University of Alberta

An Adjuvant Investigation: Chemical Synthesis and Immunological Evaluation of Natural and Unnatural Archaeal Lipids

by

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To My Supportive Family

Abstract

Liposomes formulated from the total polar lipid (TPL) extract of Archaebacteria stimulate strong humoral and cell mediated immune responses to encapsulated antigen. This study involves the synthesis and immunological evaluation of specific components of the TPL extract of *Methanosphaera stadtmanae*, *Methanobrevibacter smithii* and *Methanopyrus kandleri*. Specifically glycerol phytanyl ether lipids containing attached head groups of gentiobiose **5** and **6**, serine **7** and **8**, and choline **9**, and as well as an artificial archaeal lipid **123** containing a β-mannan trisaccharide antigen were synthesized. Murine serum obtained from a variety of liposome immunizations were evaluated for ovalbumin (OVA) specific IgM and IgG antibodies and compared to control immunizations. Liposome formulations containing serine lipids **7** and **8** and the β-mannan **123** induced strong humoral immunity with a class switch to IgG antibody, while liposomes consisting of gentiobiose or choline lipids **5**, **6**, and **9** had little to no adjuvanticity toward encapsulated antigen.

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Table of Contents

List of Tables

List of Figures

List of Schemes

List of Symbols, Nomenclature or Abbreviations

α	Alpha
A	Archaeal
AC	Archaetidylcholine
Ac	acetyl
AD	Asymmetric dihydroxylation
ADP	Adenosine diphosphate
APC	Antigen presenting cell
APS	Archaetidylserine
ATP	Adenosine triphosphate
β	Beta
BCA	Bicinchoninic acid
t-BHP	*tert*-butyl hydrogen peroxide
Bn	benzyl
BnBr	benzyl bromide
BSA	Bovine serum albumin
Bz	benzoyl
BzCl	benzoyl chloride
C	Caldarchaeal
CH_2Cl_2	Dichloromethane
D	Dextrorotatory
DCC	1,3-Dicyclohexylcarbodiimide
DCM	dichloromethane
DET	diethyl tartrate
DIBALH	Diisobutyl aluminum hydride
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
ELISA	enzyme linked immunosorbent assay
EWG	electron-withdrawing group
Glc*p*	Glucopyranoside

GlcNAc	*N*-acetylglucosamine, 2-acetamido-2-deoxy-D-glucose
HMQC	Heteronuclear multiple quantum coherence
HPLC	High pressure liquid chromatography
HRMS	High resolution mass spectroscopy
HRP	horseradish peroxidase
I	Inositol
IAD	Intramolecular aglycon delivery
IFN-γ	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
IR	Infrared
kDa	Kilo dalton
L	Levorotatory
LDA	Lithium diisopropylamine
MHC	Major histocompatibility complex
MPLC	Medium pressure liquid chromatography
MS	Mass spectroscopy
NBS	*N*-Bromosuccinimide
NIS	*N*-Iodosuccinimide
NMR	Nuclear magnetic resonance
OVA	Ovalbumin
P	Phosphate
PP_i	Inorganic pyrophosphate
PPh_3	triphenylphosphine
ppm	parts per million
pyr.	pyridine
R_f	Retention factor
RNA	Ribonucleic Acid
S	Serine
Tf_2O	Triflic anhydride
THF	Tetrahydrofuran

Th1	T helper cell subset 1
Th2	T helper cell subset 2
TLC	Thin-layer chromatography
TPL	Total polar lipid
TTBP	Tri-*tert*-butyl pyrimidine
UV	Ultraviolet
Z	benzyloxycarbonyl

Chapter 1

Introduction

Adjuvants and the Immune Response

1.1 Immune Response

The humoral arm of the immune system responds to infection by producing immunoglobulins or antibodies. B cells produce many immunoglobulin (Ig) isotypes used by the immune system to combat a variety of antigen types including polysaccharides, lipids and protein. Polysaccharide and lipid antigens are removed *via* a T cell independent mechanism, except for CD1 lipid presentation, whereas clearance of protein antigen is dependent on T helper cells. B cells can act as antigen presenting cells displaying peptide antigen to T cells. Protein antigen binds to surface immunoglobulin on B cells, it is internalized, processed into peptide fragments and displayed to T cells by the major histocompatibility complex (MHC). After the activation of T cells, cytokines are produced that cause growth and differentiation of B lymphocytes. One of the key differentiation functions mediated by cytokines is isotype switching and secretion of the antibody. The initial response to the antigen may have involved IgM but when an isotype switch occurs it changes the heavy chain region to a new isotype A, D, E or G. Isotype switching allows the humoral system to carry out its effector function, the physical elimination of antigen. Antibodies can bind to the surface of the pathogen neutralizing its invasiveness and capability to proliferate. Alternatively, antibodies bind to the pathogen initializing opsonization, which causes increased phagocytosis of the antigen. Further,

the circulation of certain immunoglobulin isotypes can stimulate complement proteins to participate in eradication of the antigen.

Uptake of antigen and presentation to T cells is not a function limited to B lymphocytes. Antigen presenting cells (APC), such as dendritic cells or macrophages, can also take up pathogens at the site of infection. Depending on the nature of the pathogen two different presentation pathways can occur. The engulfed pathogen can be compartmentalized into the cytosol or the endosome. The degraded antigen, in the form of peptide fragments is loaded onto the MHC. The cytosolic peptides are bound to MHC class I protein whereas, peptides within the endosome are bound to the MHC class II isotype. The APC, which has MHC-peptide complex displayed on its cell surface binds to a naïve T-cell. Once this recognition event occurs the T-cell population can proliferate *via* the production of cytokines. Recognition of MHC class I stimulates naïve T-cells to differentiate into $CD8^+$ cytotoxic T-cells, which are responsible for cell lysis. By comparison, the recognition of MHC class II leads to T-cell differentiation into the $CD4^+$ T-cell subsets that stimulate either cell-mediated immunity (Th1) or humoral immunity (Th2). Effector functions of these two subsets include cytokine secretion, macrophage activation, stimulation of antibody secreting B cell lymphocytes and inflammatory responses which ultimately lead to antigen clearance.

1.2 Vaccine Research

The development of vaccines has not only provided a better understanding of the intricacies of the immune system but has improved the quality of life for millions of people around the world. Vaccinology is an important area of research that evolved from the invention of the smallpox vaccine in the late 18^{th} century by Edward Jenner. Vaccine

formulations do not cause lethal infection because they consist of inactivated organisms/toxins, live attenuated pathogens or small synthetic epitopes. Vaccinations "trick" the host into believing it is being invaded by a pathogen, thereby programming the specific mechanisms needed to fight future infection. Once vaccinated against a specific disease, the host immune system can draw on its cache of trained antigen presenting cells, T-cells, cytokines and circulating antibodies to clear the pathogen when re-infection occurs.

An active area of research involves the development of synthetic vaccines based on carbohydrate antigens expressed on the surface of specific pathogens. However, the development and progress of this research has been met with varying levels of success because small carbohydrate antigens are generally non-immunogenic. Efforts to circumvent this problem include the conjugation of the synthetic antigen to a highly immunogenic protein carrier. Although this has been successful in a number of cases, there is still a demand for potent clinically acceptable adjuvants to enhance the immunogenicity of carbohydrate based vaccine preparations.

The success of a vaccine depends on a number of factors including its ability to stimulate long lasting adaptive immunity towards a live pathogen. One way to enhance the success of a vaccine is through the use of immunostimulatory compounds, called adjuvants, in vaccine formulations. An adjuvant is any substance that enhances the immune response to an antigen with which it is mixed.[1] Vaccine adjuvants are designed to stimulate the immune system in the exact same manner as natural pathogens. Therefore, when new vaccine adjuvants are designed, one must keep in mind the integral

processes of the immune system such that an effective response against the antigen is accomplished.[2,3]

Many benefits have been outlined for the use of adjuvants[4] including their ability to 1) augment the immunogenicity of otherwise weak antigens or 2) reduce the amount of antigen administered, 3) increase the promptness and duration of the immune response, 4) indirectly induce alterations in antibody structural and binding properties and 5) induce cell-mediated immunity. Adjuvants may also stimulate the immune systems of immunocompromised patients. For these reasons adjuvant research has been given considerable attention.

1.3 Examples of Adjuvants Used in Vaccine Research

Past and present efforts to design adjuvants that strongly stimulate the immune system have been hampered by the lack of information about their mechanism of action. However, many different types of adjuvants have been used in immunological tests. The most common adjuvant formulations in current use are emulsions, mineral salts, bacterial components and lipid molecules. The adjuvants below do not comprise a comprehensive list but is intended to provide a comparison between liposomes and common adjuvants used in our laboratory. There are many different types of adjuvants including bacterial components such as CpG, which is unmethylated bacterial DNA and LPS and derivatives like monophosphoryl lipid A (MPL).[4] Triterpenoid glycosides or saponins are tree extracts and are commonly known as Quil A and a less toxic form QS21.[4] ISCOMS are another type of adjuvant that combines phospholipids and cholesterol with Quil A to make liposome like particles. Others include using the effector molecules of the immune system such as antibodies and cytokines.[4]

1.3.1 Freund's Adjuvant

One of the first adjuvants developed and still used today in many experimental applications is known as Freund's adjuvant. Freund's complete adjuvant consists of a combination of mineral oil, a surfactant, and killed mycobacteria. It is injected into a subject as a water-in-oil emulsion. The emulsion acts to trap the antigen and release it over a long period of time.[5] Typically, Freund's complete adjuvant is only used in the first injection. Later booster injections employ Freund's incomplete adjuvant that is deficient of killed mycobacteria. This adjuvant system stimulates the immune system by localizing the antigen at the site of injection. APC's can then aggregate at the site of infection, facilitating antigen uptake and stimulation of the adaptive immune response. Although this adjuvant system is still widely used it does have some drawbacks. The complications arise from the inability to metabolize the mineral oil used in the emulsion and severe side reactions, caused by the mycobacterial components.[5]

1.3.2 Aluminum Adjuvants

Alum is another adjuvant that has been widely used and is currently one of the few adjuvants licensed for human use. Aluminum in the form of aluminum hydroxide or aluminum phosphate has been widely used to adsorb antigen. The mechanisms of adsorption are not quite clear. However, aluminium adjuvant activity is thought to occur *via* one of two mechanisms.[6] The first involves the depot effect and the delayed clearance of the alum-antigen from the injection site. This allows for prolonged exposure or prolonged release of antigen to the immune system. The second involves the presentation of otherwise soluble antigen in particulate form, which facilitates antigen targeting to APC's. Alum has varying effects on the stimulation of the immune system.

It can effectively stimulate IgM response with a class switch to IgG. Also, when a cytokine, IL-12, was absorbed onto alum it stimulated a Th1 response.[6] With the hypothesis that aluminum plays a specific role in the development and progression of Alzheimer's disease, the long term effects of aluminum exposure have not yet been adequately explored.[7] The uncertainty of its future use in humans opens the door for discovery of new adjuvant systems.

1.3.3 Liposomes

Glycerophospholipids are found in lipid membranes and are one of the major constituents of bacteria, plant, and animal cells. The major function of lipid membranes is to act as a protective barrier. The general structure of phospholipids can be described in terms of three major parts (Figure 1.1). A hydrophobic and a hydrophilic component that is bisected by a phosphate group. The hydrophobic end contains long acyl chains attached to the (stereospecific numbering) *sn*-1,2 positions of glycerol *via* ester linkages. The acyl chains vary in length and degree of saturation. The phosphate is attached to the 3 position of glycerol. The polar head group usually consists of choline, ethanolamine, serine or inositol. The hydrophobic and hydrophilic nature of these compounds results in an ordered orientation in aqueous solutions. Non-polar acyl chains align into a bilayer arrangement so as to minimize their contact with water, where as the polar head group is readily solvated by water and is oriented toward the aqueous environment. When these compounds form the lipid bilayer they spontaneously close into a lipid vesicle that encapsulates an aqueous compartment.

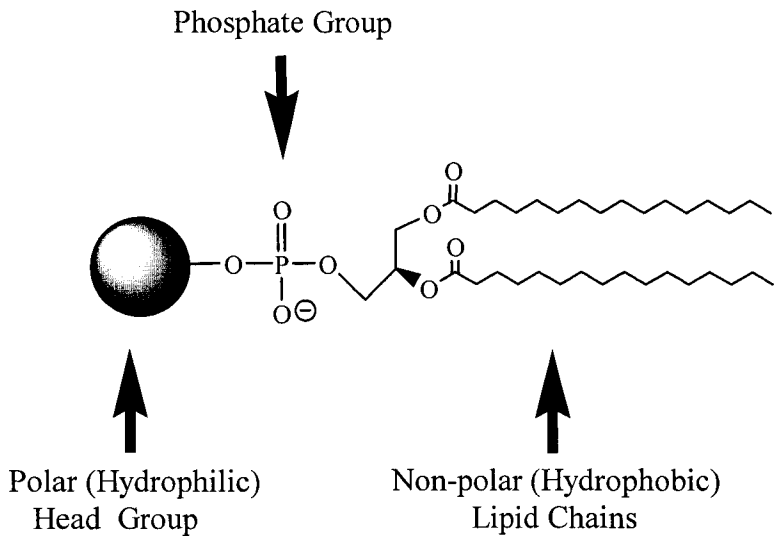


Figure 1.1: General structure of a phospholipid.

Natural and synthetic lipids hydrated in an aqueous solution can form these lipid vesicles, these structures are known as liposomes. They are very good candidates to use as adjuvants[8] because they may mimic natural pathogens. Also, some liposome forms may have long lasting circulation within the body and because of the aqueous core many different water soluble antigens may be entrapped. Liposomes were first used as immunoadjuvants when liposomes encapsulating diphtheria toxoid created higher antibody response when compared to antibody titers of antigen without liposomes.[9] From that first example, liposomes have been used extensively for biochemical and medicinal purposes. Examples include the development of a liposomal malaria vaccine for use in humans[10] and unique liposomes, called archaeosomes, made from the polar lipid extract of archaebacteria.

