of hexanes, and then was recrystallized from methanol-water solvent to give 1,3:4,6-di-*O*-benzylidene-D-mannitol (4.2). The yield was 31%.

^1H NMR (300 MHz, CDCl_3): δ 7.44 (4 H, m), 7.36 (6 H, m), 5.49 (2 H, s), 4.37 (2 H, dd, $J = 10.8, 4.8$ Hz), 4.18 (4 H, m), 3.64 (2 H, dd, $J = 10.8, 9.1$ Hz), 2.94 (2 H, s, broad); ^{13}C NMR (75 MHz, CDCl_3): δ 136.96, 129.32, 128.47, 125.92, 101.52, 80.46, 70.54, 61.57.

1,3:2,5:4,6-Tri-*O*-benzylidene-D-mannitol (4.3)

This was a byproduct of the preparation of 1,3:4,6-di-*O*-benzylidene-D-mannitol (4.2). After being recrystallized to give 1,3:4,6-di-*O*-benzylidene-D-mannitol, the mother liquid was half removed under reduced pressure and recrystallized again in methanol and water solution to give the byproduct, 1,3:2,5:4,6-tri-*O*-benzylidene-D-mannitol (4.3). The yield was 7%.

^1H NMR (300 MHz, CDCl_3): δ 7.42 (15 H, m), 5.89 (1 H, s), 5.58 (1 H, s), 5.51 (1 H, s), 4.46 (1 H, dd, $J = 10.5, 5.1$ Hz), 4.09 (2 H, m), 3.92 (3 H, m), 3.78 (2 H, m); ^{13}C NMR (75 MHz, CDCl_3): δ 137.66, 137.29, 129.00, 128.93, 128.89, 128.31, 128.24, 128.19, 126.22, 126.16, 126.13, 100.88, 100.77, 99.26, 82.30, 82.02, 69.27, 69.13, 65.99, 60.69.

1,3:4,6-Di-*O*-benzylidene-2,5-di-*O*-trimethylacetyl-D-mannitol (4.4)

Trimethylacetyl chloride (2.42 g, 20.1 mmol) was added to a solution of 1,3:4,6-di-*O*-benzylidene-D-mannitol (4.2) (1.20 g, 3.35 mmol) and DMAP (2.46 g, 20.1 mmol) in anhydrous dichloromethane (10 mL) cooled in an ice bath and the solution was stirred overnight with a drying tube. The reaction mixture was diluted with chloroform and then poured into ice water. The organic layer was washed with saturated sodium bicarbonate solution twice, water, and brine, dried over anhydrous sodium sulfate, filtered and

concentrated under reduced pressure to give the product, 1,3:4,6-di-*O*-benzylidene-2,5-di-*O*-trimethylacetyl-D-mannitol (**4.4**), without further separation. The yield was 127%.

^1H NMR (300 MHz, CDCl_3): δ 7.46 (4 H, m, Ph-H), 7.33 (6 H, m, Ph-H), 5.44 (2 H, s, Ph-CH-), 5.23 (2 H, dt, $J = 9.6, 5.4$ Hz, H-2, H-5), 4.50 (2 H, dd, $J = 10.2, 5.4$ Hz, H-1e, H-6e), 3.93 (2 H, d, $J = 9.6$ Hz, H-3, H-4), 3.61 (2 H, t, $J = 10.2$ Hz, H-1a, H-6a), 1.22 (18 H, s); ^{13}C NMR (75 MHz, CDCl_3): δ 173.95, 136.95, 128.84, 128.12, 126.00, 101.26, 76.14, 68.00, 61.46, 38.83, 27.08.

2,5-Di-*O*-trimethylacetyl-D-mannitol (4.5)

Boron trifluoride diethyl etherate (16.3 g, 115 mmol) was added dropwise to a stirred solution of 1,3:4,6-di-*O*-benzylidene-2,5-di-*O*-trimethylacetyl-D-mannitol (**4.4**) (6.07 g, 11.5 mmol) in anhydrous dichloromethane (50 mL) cooled in an ice water bath. After stirring overnight, the reaction was quenched with 5% sodium bicarbonate solution, separated, and the aqueous layer was extracted with chloroform twice. The combined organic layer was washed with water, and brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified to give the product, 2,5-di-*O*-trimethylacetyl-D-mannitol (**4.5**) by flash column chromatography on silica gel using a gradient elution from pure chloroform to chloroform/methanol 10:1. The yield was 73%.