1.4 Archaeal Lipids to Archaeosomes: An Overview

1.4.1 Archaea: Lipid Structure and Biosynthesis

The discovery of microorganisms living in extreme environments that were unlike Eubacteria led to the new classification of Archaea. Through RNA sequencing[11]

archaebacteria were discovered to be no more related to eubacteria than to eukarya.[12] There are three phenotypes within this new branch having characteristics that set them apart from bacteria: the halophiles, the methanogens and the thermophiles. One such criteria is the environment in which they live. These special microorganisms are capable of residing in extreme environments that include high salt concentrations, anaerobic and high pH and temperature conditions. Due to the nature of their surrounding environment they have developed unique structural features that allow their survival. Cultivation of these microorganisms and elucidation of their molecular composition has revealed that the lipid structure that makes up the Archaea protective lipid bilayer is very different from that found in Eubacteria.[13]

Although a glycerol backbone is common to both eubacteria and archaebacteria, the hydrophobic lipid chains are attached *via* ether linkages instead of ester linkages, at positions sn-2,3 of the glycerol moiety. These alkyl chains range in length from 16 to 32 carbons and contain numerous methyl side chains. There are typically four different types of lipids found within the membrane of archaebacteria (Figure 1.2). The open chain archaeal lipid **1** is the simplest and the one which closely resembles a phospholipid. The lipid membrane may also contain several closed chained forms, a macrocyclic **2** and caldarchaeal lipids **3** and **4**. Both the archaeal **1** and macrocyclic **2** structures are lipids that span only half the membrane whereas the caldarchaeal **3** and **4**, span it completely. The caldarchaeal lipid can also have increased structural diversity by incorporating cyclopentane rings **4** within the isoprenoid chain. It is noteworthy that even though structures **3** and **4** are also macrocycles, adopted literature nomenclature refers to **2** as the macrocyclic structure and **3** and **4** as the caldarchaeal structure.

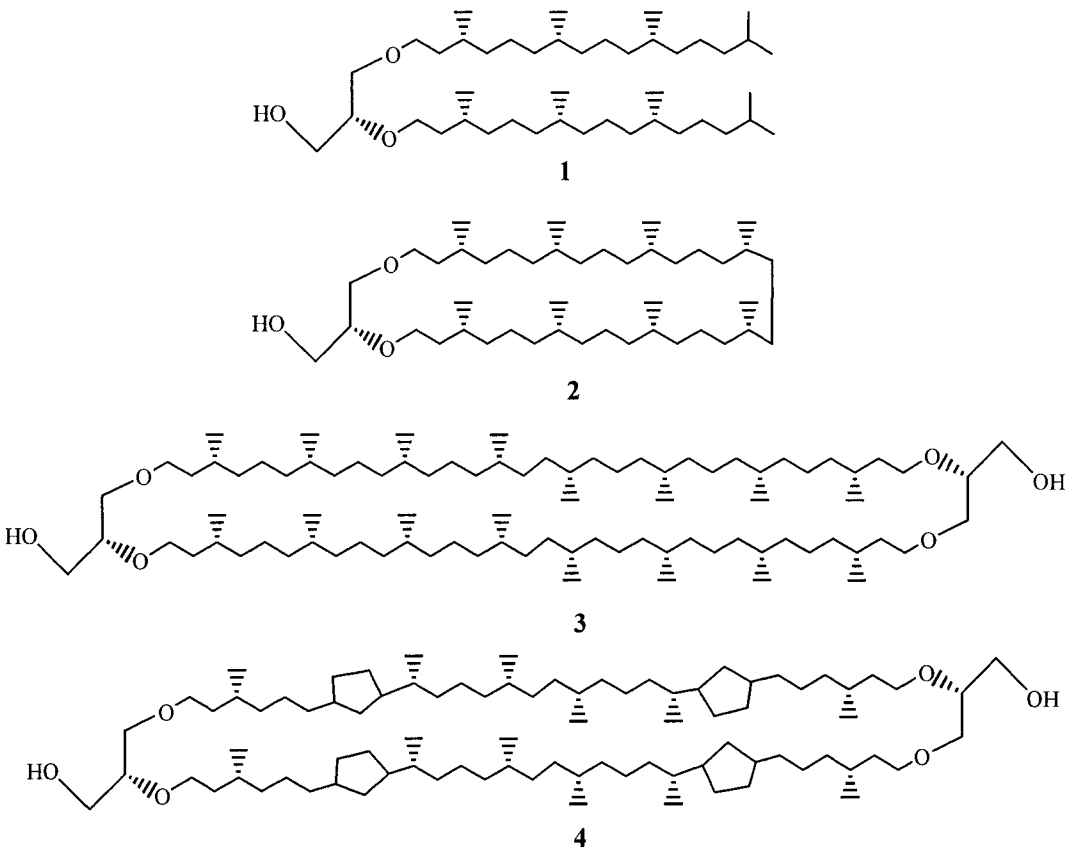


Figure 1.2: Lipid structures of Archaebacteria. 1) Archaeal 2) Macrocyclic 3) Caldarchaeal 4) Caldarchaeal (cyclopentane)

The isoprenoid chains present in these lipids serve to change the transition temperature of the lipid membrane, which acts to decrease the membrane fluidity at elevated temperatures. The transition temperature is the temperature at which the lipid undergoes a liquid crystal to liquid-gel phase transition. Structural changes, such as incorporation of cyclopentane rings as in **4** further increase the transition temperature of the membrane thereby decreasing membrane fluidity and rendering the microorganism more stable to environmental stress.

The absolute stereochemistry of the glycerol moiety in eubacteria is *S*-2,3-*O*-diacyl glycerol however in the archaea structures it has been determined to have the

opposite configuration. Both glycerol enantiomers of the archaeol lipid have been synthesized and the stereochemistry of the synthetic *R*-2,3-*O*-diphytanyl glycerol corresponded to the natural analog isolated from the species *Halobacterium cutirubrum*.[14] The stereochemistry of the branch points in the isoprenoid chains and the five membered rings have been elucidated by comparison of optical rotation of synthetic and natural compounds.[15,16]

The biosynthesis of archaebacterial membrane lipids has been extensively studied.[17,18,19,20] The biotransformation involves the production of geranylgeraniol diphosphate through prenyl transferase reactions originating from mevalonate. Glyceraldehyde-3-P is converted to glycerol-1-P, which produces the (*R*) stereochemistry commonly found in species of Archaea, through the intermediate dihydroxyacetone phosphate (Figure 1.3). This can then act as a prenyl acceptor in the transfer reaction with the geranylgeraniol diphosphate to produce di-*O*-2,3-digeranylgeranyl 1-phospho-glycerol. This substrate can then undergo polar head group functionalization[21,22] and hydrogenation[23] to produce the archaeal lipid. The caldarchaeal lipid is believed to be produced, before hydrogenation, *via* a condensation reaction between two molecules of di-*O*-2,3-digeranylgeranyl 1-phospho-glycerol.[24]

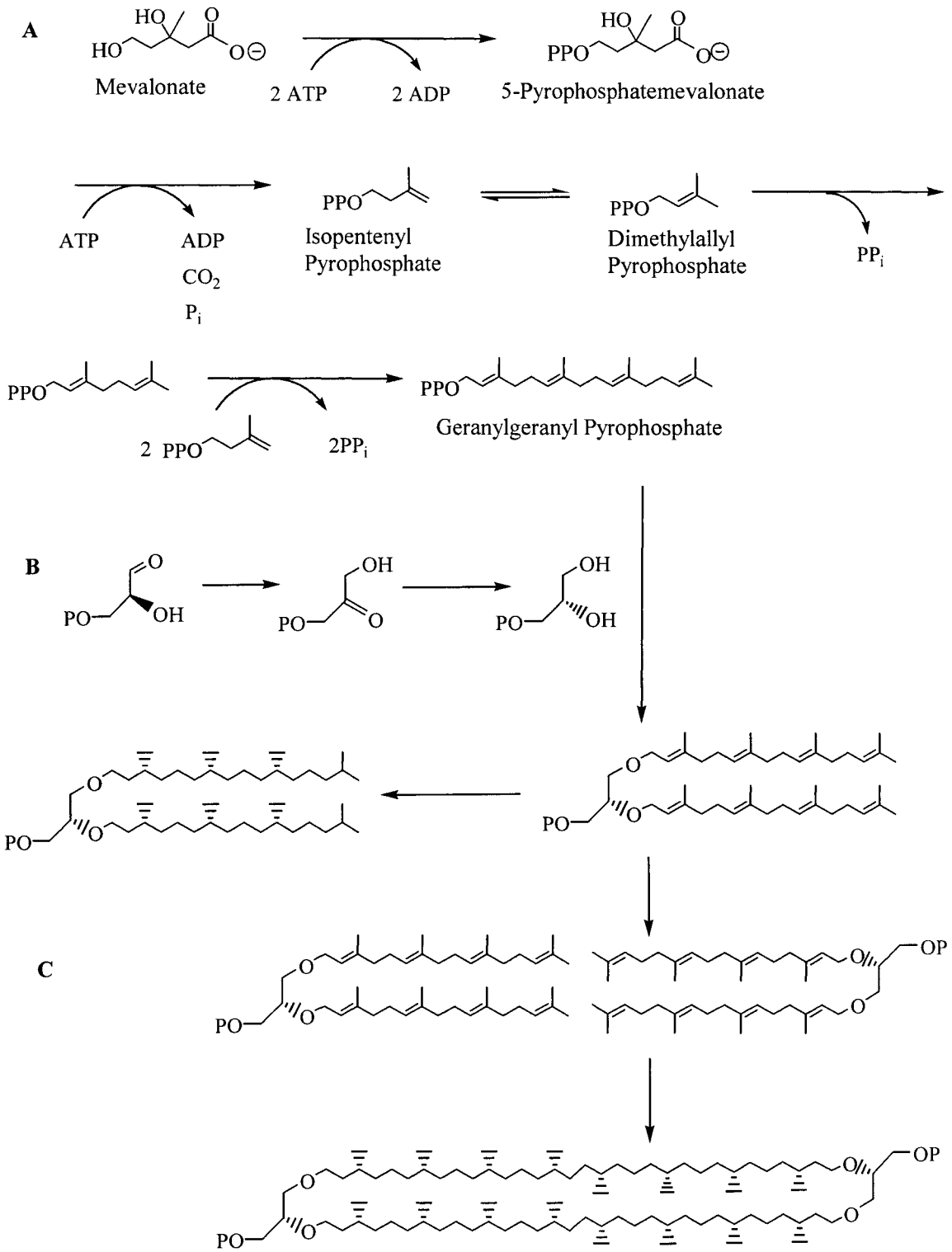


Figure 1.3 Archaeal and caldarchaeal lipid biosynthesis. A) Melavonate pathway producing geranylgeranyl pyrophosphate B) Biosynthesis of glycerol 1-phosphate and the preceeding alkyl functionalization and hydrogenation to produce the archaeal lipid C) Caldarchaeal biosynthesis.

1.4.2 Archaeosomes as Adjuvants

In the last twenty years, the Archaea have received much attention with the discovery of new organisms, structural elucidation of their membranes, and the elucidation of the biosynthetic pathways of the archaeal and caldarchaeal lipids. These discoveries have driven scientists to find new and interesting uses for these Archaea lipid membranes. Recently, archaeosomes have garnered special attention for their use in immunological and medicinal applications. Liposomal formulations made from the total polar lipid (TPL) fraction of specific Archaea demonstrate enhanced stability toward temperature, pH, and salt extremes as well as aerobic environments and therefore may be more stable *in vivo*, in comparison to their ester analogues. These structural attributes render the archaeosomes more stable *in vivo* than those constructed from the corresponding ester-linked glycerolipids, which makes them attractive as adjuvants.

Assessment of liposomal stability was made using the TPL extract of different species of archaebacteria.[25] The archaebacteria used in the study included all three groups of Archaea, the halophiles, methanogens and the thermophiles. Of special interest were two methanogen species resident in the human digestive tract, *Methanobrevibacter smithii* and *Methanospaera stadtmanae*. These archaebacteria may be good candidates for use in liposomal formulations because of their immunocompatibility with humans.[26] After extraction of the TPL and formation of unilamellar vesicles[27] called archaeosomes, the liposome formulations were tested to determine their stability. The archaeosomal stability to phospholipases, temperature, pH and serum proteins was compared to that of conventional liposomes.[25] Of particular interest, archaeosomes with high concentrations of the caldarchaeal lipids were found to have enhanced stability and were less likely to

leak substrates encapsulated within the aqueous core. Once it was established that these lipid extracts had formed stable unilamellar vesicles, these liposomes were screened for their ability to stimulate an effective immune response.

The first study to determine the immunostimulatory capability of these unique lipid vesicles involved the quantification of archaeosome and liposome uptake by phagocytes.[28] This gave promising results as liposomes consisting of archaea lipids were phagocytosized 3 to 53 times faster than liposomes consisting of ester lipids. This study was followed by the evaluation of antibody titers to encapsulated bovine serum albumin (BSA) in archaeosomes formed from *M. smithii* and *M. stadtmanae* lipid extracts.[29] The archaeosomes of *M. smithii* elicited the greastest humoral response with almost a 3-fold difference to that of *M. stadtmanae*. The study included an examination of the extracts in an attempt to link the structural characteristics to the increased humoral response. Analysis of purified lipid extracts by MS and NMR experiments revealed the presence of archaeal and caldarchaeal lipids. However, the amounts varied dramatically and the polar head groups were dissimilar in some cases (Table 1.1). The TPL extract from *M. smithii* was dominated by lipids bearing a serine head group whereas the polar head groups of *M. stadtmanae* were predominately inositol or the disaccharide gentiobiose (β-Glc*p*-(1→6)-β-Glc*p*). After establishing the production of a humoral immune response to BSA entrapped in archaeosomes in vivo, studies progressed toward quantification of cell mediated response, determination of factors influencing humoral and cell mediated responses, and the elucidation of mechanistic aspects of archaeosomal activation of the immune system.

Table 1.1: Comparison of lipid structures found in two species of archaebacteria.

Structure	*Methanobrevibacter smithii*	*Methanosphaera stadtmanae*
β-Glc*p*-(1→6)-β-Glc*p*-C-PI β-Glc*p*-(1→6)-β-Glc*p*-C-P	34 mol % combined	8 mol % combined
glucose-C-P	3 mol %	0 mol %
β-Glc*p*-(1→6)-β-Glc*p*-A	12 mol %	36 mol %
P-A	8 mol %	1 mol %
IP-A	<8 mol %	50 mol %
SP-A	30 mol %	minor

A-Archaeal, C-Caldarchaeal, I-Inositol, P-Phosphate, S-Serine

To address these issues, archaeosomes encapsulating protein antigen were formulated from a number of different species of Archaea, including *M. smithii.* The archaeosomes were injected into mice and the humoral and cell-mediated responses were quantified by measuring antibody and cytokine production.[30] In all immunizations with archaeosomes, IgM antibodies were produced with a strong class switch to IgG isotypes. This indicates a cell mediated activation of a strong humoral response to the encapsulated antigen. Spleen cells from immunized mice were cultured to determine cytokine production, cytokines IFN-γ and IL-4 were present, indicative of T-helper cell subset Th1 and Th2. In order to investigate factors that influence the immune responses, the protein antigen, the amount of protein/lipid used in a single injection and the lipid compositon (increasing amounts of ester lipids) were varied.[30] Adjuvant activity dropped with the incorporation of ester lipids and lipid quantity per dose had a pronounced affect on anti-BSA antibody titres, whereas the nature of the protein antigen had little effect.

To gain information about the adjuvant activity, a thorough understanding of the presentation pathway, by MHC I or MHC II, is required. In fact, both pathways have been proven to be at work in this archaeosome system, especially when *M. smithii* is used.[31,32] Archaeosomes with entrapped ovalbumin (OVA) were found to stimulate MHC I presentation pathway, *via* quantification of cytokine production and inhibition assays.[31] The activation of APC's *in vitro* by archaeosomes resulted in up regulation of stimulatory molecules that led to the proliferation of naïve T-cells and the production of MHC II proteins. They also enhanced cytokine production including recruitment and activation of APC's.[32] Strong activation of APC's leads to a potent T-cell response and immunological memory. Archaeosomes encapsulating ovalbumin have also been tested for their ability to protect mice against ovalbumin expressing tumors. This treatment was found to be effective. Naïve mice developed large tumors within 12-15 days but mice that were immunized with ovalbumin-archaeosomes exhibited protection, remaining tumor free at day 20.[33a]

With the establishment of such an effective immune response to soluble antigen encapsulated in archaeosomes efforts turned towarded determining the safety of this adjuvant system.[33b] Since the polyether lipids in archaebacteria should be less susceptible to metabolic degradation than comparable ester lipids there is safety concerns needed to be addressed. Four groups of mice were injected subcutaneously on day 0 and day 21 with either phosphate buffered saline (PBS), ovalbumin in PBS, archaeosomes without encapsulated antigen or archaeosomes encapsulating ovalbumin. Injection site reactions, body weight, temperature and clinical signs of adverse reactions were monitered. Sera were also collected on days 1, 2, 22, and 39 and assayed for production

of creatine phosphokinase (CPK) and anti-lipid antibodies. Elevated CPK, which is an enzyme found in heart, brain and skeletal muscle, indicates trauma to one or more of these organs.[33b] On day 39 the mice were sacrificed and all major organs (liver, spleen, kidneys, heart, lungs) were examined and weighed. There were no signs of redness, swelling and abscess formation, also normal temperature, body weight and absense of behavioral changes all indicate that this adjuvant system appears to be safe. No anti-lipid antibodies were detected in sera, organs were normal in appearance when compared to the two control groups (PBS and OVA/PBS), there was no clinically significant increase in CPK levels and the hemolytic properties of archaeosomes do not pose a safety concern.[33b] Archaeosome adjuvants therefore appear to be safe in the mouse model and need to be evaluated for human use.

In summary, studies clearly demonstrate that archaeal lipids formulated into liposomes stimulate the aggregation of APC's followed by strong humoral and cell-mediated immunity with long lasting protection in a superior manner to that of conventional liposomes and well known adjuvants. Two species that have been used throughout this work are *M. smithii* and *M. stadtmanae*. *M. smithii* archaeosomes, which have a high concentration of serine as a polar head group and the caldarchaeal lipid, elicits the immune response described above. Whereas the archaesomes constructed from *M. stadtmanae*, consisting mostly of carbohydrate head groups and archaeal lipids, do not improve the antigenicity of encapsulated protein. It is therefore of interest to analyze specific components of the TPL extract in order to determine their adjuvanticity. These compounds are most readily available *via* chemical synthesis. The synthesis and evaluation of some of these molecules is described in chapters 3, 4 and 5.

Chapter 2

An Analysis of the Synthetic Transformations Required to Construct Archaeal Glycolipids and Phospholipids

2.1 Scope of Project

Liposomes constructed of lipids from archaebacteria induce strong humoral and cell mediated immune reponses to encapsulated antigen. Over the years, studies concentrated on a number of species of archaebacteria. Of note, the TPL extract of *Methanobrevibacter smithii* is rich in the archaeal and caldarchaeal lipid structures with serine polar head groups and displays superior adjuvanticity to archaesomes consisting of the lipids from *Methanosphaera stadtmanae*. Therefore, Sprott *et al.* has suggested that both the lipid structures and polar head groups are important structural neccessities that are essential for the biological activity. In light of these findings, it is of importance to determine whether the individual components of the TPL extract from these species elicit different immunogenecity to encapsulated antigen.

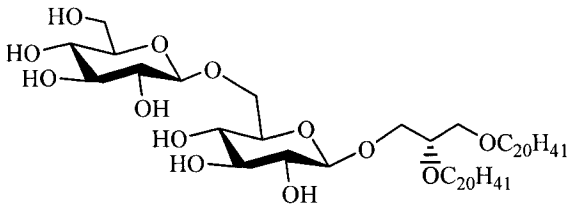
2.2 Synthetic Targets

As previously suggested, the caldarchaeol lipids may be a structural necessity for increased adjuvanticity however at the early stages in this project we thought it was best to limit ourselves to the more easily synthesized archaeol type. One problem envisioned with construction of the caldarchaeal lipid is that chemical synthesis would not yield sufficient quantities for immunological tests. So instead of directing the effort on the

caldarchaeal lipid, we could synthesize the archaeal lipid containing specific polar head groups recognized by receptors on the surface of antigen presenting or phagocytic cells.

One class of receptors that can be targeted is carbohydrate-binging proteins called lectins, which serve as first defense against bacteria, fungi and viruses. APC's display surface receptors that recognize glucan and mannan carbohydrate structures. Activation of these APC's stimulates phagocytosis and secretion of cytokines. For example, linear (1→3)-β-glucan oligosaccharides, a fungal cell wall component, stimulates the induction of an innate immune response *in vivo via* toll-like receptor activation.[34]

Another targeted receptor is one that recognizes apoptotic cells. Apoptosis or programmed cell death is a normal event in the cycle of a cell, which is meant to maintain homeostasis of biological systems.[35] This involves efficient clearance of the apoptotic cell *via* receptor-mediated phagocytosis. During apoptosis, a cell undergoes certain changes in cellular physiology. The initial stage of apoptosis involves membrane phospholipid scrambling or enhanced transbilayer movement.[35] This scrambling causes phosphatidylserine, which is usually exposed to the internal core, to be displayed externally on the cell surface. This event stimulates macrophage-mediated clearance of apoptotic cells displaying phosphatidylserine. Studies have proven that macrophages use a phosphatidylserine specific receptor to engulf apoptotic cells. Sprott has attempted to make a connection between this serine acceptor and the immunogenicity of the archaeosomes.[36,37]



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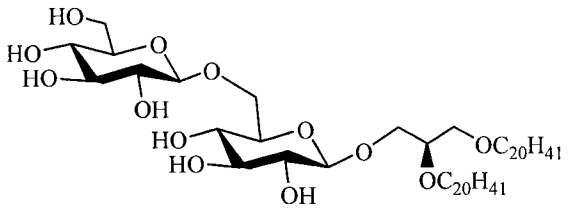

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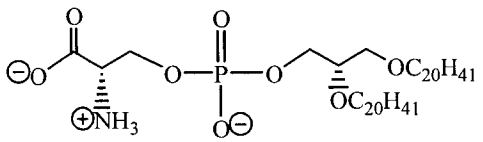

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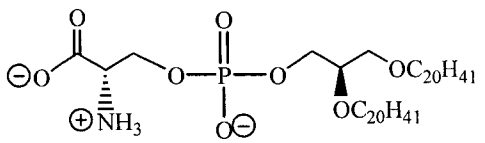

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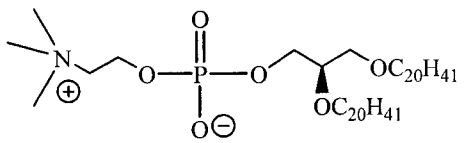

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Figure 2.1: Synthetic targets **5-9**.

Components of the TPL extract of *M. stadtmanae* and *M. smithii*, namely the β-Glc*p*-(1→6)-β-Glc*p*-archaeal (**5**) and the serine phosphoarchaeal (**7**) lipids and their unnatural glycerol epimers (**6**) and (**8**), will be synthesized (Figure 2.1). Also included will be the unnatural glycerol epimer of archaetidylcholine (**9**) from *Methanopyrus kandleri*.[38] The adjuvanticity of archaetidylcholine **9** has not been established whereas liposomal formulations containing large quantities of **5** have been found to be poor

adjuvants. In contrast, archaeosomes containing the polar head group serine such as **7** have very strong immunogenecity.

In addition to synthetic targets **5-9**, it would be of interest to attempt to mount an immune response against a β-mannan carbohydrate antigen that is associated with a liposome formulation. The strategy includes synthesis of a nonnatural structure that would incorporate the phosphoarchaeal lipid linked to the carbohydrate antigen *via* a spacer X as shown in Figure 2.2

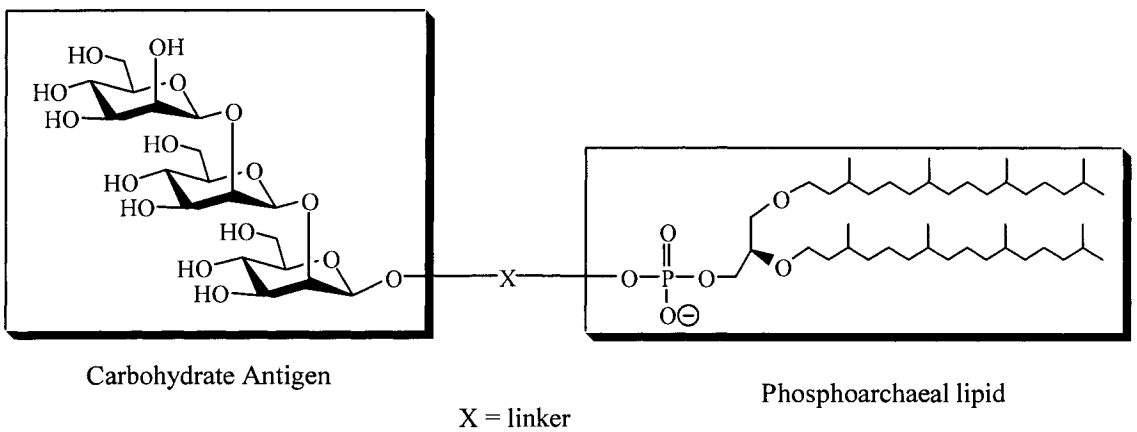


Figure 2.2: The target β-mannan neoglycolipid.

2.3 Archaeal Lipid Synthesis

The synthesis of archaeal[14,39,40] and caldarchaeal[15,41,42] lipids have been attempted previously. The caldarchaeal lipid features 18 stereogenic centers and a 72 atom macrocycle. Clearly, both complex stereochemical challenges and the entropic hurdle associated with large cyclic systems must be overcome to complete any synthesis. A ring closure would likely occur at the end of the synthesis when synthetic intermediates are very valuable. Also, to obtain sufficient amounts of the caldarchaeal lipids the synthesis

must be amenable to scale up. If the goal of synthesizing the caldarchaeal lipid was to incorporate into liposomes, one would also have to functionalize the primary alcohol of glycerol to mimic the natural lipid. The construction of the caldarchaeal lipid is a huge task and maybe best if left to enzymatic synthesis by the living microorganisms, followed by extraction.

The archaeal lipids are less structurally diverse and therefore are somewhat easier to synthesize. A retro-synthetic analysis in Figure 2.3 shows that the general structure of the archaeal lipid can be divided into four major synthetic precursors, the polar head group, a phosphate, glycerol, and the isoprenoid (phytanyl) chain.

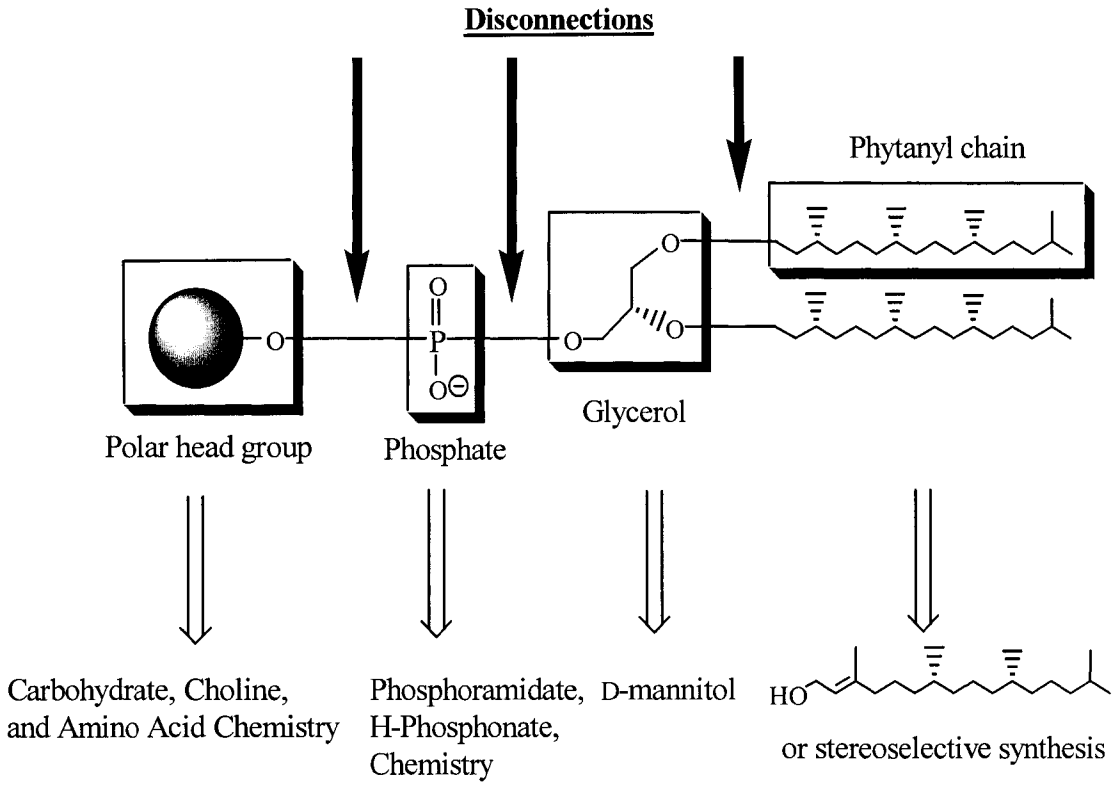


Figure 2.3: Retrosynthetic analysis of archaeal lipids.