^1H NMR (300 MHz, CDCl_3): δ 4.99 (2 H, m), 4.68 (2 H, dd, $J = 4.2, 1.8$ Hz), 4.00 (2 H, dd, $J = 9.3, 6.9$ Hz), 3.74 (2 H, dd, $J = 9.3, 6.9$ Hz), 1.22 (18 H, s); ^{13}C NMR (75 MHz, CDCl_3): δ 177.98, 80.12, 73.33, 70.49, 38.75, 27.15.

(2R,3S)-1,3-*O*-Benzylideneerythritol (4.9)

Sodium borohydride (5.28 g, 0.139 mol) was added in small portions to a stirred mixture of (2R,3R)-2,4-*O*-benzylideneerythritol (**4.8**) (29.3 g, 0.141 mol) in tetrahydrofuran (250 mL) and water (250 mL) cooled at $\sim 0^\circ\text{C}$. After one hour, ethanol (200 mL) was added, and the solution was adjusted to about pH = 8 through addition of glacial acetic acid. THF and ethanol were removed under reduced pressure. The remaining reaction mixture was extracted with ethyl acetate three times. The combined organic layer was washed with sodium bicarbonate solution, water, and brine, dried over anhydrous sodium bisulfate, filtered, dried under reduced pressure, and recrystallized from ether to give the pure product, (2R,3S)-1,3-*O*-benzylideneerythritol (**4.9**). The yield was 80%.

mp 136.5-137.0 $^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3): δ 7.46 (2 H, m, Ar-H), 7.35 (3 H, m, Ar-H), 5.51 (1 H, s, PhCH-), 4.30 (1 H, dd, $J = 10.5, 5.4$ Hz, H-1e), 3.92 (3 H, m, H-2, H-4a, H-4b), 3.69 (1 H, dt, $J = 9.0, 4.5$ Hz, H-3), 3.61 (1 H, t, $J = 10.5$ Hz, H-1a), 2.03 (2 H, s, broad, OH); ^{13}C NMR (75 MHz, CDCl_3): δ 137.32, 129.15, 128.32, 126.09, 101.07, 81.13, 70.85, 62.72, 62.56.

(2R,3S)-1,3-*O*-Benzylidene-4-*O*-triphenylmethylethritol (4.10)

Triphenylmethyl chloride (32.4 g, 0.116 mol, 1.1 equivalents) was added to a stirred solution of (2R,3S)-1,3-*O*-benzylideneerythritol (**4.9**) (22.2 g, 0.106 mol) and anhydrous pyridine (9.20 g, 0.116 mol, 1.1 equivalents) in anhydrous dichloromethane (500 mL). After the reaction was refluxed at 40 °C overnight with a drying tube, the solution was washed with water three times, then with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give the product, (2R,3S)-1,3-*O*-benzylidene-4-*O*-triphenylmethylethritol (**4.10**). The yield was 120 %.

¹H NMR (300 MHz, CDCl₃): δ 7.50-7.37 (20 H, m, Ar-H), 5.51 (1 H, s, PhCH-), 4.32 (1 H, dd, J = 10.5, 4.2 Hz, H-1e), 3.85 (2 H, m), 3.64 (2 H, m), 3.41 (1 H, dd, J = 9.3, 6.3 Hz, H-4a), 2.93 (1 H, s, broad, -OH); ¹³C NMR (75 MHz, CDCl₃): δ 146.86, 143.30, 137.50, 128.90-126.12 (many peaks), 100.96 (PhCH-), 87.62 (Ph₃C-), 79.14, 70.59, 65.70, 65.19.

(2R,3S)-1,3-*O*-Benzylidene-2-*O*-trimethylacetyl-4-*O*-triphenylmethylethritol (4.11)

Trimethylacetyl chloride (25.6 g, 0.212 mol) was added slowly to a stirred solution of (2R,3S)-1,3-*O*-benzylidene-4-*O*-triphenylmethylethritol (**4.10**) (48.0 g, 0.106 mol) and DMAP (25.9 g, 0.212 mol) in anhydrous dichloromethane (200 mL) cooled in an ice bath. After stirring overnight with a drying tube at room temperature, the reaction solution was refluxed at 45 °C for 10 hours, then washed with water six times,

then with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give the product, (2R,3S)-1,3-*O*-benzylidene-2-*O*-trimethylacetyl-4-*O*-triphenylmethylethritol (**4.11**). The yield was 98%.