2.3.1 Isoprenoid Alkyl (Phytanyl) Chains

As indicated in Figure 2.3, the glycerolipid portion of the molecule can be constructed from glycerol and two alkyl chains. Unlike its acyl counterparts, the alkyl chain is fully saturated and is branched at positions 3, 7, 11 and 15 with positions 3, 7 and 11 all having defined (*R*) stereochemistry. This is the only structural characteristic that needs to be considered in its synthesis. Several methods for the construction of the alkyl chains are outlined in Figure 2.4.

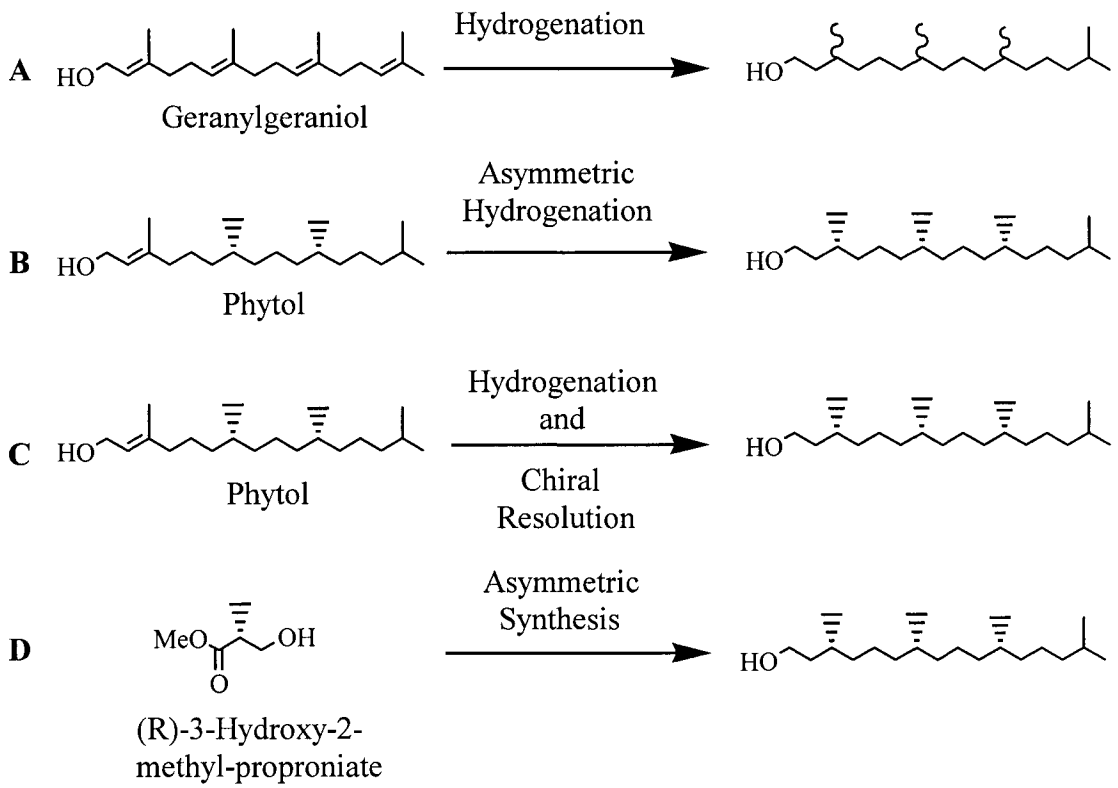
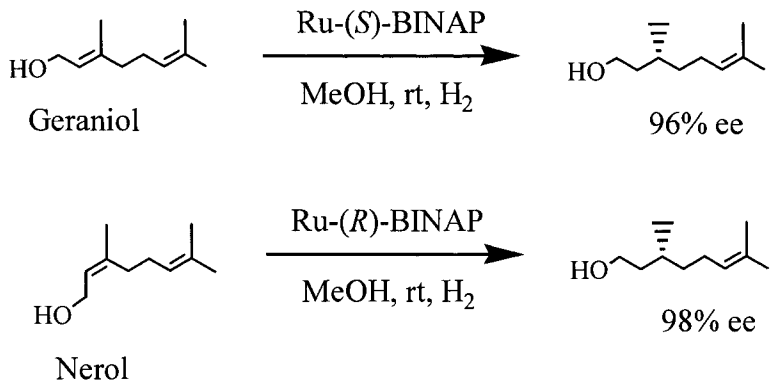


Figure 2.4: Synthetic strategies for (3*R*, 7*R*, 11*R*)-3,7,11,15-tetramethylhexadecan-1-ol or phytanyl alcohol.

One could imagine synthesis from costly geranylgeraniol (Figure 2.4, **A**), however hydrogenation of this substrate would lead to as many as eight diastereomeric alcohols. A second method to obtain optically pure phytanyl alcohol is to use a strategy (Figure 2.4, **B**) of asymmetric hydrogenation. This method orients an allylic alkene in a

chiral environment such that two atoms of hydrogen are delivered stereospecifically to one face. Noyori and coworkers have developed such a strategy using a chiral ruthenium catalyst.[43,44,45,46] Scheme 2.1 outlines the initial study employing geraniol and nerol as test substrates, and the resulting hydrogenated product was obtained in high yield and with high enantiostereoselectivity. It is important to note that only the allylic double bond was reduced.

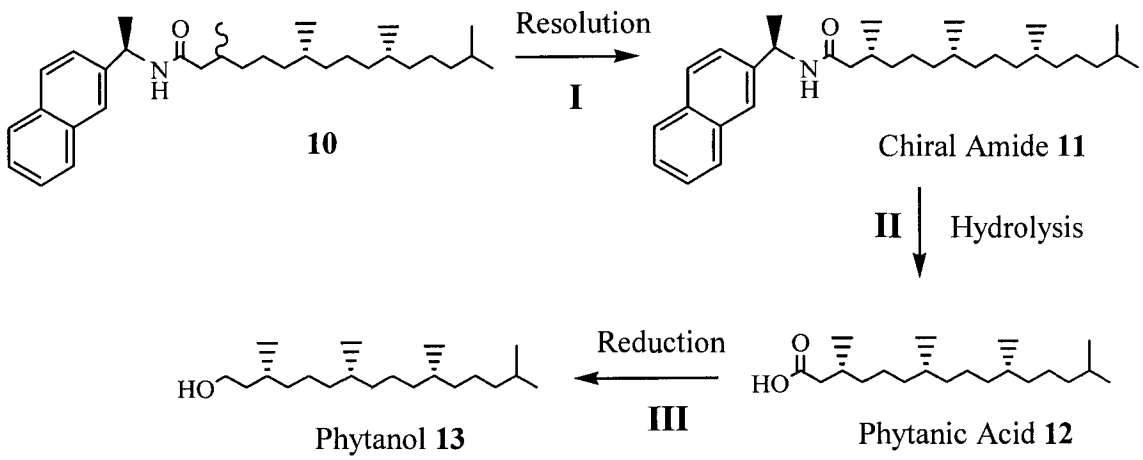


Scheme 2.1: Asymmetric hydrogenation of allylic olefins, geraniol and nerol.

The BINAP-ruthenium complex is readily synthesized and was applied to the diastereoselective hydrogenation of *E*-phytol.[47] The resulting reaction mixture was examined to determine the diastereomeric ratio of the products by conversion of the primary alcohol to the carboxylic acid and subsequent condensation with (*R*)-(+)-1-(1-naphthyl)ethylamine produced diastereomeric amides (**10**, Scheme 2.2) that are separable by MPLC or HPLC. The diastereomeric ratio from this synthetic transformation was determined to be >100:1. This represents a quick and efficient route to (3*R*, 7*R*, 11*R*)-3,7,11,15-tetramethylhexadecan-1-ol of high purity. Sita suggests that reducing the commercially available phytol using a standard hydrogenation procedure would produce a 50:50 mixture of the (3*R*) desired product along with the (3*S*) diastereomer. And that subsequent resolution of this mixture using the chiral amide approach represents another

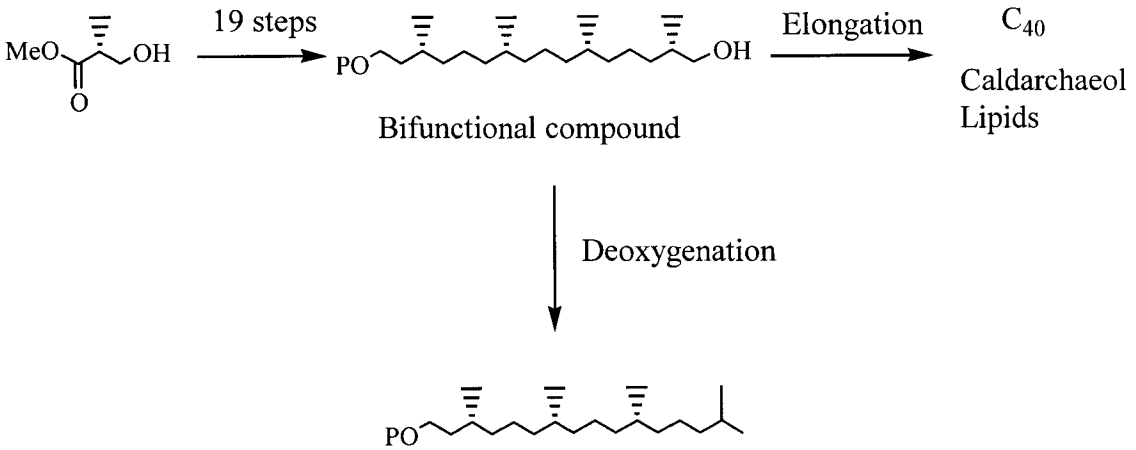
method of obtaining the chiral substrate, (3*R*, 7*R*, 11*R*)-3,7,11,15-tetramethylhexadecan-1-ol.

This approach (Figure 2.4, **C**) was attempted but was fraught with difficulties.[48] The hydrolysis (**II**, Scheme 2.2) of the chiral amide **11** after resolution (**I**, Scheme 2.2) was problematic, standard hydrolysis conditions with concentrated sulfuric acid produced the primary amide or the desired product at very low yields. Further attempts with *t*-butoxide and DIBALH returned only starting material. Finally, sodium nitrite was used to form an *in situ* *N*-nitrosamide, which weakens the amide bond making it susceptible to lithium hydroperoxide hydrolysis conditions. However, the overall yield was only 22% from the racemic (3*R/S*, 7*R*, 11*R*)-3,7,11,15- tetramethylhexadecan-1-ol to the optically pure phytanic acid **12**. In light of this poor yield and the high cost of the chiral auxillary, and the fact that the phytanic acid has to be reduced (**III**, Scheme 2.2) after the resolution/hydrolysis sequence, this method does not represent a convenient route to the desired substrate **13**.



Scheme 2.2: Synthesis of (3*R*, 7*R*, 11*R*)-3,7,11,15-tetramethylhexadecan-1-ol *via* chiral resolution.

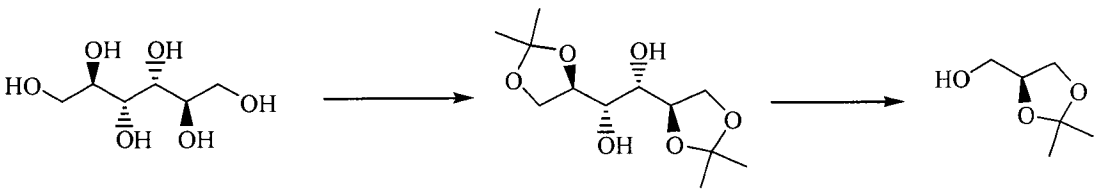
Lastly, precursors of (3*R*, 7*R*, 11*R*)-3,7,11,15-tetramethylhexadecan-1-ol can be obtained from asymmetric synthesis (Figure 2.4, **D**)[49] Chiral precursors such as (*R*)-3-hydroxy-2-methyl-propionate and (*R*)-citronellol have been employed to construct a bifunctional variant of (3*R*, 7*R*, 11*R*)-3,7,11,15-tetramethylhexadecan-1-ol.[40] Although the synthesis of this involves 19 synthetic steps the bifunctional nature of the compound allows for elongation to the C_{40} unit found in the caldarchaeol lipids (Scheme 2.3).



Scheme 2.3: Asymmetric synthesis of a bifunctional isoprenoid, which is further manipulated to yield either phytanyl alcohol or the caldarchaeal lipids.

2.3.2 Glycerol

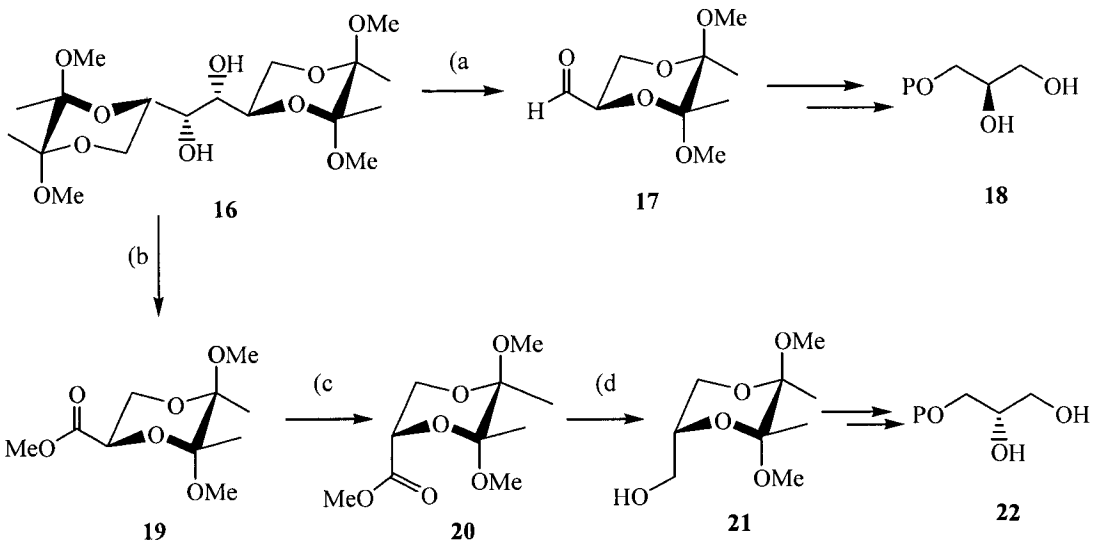
Compounds that contain 1,2-*sn* or (*S*) glycerol and 2,3-*sn* or (*R*) glycerol configurations exist in nature. One of most effective methods to obtain optically pure 1,2-*sn* glycerol is from the manipulation of D-mannitol,[50] whereas 2,3-*sn* glycerol can be derived from L-ascorbic acid,[51] L-serine[52] or L-mannonic-γ-lactone.[53,54] These routes take advantage of the stereochemistry that is already present in readily available natural products. Also, with advances in asymmetric synthesis there are numerous ways of functionalizing olefins to obtain *S*- or *R*-glycerol.



13 14 15

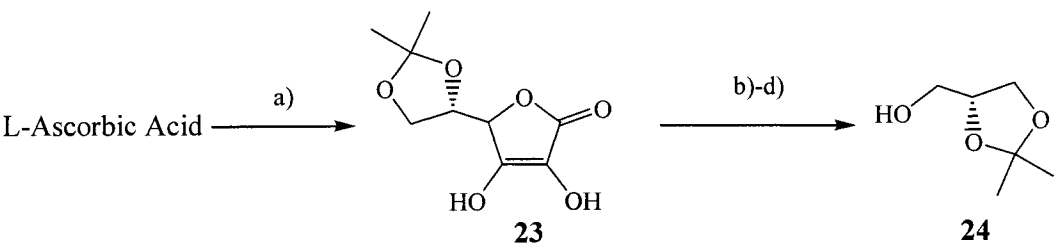
Scheme 2.4: Synthesis of (*S*)-glycerol acetonide *via* D-mannitol.

The most common and inexpensive route to obtain (*S*)-glycerol acetonide is from D-mannitol (Scheme 2.4). D-mannitol **13** is easily converted into the 1,2:5,6 diacetonide **14** which can then undergo oxidative cleavage of the diol followed by reduction of the resultant aldehyde to afford the enantiomerically pure product. From (*S*)-glycerol acetonide **15**, the opposite glycerol enantiomer can be indirectly obtained *via* a protection-deprotection strategy or through a C2 inversion reaction such as a Mitsunobu reaction. Scheme 2.5 outlines another route, recently developed by Ley, that utilizes D-mannitol, butanediacetal protecting groups and a lithium diisopropylamine (LDA) induced inversion of the C2 position.[55,56] Sodium periodate oxidation of the diol of **16** produces **17**. However, periodate oxidation in the presence of methanol leads to generation of an *in situ* methyl hemiacetal, which is converted to the methyl ester **19** by bromine oxidation. Inversion of methyl ester **19** with LDA yielded **20**. Reduction of **17** or **20** to the primary alcohol and subsequent protection can lead to the desired enantiomerically pure glycerol diols **18** and **22** after deprotection of the butanediacetals. This synthetic scheme represents a procedure that produces both enantiomers of glycerol in an efficient manner.



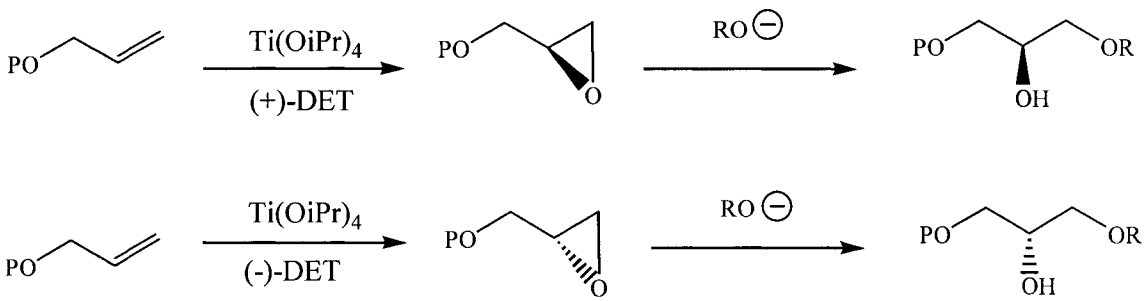
Scheme 2.5: a) $NaIO_4$, CH_2Cl_2, b) $NaIO_4$, $MeOH/H_2O$ then $NaHCO_3$, Br_2, c) LDA, THF, -78 °C then *t*-BuOH, d) $LiAlH_4$, THF.

Many other direct methods to obtain the 2,3-*sn* or (*R*)-glycerol have been reported in the literature. For example, after fours steps, L-ascorbic acid[51] was converted into the desired (*R*)-glycerol acetonide (Scheme 2.6). The terminal diol of L-ascorbic acid was protected as the isopropylidene acetal **23** then treated with sodium borohydride to reduce the ene-diol functionality. The reaction mixture was then treated with sodium hydroxide and the solution was neutralized. The corresponding crude acyclic acetonide was oxidized to (*S*)-glyceraldehyde acetonide using lead tetraacetate. The final enantiomerically pure (*R*)-glycerol acetonide **24** was obtained by reduction of the aldehyde using sodium borohydride.

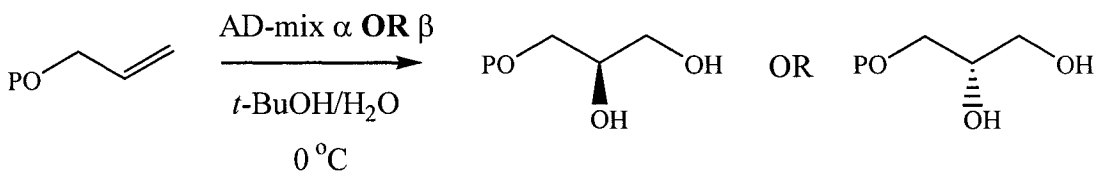


Scheme 2.6: a) $ZnCl_2$, Acetone, b) i) $NaBH_4$, EtOH ii) NaOH for 8-10 h and then neutralization with HCl, c) $Pb(OAc)_4$, EtOAc, 0 °C, d) $NaBH_4$, EtOH.

The invention of unique methods to functionalize olefins such as alkene oxidation, to yield epoxides (Scheme 2.7) or diols (Scheme 2.8), could also be a viable route to optically pure glycerol. The methods developed by Sharpless using AD-mix for asymmetric dihydroxylation or allylic epoxidation *via* titanium catalysts can directly lead to the desired products or to synthetic precursors that can be manipulated to afford them.



Scheme 2.7: Allylic epoxidation and opening with O-nucleophile.



Scheme 2.8: Sharpless asymmetric dihydroxylation.

2.3.3 Phosphate

As so many biological molecules contain phosphate moieties there is a demand for effective phosphorylation chemistry. The drive to simplify oligonucleotide synthesis has produced four major coupling strategies that can be used to construct phosphomonoester and diester linkages for use in oligonucleotide, phospholipid and carbohydrate chemistry. The different coupling methods are divided into two groups based on their oxidation states.[57]

The first group involves phosphorus that is tetracoordinate and has an oxidation state of +5 (Figure 2.5). This class of compounds is attractive because they are very

stable, however as a consequence, they are less reactive. These compounds tend to be electrophilic and react with a variety of nucleophiles. The phosphodiester (1) and phosphotriester (2) approaches were developed in the infancy of phosphorylation chemistry.

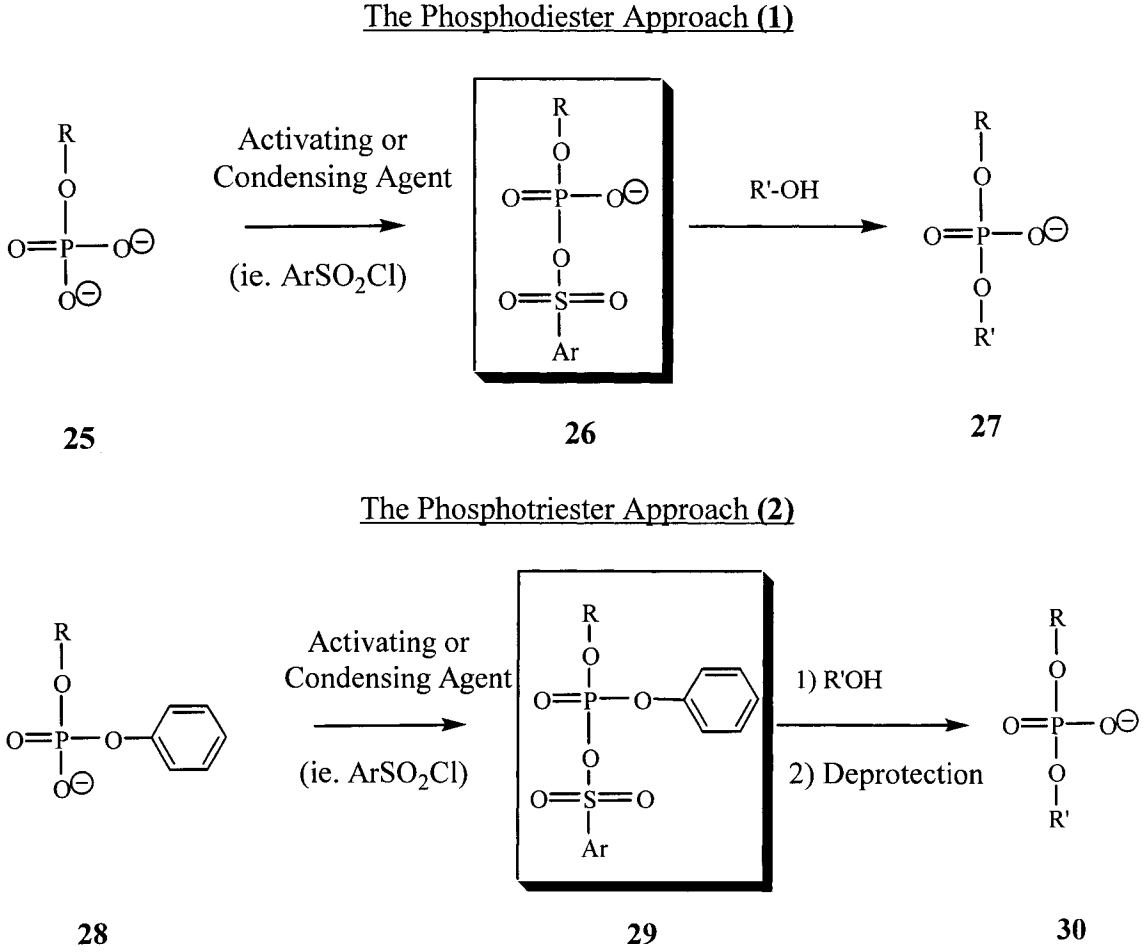


Figure 2.5: Phosphorus (V) phosphorylation strategies.

In the phosphodiester approach, the activation of the phosphomonoester with an activating agent such as dicyclohexylcarbodiimide or arylsulfonyl chloride produces intermediate **26**. This is followed by nucleophilic attack by R'-OH to yield the phosphodiester **27**. The drawbacks of this method outweigh the benefits, as the condensation reaction is low yielding, it requires long reaction times and the use of many

equivalents of reactants especially with the increase in nucleotide chain length.[58] The reaction mixture was also difficult to purify. The phosphotriester approach is similar to the one previously described except it begins with a phosphodiester that is functionalized with a protecting group. This phosphodiester **28** is activated and undergoes a condensation reaction to produce an intermediate phosphotriester **29**. Once the protecting group is removed, after chain elongation, the phosphodiester linkage of **30** is unmasked. Benefits of this sequence include easier purification due to the neutral nature of the intermediate triester **29**, amenable to large-scale synthesis and shorter reaction times.[58]

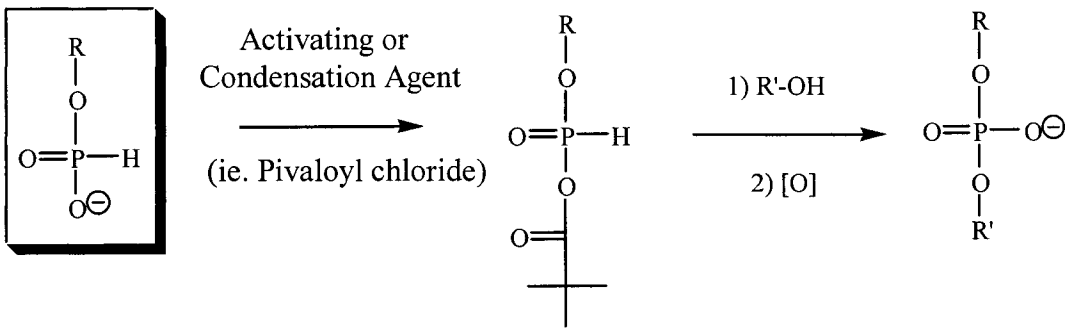
The second group outlines more recent approaches to phosphitylation chemistry, which includes the use of *H*-phosphonates (3) and phosphoramidates (4) (Figure 2.6). The inherent advantage of these methods is that these compounds have very different reactivity when compared to the phosphate derivatives used in the two previously described coupling strategies. The oxidation state of these phosphorus compounds is +3 and they have either tetrahedral or trigonal pyramid geometry.

The *H*-phosphonates **31** have very unique chemistries in that their electrophilic and nucleophilic behavior can be tuned.[57] These phosphorylating agents are activated by condensing agents such as pivaloyl chloride or chlorophosphate derivatives in the presence of pyridine and acetonitrile. The activated compound **32** can then undergo nucleophilic attack by a hydroxylic compound to produce an *H*-phosphonate diester, which upon oxidation forms the phosphodiester compound **33**.