¹H NMR (300 MHz, CDCl₃): δ 7.62-7.26 (20 H, m, Ar-H), 5.62 (1 H, s, PhCH-), 4.98 (1 H, dt, J = 9.9, 5.4 Hz, H-2), 4.40 (1 H, dd, J = 10.5, 5.4 Hz, H-1e), 4.02 (1 H, ddd, J = 9.9, 5.1, 2.4 Hz, H-3), 3.59 (1 H, t, J = 10.5 Hz, H-1a), 3.22 (2 H, m, H-4a, H-4b), 1.25 (9 H, s, CH₃); ¹³C NMR (75 MHz, CDCl₃): δ 176.95, 143.80, 137.70, 128.96-126.36 (many peaks), 101.03, 86.41, 78.89, 68.13, 63.12, 62.86, 38.60, 26.52. HRMS Exact mass: calcd for C₃₅H₃₅O₅ [M-1]⁺, 535.2485. Found 535.2486.

(2R,3S)-1,3-*O*-Benzylidene-2-*O*-trimethylacetylerythritol (4.12**)**

Trifluoroacetic acid solution (19.0 mL, 90%) was added to a stirred solution of (2R,3S)-1,3-*O*-benzylidene-2-*O*-trimethylacetyl-4-*O*-triphenylmethylethritol (**4.11**) (39.0 g, 73.0 mmol) in dichloromethane (300 mL). Five minutes later, the reaction was diluted through addition of dichloromethane (200 mL), washed with saturated sodium bicarbonate solution, water, and brine, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The pure product was obtained by flash column chromatography on silica gel using a gradient elution from pure chloroform to chloroform/methanol 10:1. The yield was 46%.

H.N.

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^1H NMR (300 MHz, CDCl_3): δ 7.40 (5 H, m, Ar-H), 5.57 (1 H, s, PhCH-), 4.94 (1 H, dt, J = 9.9, 5.4 Hz, H-2), 4.42 (1 H, dd, J = 10.5, 5.4 Hz, H-1e), 3.89 (1 H, ddd, J = 9.9, 5.4, 2.4 Hz, H-3), 3.81 (1 H, dd, J = 8.1, 2.4 Hz, H-4a), 3.71 (1 H, dd, J = 8.1, 5.4 Hz, H-4b), 3.67 (1 H, t, J = 10.5 Hz, H-1a), 1.24 (9 H, s, CH_3); ^{13}C NMR (75 MHz, CDCl_3): δ 174.02, 137.05, 129.30, 128.37, 126.21, 101.41, 79.68, 67.85, 62.57, 61.99, 38.82, 27.10.

(2R,3S)-3-*O*-Benzoyl-2-*O*-trimethylacetylerythritol

Boron trifluoride diethyl etherate (0.21 g, 1.5 mmol) was added to a stirred solution of (2R,3S)-1,3-*O*-benzylidene-2-*O*-trimethylacetyl-4-*O*-triphenylmethylethritol (**4.11**) (0.20 g, 0.37 mmol) in anhydrous dichloromethane (1 mL). After stirring overnight, the reaction solution was quenched with water, and diluted with dichloromethane. The organic layer was washed with sodium bicarbonate solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product mixture was purified to give (2R,3S)-3-*O*-benzoyl-2-*O*-trimethylacetylerythritol as the dominant product by flash column chromatography on silica gel using a gradient elution from pure chloroform to chloroform/methanol 1:1. A small amount of (2R,3S)-2-*O*-trimethylacetylerythritol (**4.13**) was also obtained.

^1H NMR (300 MHz, CDCl_3): δ 8.02 (2 H, m, Ar-H), 7.56 (1 H, m, Ar-H), 7.43 (2 H, m, Ar-H), 5.56 (1 H, q, J = 5.4 Hz, H-3), 5.39 (1 H, q, J = 5.4 Hz, H-2), 4.20 (1 H, dd, J = 10.2, 5.4 Hz, H-4a), 4.17 (1 H, dd, J = 9.6, 5.4 Hz, H-1a), 3.97 (1 H, dd, J = 10.2, 5.4

Hz, H-4b), 3.83 (1 H, dd, $J = 9.6, 5.4$ Hz, H-1b), 1.20 (9 H, s, CH_3); ^{13}C NMR (75 MHz, CDCl_3): δ 175.20, 165.59, 138.88, 133.40, 129.21, 128.50, 72.14, 71.52, 70.57, 70.39, 38.60, 27.10.