The phosphoramidite method is a variant of the phosphite triester approach also used in the infancy of phoshorylation chemistry. In the phosphite triester method, dichlorophosphine is used in the condensation reaction of two nucleotides, to produce a

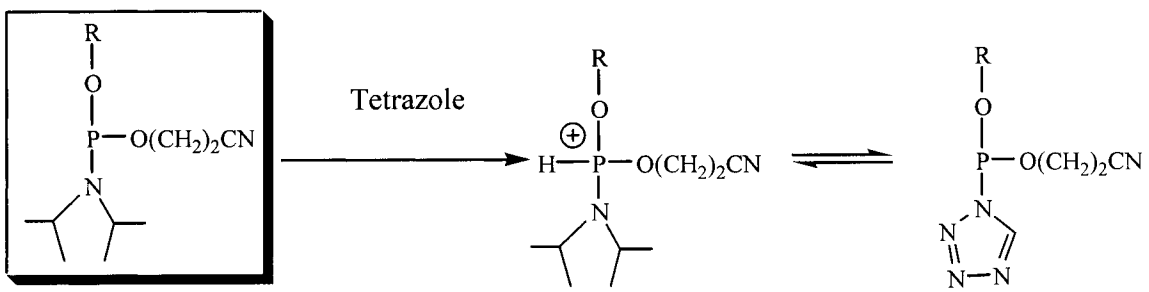
dinucleotide. However, dichlorophosphines are difficult to handle because of their instability toward both hydrolysis and oxidation. This problem could be resolved with low reaction temperature and inert reaction environment, however these conditions generally are not suitable for automation.[59]

The *H*-Phosphonate Approach **(3)**



31 **32** **33**

The Phosphoramidite Approach **(4)**



34 **35** **36**

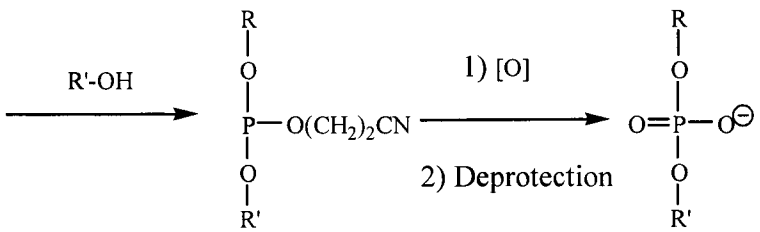
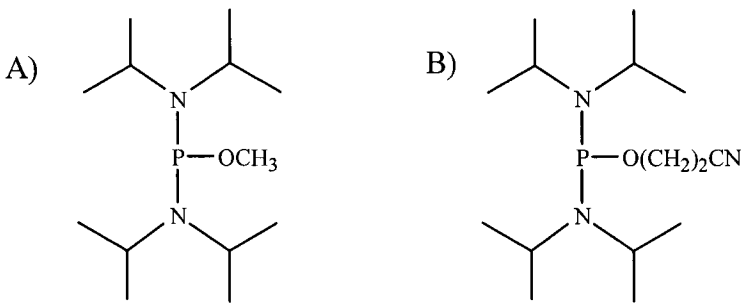


Figure 2.6: Phosphorus (III) phosphorylation strategies.

It would be beneficial to have a compound with similar reactivity to the dichlorophospines that is also shelf stable. This led to the development of bis-diisopropylaminoethoxyphosphine **37** and bis-diisopropylaminocyanoethoxyphosphine **38** (Figure 2.7). The condensation reaction (Figure 2.6) involves activation of a phosphoramidite **34** by tetrazole, *via* formation of either a protonated amidite **35** or the corresponding tetrazolide **36** followed by nucleophilic attack by the R'-OH.[59] Upon oxidation and deprotection the corresponding phosphodiester is formed.



37 **38**

Figure 2.7: Phosphorylating agents used in the phosphoramidite approach.

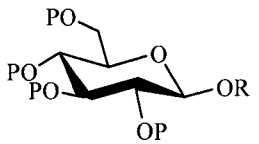
The latter P(III) methods, although developed for the synthesis of oligonucleotides, are general and can be applied to the synthesis of carbohydrates and lipids that contain phosphoesters.

2.3.4 Polar Head Group

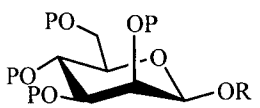
The polar head groups of lipids contain glycerol, choline, ethanolamine, amino acids such as serine and carbohydrates such as inositol, *N*-acetyl glucosamine, glucose, mannose, galactose and complex oligosaccharides. Structures such as serine, choline and the monosaccharides listed are all relatively easy to incorporate as the polar head group. These compounds or their precursors are commercially available. The only consideration needed is that of a protecting group strategy, for example, so that the correct hydroxyl of

a monosaccharide is phosphorylated or if a specific anomeric conformation (α or β) is required.

Glycoproteins and glycolipids found in natural systems have much more complex carbohydrate structures than simple monosaccharide components. Therefore, the synthetic complexity is enhanced by the various glycosidic linkages found in the carbohydrate component. From a synthetic perspective there are four different types of glycosidic linkages: *trans*-1,2-α, *trans*-1,2-β, *cis*-1,2-α, and *cis*-1,2-β. For the purpose of this study, the overview will strictly focus on the synthesis of *trans*-1,2-β and *cis*-1,2-β linkages (Figure 2.8).



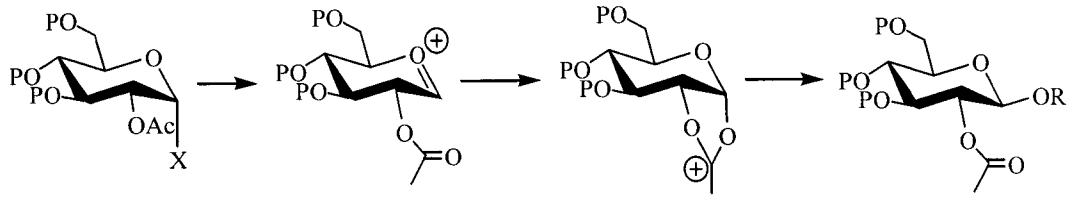
Trans-1,2-β



Cis-1,2-β

Figure 2.8: Two common glycosyl linkages found in natural products.

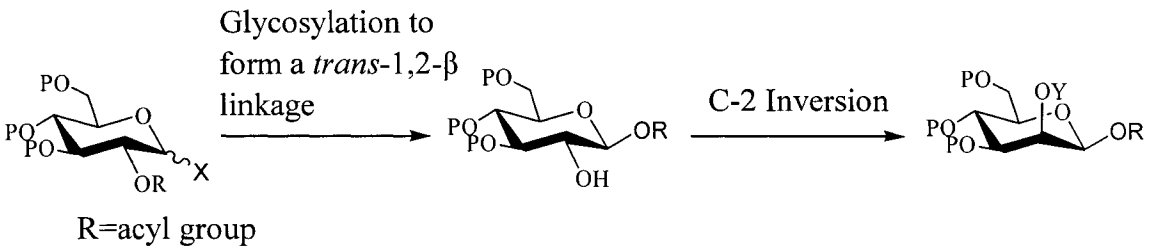
Trans-1,2-β linkages are one of the simplest linkages to form in carbohydrate chemistry. There are many ways to achieve this linkage and they are based on a glycosyl donor **39** (X = halide, sulfide, or imidate) with an acyl protecting group at C-2 (Scheme 2.9). The acyl group at the C-2 position plays an essential role in the stereochemical outcome of the glycosylation/glycosidation reaction. In the presence of a promoter the glycosyl donor will form an oxacarbenium ion **40**. The ester group participates by forming an intermediate acetoxonium ion **41** that is opened by equatorial attack at the anomeric position by an incoming nucleophile to afford **42**.



39 40 41 42

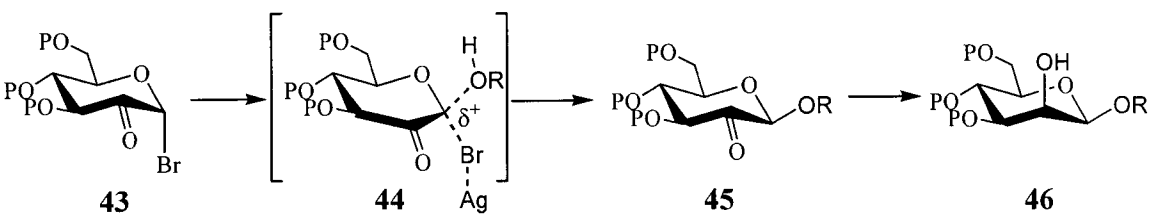
Scheme 2.9: Formation of *trans*-1,2-β linkage.

The *cis*-1,2-β linkages are much more challenging to synthesize in a stereospecific manner. The most common methods use either glucose or mannose to form the desired *cis*-1,2-β linkage. The first synthetic strategy to form the desired linkage represents an indirect approach because it starts from glucose. This route takes advantage of ease of formation of a *trans*-1,2-β glucosyl linkage followed by C-2 epimerization (Scheme 2.10). Epimerization strategies include converting the C-2 hydroxyl into a leaving group followed by axial attack of a suitable nucleophile.[60,61,62] However, this method suffered from complications such as the unreactive nature of C-2 toward nucleophilic substitution and ring contraction when a double inversion was attempted at C-2 and C-4 of galactose.[63] Nevertheless, the approach has recently been successfully used to produce a thio-mannan analog of the *β*-mannan yeast cell wall, with quite good success considering that inversion was done on a trisaccharide.[64] The alternative approach to inversion of C-2 is *via* an oxidation/reduction inversion sequence of C-2.[65] The typical inversion method would consist of oxidation *via* a Swern reaction to a β-glycosid-2-ulose. The reduction could then be carried out stereospecifically to invert the C-2 by reduction with $NaBH_4$ or by L-selectride depending on the substrate. This inversion sequence is used in the synthesis of number of substrates including mono-,[66] di-,[67,68,69] tri-[70,71] and oligosaccharides[72] containing *cis*-1,2-β linkages.



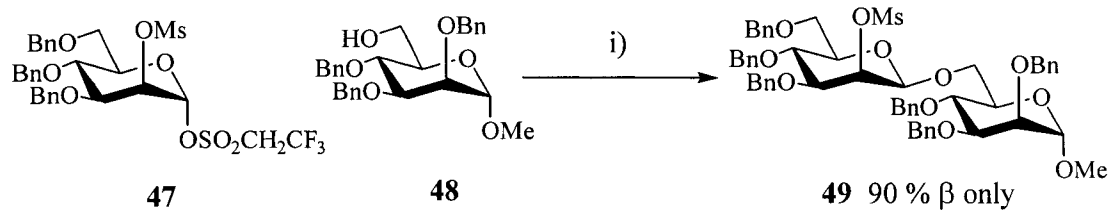
Scheme 2.10: Synthetic strategy starting from glucose involves selective *trans*-1,2-β linkage followed by C-2 inversion.

A variant of this method uses an α-bromo glycosid-2-ulose **43** as a glycosyl donor in the glycosylation reaction. This Koenigs-Knorr type glycosylation proceeds through transition state **44** to thereby acheiving β-selectivity (Scheme 2.11, **43-46**).[73] Because the glycosyl donor already has the 2-keto functionality there is no need for an oxidation reaction, and the mannose configuration of **46** can be directly obtained with reduction of **45**. The usefulness of this glycosylation strategy has been established with the synthesis of a trisaccharide component of *Hyriopsis schlegelii* glycosphingolipid[74] and the synthesis of the di- to hexasaccharide (1→2)-linked β mannan oligomers of the phosphomannan antigen of *Candida albicans*. [64,66]



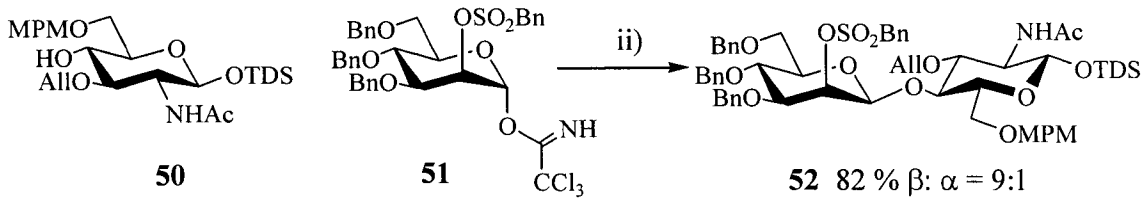
Scheme 2.11: Ulosyl bromide glycosylation strategy.

a)



47 48 49 90 % β only

b)



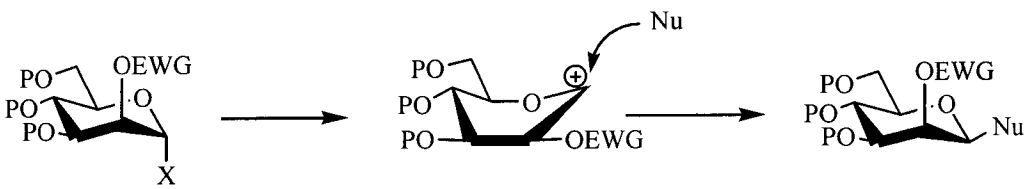
50 51 52 82 % β: α = 9:1

Scheme 2.12: Synthetic examples of *cis*-1,2-β linkage formation using a 2-*O* electron withdrawing group. i) acetonitrile, 18 hrs ii) TMSOTf, CH_2Cl_2.

The second set of synthetic strategies uses mannose with special protecting group considerations and glycosylation conditions to achieve the correct anomeric stereochemistry. To favour β-selectivity, early methods use insoluble promoters such as silver silicate or silver zeolite[75] with an α-mannosyl halide containing a nonparticipating group at C-2. The stereoselective synthesis of *cis*-1,2-β linkage is also achieved using a nonparticipating strongly electron withdrawing group at C-2.[76,77] Srivastava suggests that the incorporation of a strong electron withdrawing group at C-2 combined with a highly reactive electronegative leaving group on C-1 controls the transition state structure during glycosylation. Following the rationale set forth by Srivastava and Schuerch, having a strong electron withdrawing group at C-2 enhances β selectivity in two ways. First, if the reaction proceeds *via* an ion pair then a 2-EWG would increase the dipole of the C2-O2 bond favouring an α ion pair intermediate and subsequent β nucleophilic attack. Secondly, increasing the dipole of C2-O2 increases S_N2 character, by strengthening the

C1-X bond and the incoming nucleophile approaches more closely from the β face.[76] This theory was tested using a glycosyl donor **47** and glycosyl acceptor **48,** the reaction proceeded over 18 h in acetonitrile to afford exclusively the β-linked disaccharide **49** in 90% yield (Scheme 2.12a).

Schmidt has recently elaborated on this strategy and suggests that the strong dipole caused by a C-2 electron withdrawing group forces the transition state structure to adopt a flat twist-boat (Scheme 2.13) which switches bias to the β anomer.[78] Outlined in Scheme 2.9b is the test glycosylation of the 4-OH position of GlcNAc to produce part of the *N*-glycan core structure of glycoproteins. The synthetic transformation of this test reaction is much more relevant compared to that completed by Srivastava and Scheurch because it involves glycosylation of a secondary hydroxyl that is notoriously unreactive. GlcNAc acceptor **50** was glycosylated with 2-*O*-benzylsulfonyl mannosyl imidate **51** and TMSOTf to produce disaccharide **52** in a 1:9 α:β ratio (Scheme 2.12b). Schmidt also investigated the impact on α:β selectivity when a 2-*O*-benzylsulfonyl mannosyl donor containing a 4,6-*O*-benzylidene group was employed, selectivity dropped only slightly to an α:β ratio of 1:8.

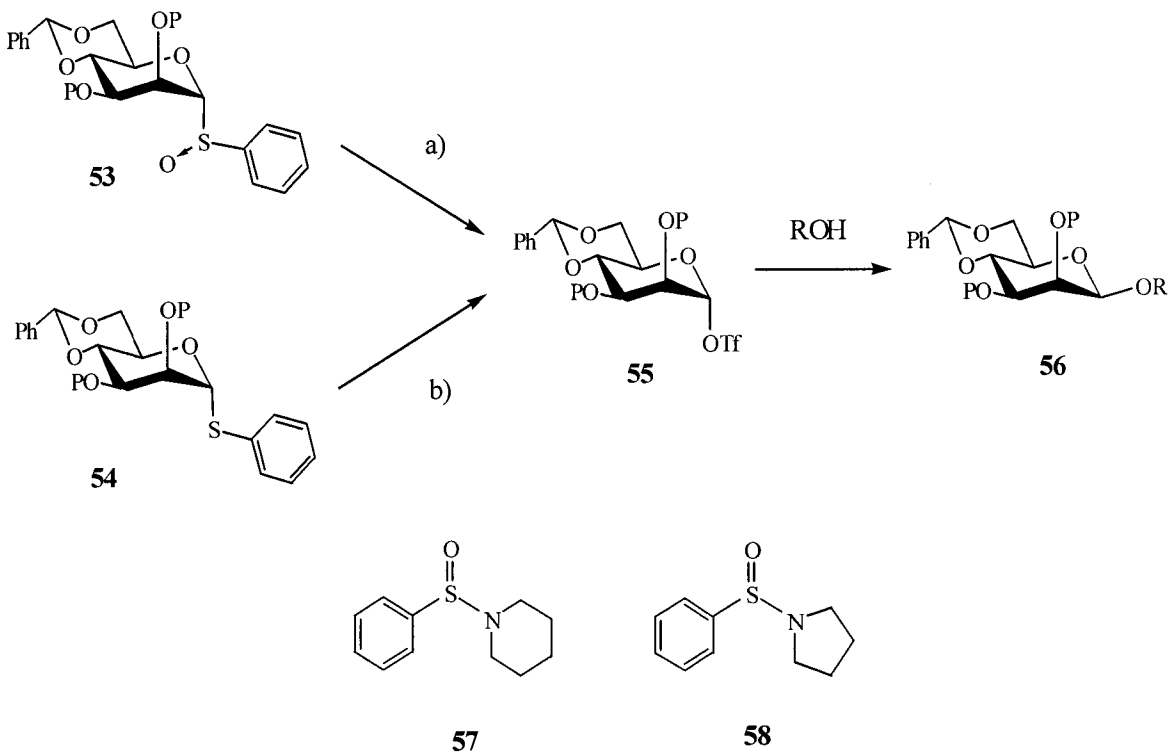


Flat Twist-Boat

Scheme 2.13: Proposed transition state structure.

The 4,6-*O*-benzylidene group and other conformationally restricting protecting groups have been used extensively to influence the stereoselective outcome of glycosylation reactions. One such study has used this protecting group in combination

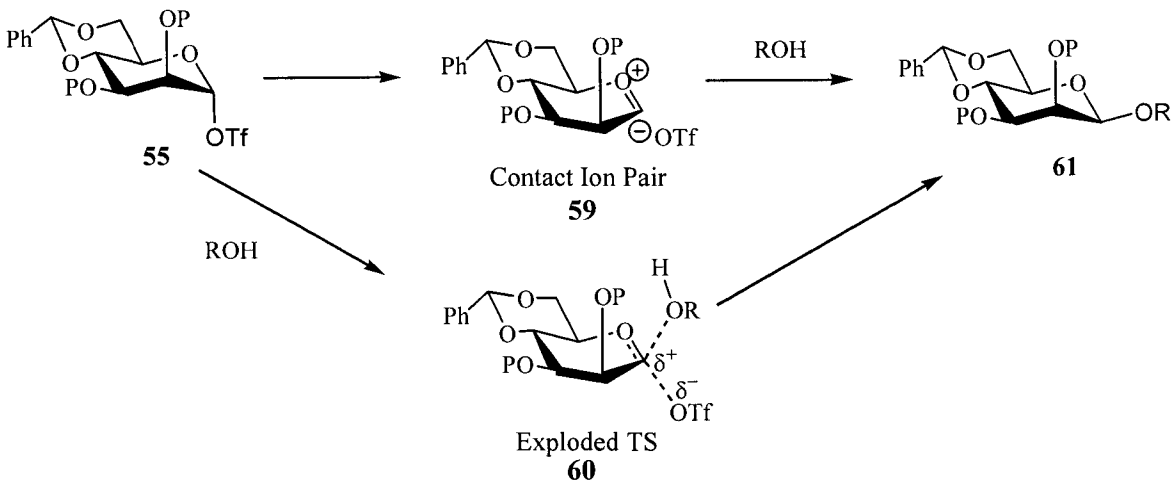
with the sulfoxide glycosylation strategy. First reported by Kahne,[79] Crich[80] has exploited this strategy to produce *cis*-1,2-β linked mannosides in high anomeric selectivity. The general procedure outlined in Scheme 2.14 uses a glycosyl sulfoxide **53** that is activated by triflic anhydride to produce an α-mannosyl triflate **55**[80] which is transformed into the *cis*-1,2-β linked mannoside **56** in the presence of a glycosyl acceptor. Alternatively, conditions have been discovered such that a thioglycoside **54** can be directly activated without oxidation to glycosyl sulfoxide.[81] Both reaction conditions are unique in that they do not require a metal promoter, the 4,6-*O*-benzylidene group on the glycosyl donor stabilizes the α-triflate and is required for high β-selectivity.



Scheme 2.14: General reaction conditions a) the sulfoxide method: Tf_2O, TTBP, -78 °C b) Crich's revised method: Tf_2O, TTBP, **57** or **58**, -60 °C or –78 °C.

A mechanism for the transformation originally proposed that the displacement of the anomeric triflate of **55** by the O-nucleophile proceeded by an S_N2 type reaction.

However, recent kinetic isotope studies reveal that the reaction proceeds through a dissociative mechanism.[82] The *cis*-β-1,2 linkage is made either *via* a contact ion pair **59** where the triflate anion blocks the α-face of the oxacarbenium transition state or *via* what Crich calls an exploded transition state **60** (Scheme 2.15).

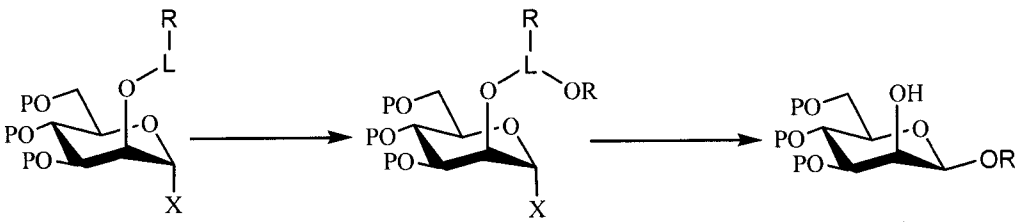


Scheme 2.15: Mechanistic rationale of glycosyl triflate glycosylation reaction.[82]

The procedure has been used extensively to produce oligosaccharides of biological importance with β-(1→2), β-(1→4), and an alternating β-(1→3)-β-(1→4) series.[83,84,85] However, this system is not limited to activation of 4,6-*O*-benzylidene protected mannosyl sulfoxides or thiomannosides, in fact similarly protected mannosyl trichloroacetimidate donors[86] activated by TMSOTf and 1-hydroxy mannosides[87] activated by Gin's dehydrative coupling strategy[88] confer comparable selectivities and yields.

One last method utilizes a specially protected mannosyl donor to enhance β-selectivity during glycosidation. The C-4 position of the mannosyl donor is protected with an ester or thioester moiety that is able to exert remote assistance and is able to improve α:β selectivity in some cases depending on the acceptor and activating agent.[89]

A glycosylation method based on intramolecular aglycon delivery (IAD), that addresses the difficulty of forming the *cis*-1,2-β linkage, has been developed but has not found general use (Scheme 2.16). This strategy employs a spacer arm **L** attached to the 2-OH of mannose donor **62**. Due to the electropositive nature of **L** the glycosyl donor **62** and a glycosyl acceptor ROH can form a transient species **63**. Furthermore, when the donor X is activated the acceptor can attack the oxacarbenium ion from the β face to form the *cis*-1,2-β linked product **64**. A number of linkers has been employed such as a vinyl ether,[90] a chlorodimethylsilyl ether,[91] *p*-methoxybenzyl ether,[92, 93] and isophthaloyl and *m*-xylylene spacers.[94]



Scheme 2.16: Intramolecular aglycon delivery.

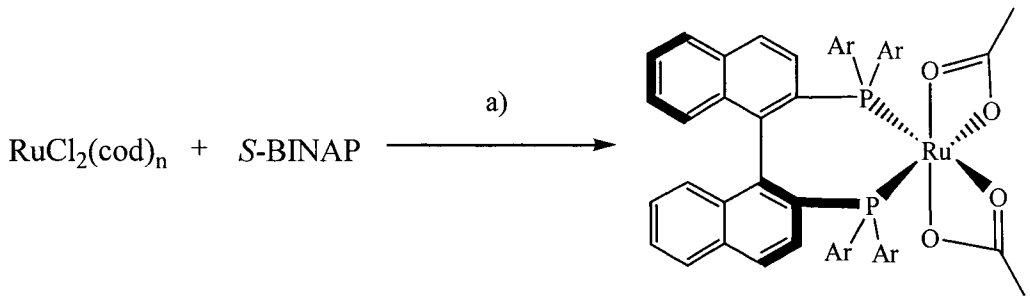
Chapter 3

The Synthesis of Archaeal Gentiobiosyl Glycerolipids, Archaetidylcholine and Archaetidylserine

3.1 Synthesis of Phytanol

One of the main issues related to the synthesis of archaea lipids is the generation of the alkyl chains of the lipid structure. As mentioned in Chapter 2 the stereospecific reduction of isopreniod elements can be achieved with BINAP ruthenium (II) catalysts.

The synthesis of stereochemically pure phytanol was first attempted using the *S*-BINAP-ruthenium(II) dicarboxylate complex. The catalyst **65**, reduces the allylic alkene of phytol in a stereospecific manner depending on which isomer, *E* or *Z*, is present in the reaction. To achieve the proper stereochemical outcome in the asymmetric hydrogenation reaction, one pure stereoisomer of phytol has to be matched with the correct enantiomer of the catalyst.[46] Phytol is available from a number of commercial sources as a mixture of *E* and *Z* stereoisomers. The major stereoisomer in this mixture is the *E*-alkene therefore according to Takaya, it should be matched with $Ru[OCO(CH_3)]_2[(S)$-BINAP] to produce the natural *R* stereochemistry at C3. This complex is not commercially available and the synthesis of **65** was done under inert atmosphere according to the literature protocol (Scheme 3.1).[44]

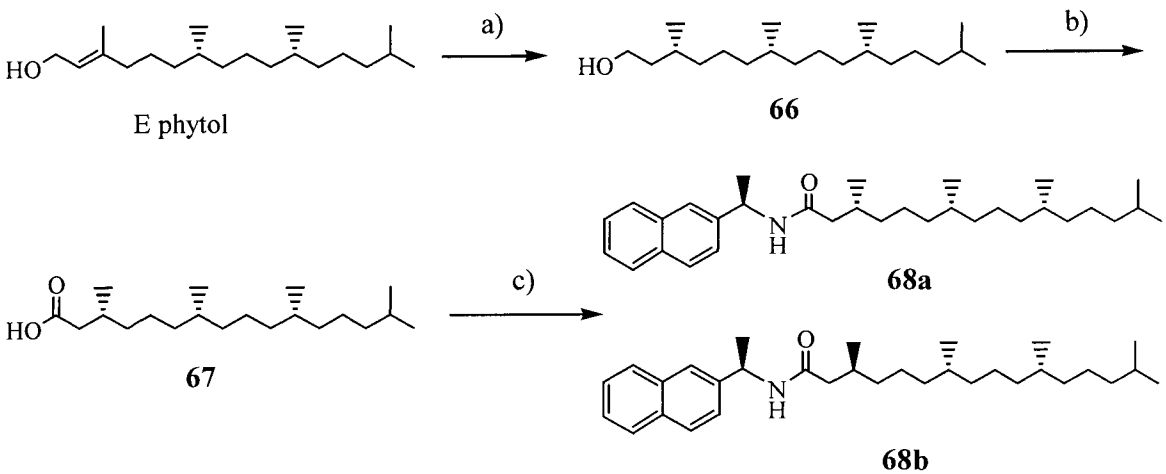


65

Scheme 3.1: a) i) triethylamine, toluene, reflux ii) NaOAc (anhydrous), *tert*-butyl alcohol, reflux.