(2R,3S)-2-*O*-Trimethylacetylerythritol (4.13)

Boron trifluoride diethyl etherate (2.99 g, 21.1 mmol) was added dropwise to a stirred solution of (2R,3S)-1,3-*O*-benzylidene-2-*O*-trimethylacetylerythritol (4.12) (1.55 g, 5.27 mmol) in anhydrous dichloromethane (10 mL) and the solution was stirred overnight. The reaction was quenched with 5% sodium bicarbonate solution and was then extracted with chloroform for three times. The combined organic layer was washed with water, and then with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give the product, (2R,3S)-2-*O*-trimethylacetylerythritol (4.13). The yield was 71%.

^1H NMR (300 MHz, CDCl_3): δ 5.09 (1 H, dt, $J = 5.7, 3.9$ Hz, H-2), 4.44 (1 H, q, $J = 5.7$ Hz, H-3), 4.06 (1 H, dd, $J = 10.2, 5.7$ Hz, H-1a), 3.95 (1 H, dd, $J = 9.3, 5.7$ Hz, H-4a), 3.79 (1 H, dd, $J = 10.2, 3.9$ Hz, H-1b), 3.68 (1 H, dd, $J = 9.3, 5.7$ Hz, H-4b), 1.85 (3 H, s, broad, -OH), 1.23 (9 H, s, $-\text{CH}_3$); ^{13}C NMR (75 MHz, CDCl_3): δ 177.51, 73.48, 72.28, 71.16, 70.80, 38.96, 27.18.

2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl bromide (4.14)

Perchloric acid (0.6 mL, ~70%) was added dropwise to a solution of acetic anhydride (100 mL) cooled in an ice-water bath. The solution was warmed to room temperature and anhydrous D-glucose (25 g) was added to the stirred mixture at such a rate to keep the reaction temperature between 30 and 40 °C. Red phosphorus (7.5 g) was added slowly after cooling the reaction mixture to 20 °C (caution: fire!), followed by addition of bromine (45 g) at such a rate to keep the temperature below 20 °C. Water (9 mL) was added dropwise to the continuously stirred and cooled mixture to prevent the temperature from rising above 20 °C. After the reaction mixture was kept for three hours at room temperature, chloroform (75 mL) was added and the mixture was filtered through a filter-bed of fine glass wood. The reaction flask and the filter funnel were washed with chloroform (15 mL). The filtrate was washed with cold water twice, and the aqueous layer was extracted with chloroform. The combined organic layer was washed with aqueous sodium bicarbonate solution and then concentrated under reduced pressure. Recrystallization from ethyl ether gave the product, tetra-*O*-acetyl- α -D-glucopyranosyl bromide (4.14). The yield was 46%.

mp 88-89 °C. ^1H NMR (300 MHz, CDCl_3): δ 6.59 (1 H, d, J = 4.5 Hz, H-1), 5.54 (1 H, dd, J = 9.9, 9.3 Hz, H-3), 5.15 (1 H, dd, J = 11.1, 9.3 Hz, H-4), 4.82 (1 H, dd, J = 9.9, 4.5 Hz, H-2), 4.29 (2 H, m, H-6), 4.11 (1 H, dt, J = 11.1, 1.8 Hz, H-5), 2.09 (3 H, s), 2.08 (3 H, s), 2.04 (3 H, s), 2.02 (3 H, s); ^{13}C NMR (75 MHz, CDCl_3): δ 170.31, 169.66, 169.60, 169.28, 86.40, 71.96, 70.44, 69.99, 67.00, 60.80, 20.49, 20.48, 20.45, 20.38.

D-Glucosylbenzene (4.15)

Tetra-*O*-acetyl- α -D-glucopyranosyl bromide (4.11 g, 10.0 mmol) dissolved in anhydrous ether (50 mL) was added to a stirred solution of benzyl magnesium bromide (40 mL, 3 M in ethyl ether) for about one hour. After refluxing for five hours, ether (50 mL) was added, and the ether layer was decanted carefully and extracted with water (60 mL). Ethanol (60 mL) was added dropwise to the residue and then was evaporated under reduced pressure. Water (100 mL) was added slowly to the residue and the combined aqueous solution was filtered to remove the solid, 1, 1-diphenylethanol. The filtrate was washed with chloroform and concentrated under reduced pressure to give the crude product, D-glucosylbenzene (4.15), without any separation. The yield was 93%.

^1H NMR (300 MHz, D_2O): δ 7.25 (5 H, s), 4.12 (1 H, m), 3.64 (2 H, m), 3.42 (4 H, m); ^{13}C NMR (75 MHz, D_2O): δ 137.32, 128.84, 128.70, 127.89, 81.65, 79.94, 77.02, 73.78, 69.59, 60.70.

2,3,4,6-Tetra-*O*-acetyl-D-glucosylbenzene (4.16)

A mixture of D-glucosylbenzene (4.15) (22.3 g, 93.0 mmol) and sodium acetate (5.0 g) in acetic anhydride (150 mL) was heated at 100 °C overnight. The acetylation mixture was cooled, poured into ice water and then stirred for six hours to hydrolyze the excess anhydride. The solution was extracted with ether for three times. The combined

ether layer was washed with saturated sodium bicarbonate solution, water, and brine, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to give the crude product, followed by recrystallization in acetone to give the pure product, tetra-*O*-acetyl-D-glucosylbenzene (**4.16**). The yield was 62%.

^1H NMR (300 MHz, CDCl_3): δ 7.33 (5 H, m), 5.34 (1 H, t, $J = 6.0$ Hz), 5.24 (1 H, t, $J = 6.0$ Hz), 5.14 (1 H, t, $J = 6.0$ Hz), 4.40 (1 H, d, $J = 6.0$ Hz), 4.29 (1 H, dd, $J = 7.5$, 2.7 Hz), 4.16 (1 H, dd, $J = 7.5$, 1.2 Hz), 3.84 (1 H, ddd, $J = 6.0$, 2.7, 1.2 Hz), 2.09 (3 H, s), 2.06 (3 H, s), 2.00 (3 H, s), 1.80 (3 H, s); ^{13}C NMR (75 MHz, CDCl_3): δ 170.60, 170.25, 169.36, 168.70, 136.08, 128.77, 128.30, 127.00, 80.11, 76.02, 74.12, 72.48, 68.43, 62.22, 20.63, 20.51, 20.49, 20.22.

2,3,4,6-Tetra-*O*-acetyl-1-*C*-phenyl- α -D-glucopyranose (4.20**)**

Bromine (48 μL , 0.96 mmol) was added to a stirred mixture of tetra-*O*-acetyl-D-glucosylbenzene (**4.16**) (0.10 g, 0.24 mmol) in carbon tetrachloride (6 mL) and water (4 mL) cooled at $\sim 20^\circ\text{C}$, and the reaction mixture was irradiated with a tungsten lamp (150 W) at a distance of ~ 5 cm. After 3.5 hours, the reaction was complete. Saturated sodium bicarbonate solution was added, and the reaction mixture was extracted with dichloromethane three times. The combined organic solution was washed with water, then with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give the product, 2,3,4,6-tetra-*O*-acetyl-1-*C*-phenyl- α -D-glucopyranose (**4.20**). The yield was 70%.

^1H NMR (300 MHz, CDCl_3): δ 7.52 (2 H, m), 7.35 (3 H, m), 5.60 (1 H, t, $J = 9.9$ Hz, H-3), 5.27 (1 H, t, $J = 9.9$ Hz, H-4), 5.06 (1 H, dd, $J = 9.9, 1.5$ Hz, H-2), 4.41 (1 H, t, $J = 9.9, 4.2, 2.4$ Hz, H-5), 4.31 (1 H, dd, $J = 12.0, 4.2$ Hz, H-6), 4.18 (1 H, dd, $J = 12.0, 2.4$ Hz, H-6'), 2.98 (1 H, d, $J = 1.5$ Hz, -OH), 2.10 (3 H, s), 2.05 (3 H, s), 1.96 (3 H, s), 1.88 (3 H, s); ^{13}C NMR (75 MHz, CDCl_3): δ 170.86, 170.21, 169.63, 168.97, 139.70, 129.65, 128.40, 125.60, 97.22, 73.55, 71.50, 68.75, 68.71, 62.20, 20.83, 20.68, 20.66, 20.41.

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