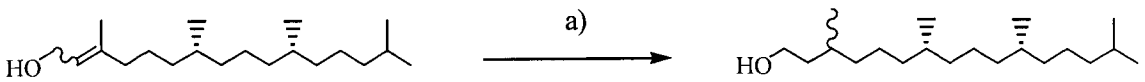
Before the utility of **65** as an asymmetric hydrogenation catalyst was assessed, the *E* and *Z* isomers of phytol had to be separated into the *E* and *Z* isomers. Sita has reported that both isomers are separable by column chromatography.[47] However, using the reported solvent system did not give appreciable separation of the two isomers. A variety of eluent systems were tried and the most effective for this purpose was 10:1 heptane:diethyl ether. However even with this solvent combination the purification of the *E*-alkene proved difficult. The minor *Z*-isomer eluted first, followed by a mixture of the *E*/*Z* alkenes, and finally pure *E*-phytol. The best separation was achieved by collecting the small portion of the purified *E*-alkene at the end of the elution sequence. Increasing the length of the glass columns and incorporating gradient elution sequences had no affect on the outcome of the purification. The *E*-alkene was obtained in gram quantities by repeated column chromatography. The purity of *E*-phytol was assessed by NMR,[95] and no *Z*-alkene was detected as an impurity. The hydrogenation of *E*-phytol in the presence of **65** proceeded at 1600 psi for 4 days and produced (3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecan-1-ol **66** in 96% yield. The stereochemical outcome of the reaction was quantified by the conversion of **66** into the corresponding chiral amides **68a** and **68b**.[46,47] The reaction sequence involves the oxidation of **66** to the aliphatic carboxylic

acid **67** using Jones' reagent (Scheme 3.2). *In situ* formation of the acid chloride by reaction with oxalyl chloride and catalytic amounts of DMF in ether and subsequent coupling with the chiral amine provided compounds **68a** and **68b**. The enantiomeric excess of the reaction was calculated using the isolated mass of **68a** and **68b** after purification of the reaction mixture by HPLC. The diastereomeric (d.e.) excess was found to be no greater than 90%. This result was unlike that obtained by Sita; the poor stereoselectivity maybe attributed to the long reaction times or slight catalyst impurity. The goal was to use **66** to alkylate *R* and *S* glycerol to obtain the natural and unnatural archaeol lipids in their stereomerically pure forms. However, because **66** was obtained in poor d.e. the resulting dialkylated glycerol was not representative of the natural and unnatural archaeol lipids. In addition, due to the difficult and costly separation of commercially available *E/Z* phytol this does not represent an effective route to the desired compound.



Scheme 3.2: a) **65**, H_2, MeOH, 1600 psi, 96% b) Jones' reagent, acetic acid, acetone, water, 68% c) *R*-(+)-1-(1-naphthyl)ethylamine, DMF, oxalyl chloride, Et_2O, 81%.

The fact that stereomerically pure phytanol was not obtained from the asymmetric ruthenium catalyst led to the exploration of other synthetic avenues. An alternate synthetic route to **66** involved the reduction of the *E/Z*-phytol *via* conventional methods (Scheme 3.3) followed by coupling with a chiral auxiliary, chromatographic separation and deprotection of the chiral auxiliary. The reagent used to determine the diastereomeric excess is a very good template for chiral resolution. The chiral amides **68a** and **68b** are separable on TLC and they have different physical properties such as crystallinity and mobility on solid supports.[47]



E/Z mixture **69**

Scheme 3.3: a) Adam's Catalyst, H_2, EtOAc or Raney-Ni, H_2, EtOH, both are quantitative.

However, as previously discussed many difficulties were encountered when this method was applied to resolution of the hydrogenated mixture **69**. Not only was the hydrolysis of the chiral amide extremely difficult, but it proceeded in low yield.[48] The chiral amine used to make **68a** and **68b** is also very expensive and the sequence involved a reduction step after resolution and hydrolysis. To overcome the predicament encountered with hydrolysis of the amide, one may envision using a chiral auxiliary that forms an ester linkage which is much more susceptible to hydrolysis when compared to an amide bond.

Following strategy 1 outlined in Figure 3.1, racemic mixture **69** was converted into the carboxylic acid in an analogous fashion as before. Mixture **70** was activated using DCC in the presence of a chiral alcohol in dichloromethane. It was thought that after the coupling, if there was a definitive difference in R_f of the two diastereomeric

esters as determined by TLC, then it would be a good candidate to use as a resolving agent.

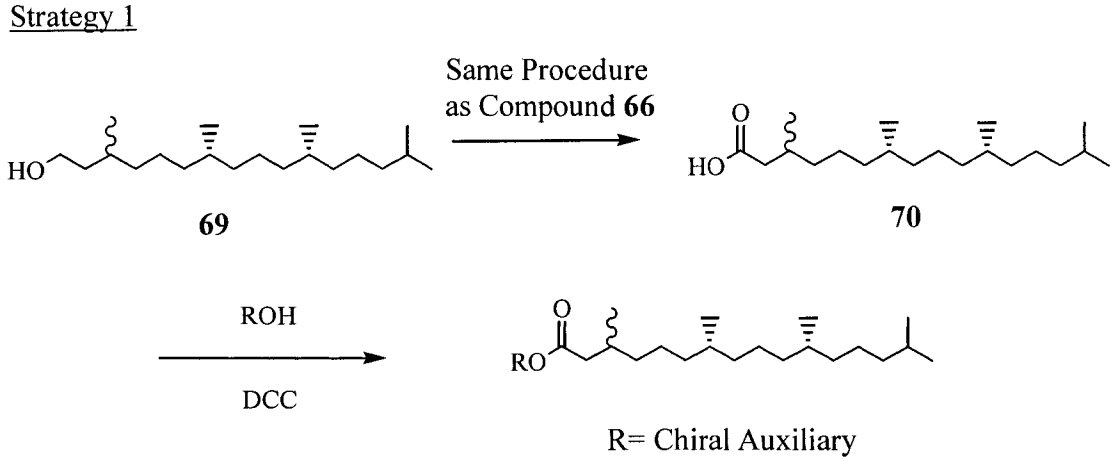
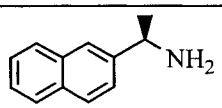
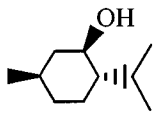
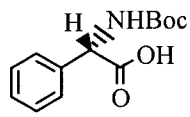
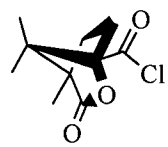
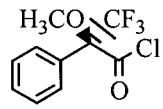
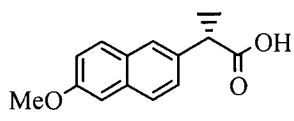


Figure 3.1: Chiral Resolution of **69**.

The chiral auxiliary (1*R*,2*S*,5*R*)-(−)-menthol (Table 3.1, Entry 2) was used to test strategy 1 but based on TLC this approach did not effectively resolve the phytanol. So, pursuing the same theme, strategy 2 was envisioned because of its inherent benefit over strategy 1 in that oxidation of phytanol and subsequent reduction after hydrolysis of the chiral auxiliary are unnecessary. The chiral carboxylic acid, *N*-Boc phenylalanine (Table

Table 3.1: Attempted chiral resolution of **69**.

Entry	RX X=NH_2 or OH		Coupling Reagent	TLC	Utility as Resolving Agent
1		**70**	N/A	Two spots	Limited
2		**70**	DCC	One spot	None
3	**69**		DCC	One spot	None
4	**69**		N/A	One spot	None
5	**69**		N/A	One spot	None
6	**69**		DCC	One spot	None
7	**Acetate of 69**	N/A	N/A	One spot	None

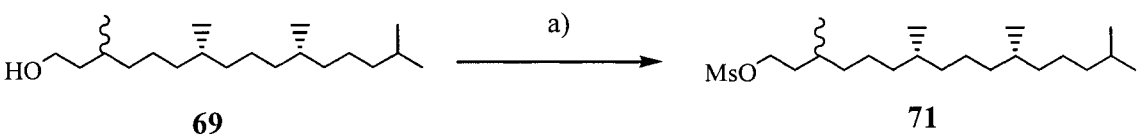
3.1, Entry 3) was reacted with DCC in dichloromethane and the alcohol of **69** was used as a nucleophile. Similarily, racemic mixture **69** was converted into the chiral ester using the commercially available acid chlorides (Table 3.1, Entries 4 and 5), (1*S*)-(-)-camphanic chloride and Mosher's acid chloride. However, entries 3-5 displayed no signifigant difference in mobility on silica gel. Another promising chiral carboxylic acid used was structurally similar to the chiral amine, *R*-(+)-1-(naphthyl)ethylamine. Naproxen, an anti-inflammatory drug, was coupled with **69** (Table 3.1, Entry 6), however TLC analysis provided one spot and it was concluded that it had no use as a resolving agent. This difference may be caused by the fact that the chiral amide has restricted rotation N-C(O) bond due to its sp^2 character, which is one characteristic that an ester linkage does not share.

The last strategy (Figure 3.1, strategy 3) attempted for resolution of the mixture of diastereomers involved an enzymatic reaction. It's well established that lipase enzymes can be used to resolve mixtures of acetylated alcohols. So compound **69** was acetylated under standard conditions using pyridine and acetic anhydride. The acetylated derivative was then subjected to enzymatic deprotection in the hope that one diastereomer would be selectively deacetylated (Table 3.1, Entry 7). However, the reaction never proceeded to completion and the deacetylation product was a mixture as determined by the chiral amide resolution technique.

Unfortunately the methods used to resolve the diastereomers of **69** were unsuccessful. This is a challenging problem because compound **69** is a long alkyl chain that contains little structural diversity to influence the outcome of the chiral resolution.

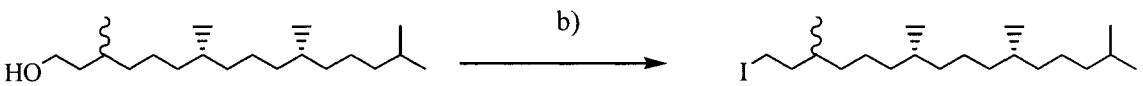
Therefore, the C3 racemic form of 3,7,11,15-tetramethylhexadecan-1-ol was used in the synthesis of the archaeal lipids.

Alcohol **69** was further functionalized so that it could undergo a Williamson ether reaction with the suitably protected enantiomers of glycerol to yield the natural and unnatural archaeal lipids (Scheme 3.4 and Scheme 3.5). The hydroxyl group of compound **69** was converted in quantitative yield to the mesylate leaving group by treatment with methylsulfonyl chloride in the presence of pyridine as a base. It was also converted to 1-iodo-(3*R*/*S*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecane **72** *via* activation of the primary alcohol by phosphonium salt and subsequent displacement by an iodide anion. The corresponding phytanyl iodide was obtained in 90% yield.



69 **71**

Scheme 3.4: Synthesis of **71**; a) MsCl, pyridine, CH_2Cl_2, 0 °C, quantitative.



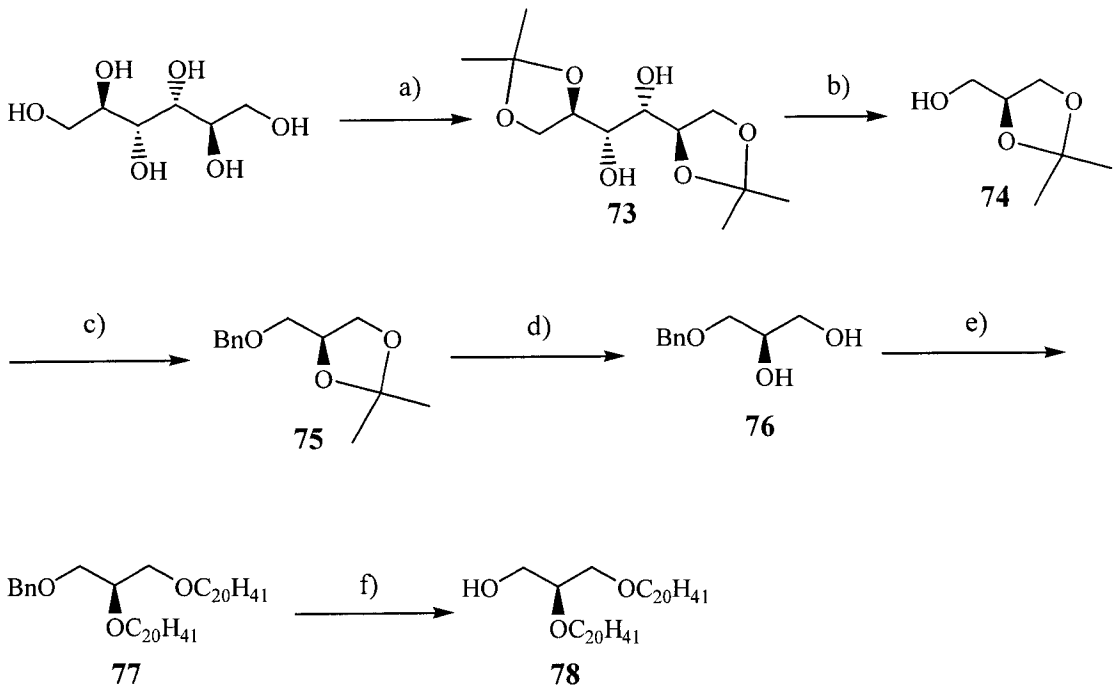
69 **72**

Scheme 3.5: Synthesis of **72**; a) I_2, imidazole, PPh_3, CH_2Cl_2, 0 °C, 90%.

3.2 Synthesis of (*R*) and (*S*) Archaeol Lipids

The simplest and most efficient method to synthesize unnatural (*S*)-archaeal lipid **78**, is from D-mannitol. The synthesis begins with the diisopropylidenation of D-mannitol, which has been acheived using a number of techniques including

acetone/$ZnCl_2$ (Scheme 3.6), 2,2-dimethoxypropane/H^+, or 2-methoxypropene/H^+. The Lewis acid zinc chloride mediated the formation of the diacetonide **73** in the presence of acetone and has been found to be one of the best methods with minimal formation of isomeric diacetals.[96] Compound **73** was then subjected to sodium periodate oxidative diol cleavage to produce two equivalents of (*R*)-glyceraldehyde acetonide. The aldehyde was immediately reduced, to the common synthetic precursor **74,** using sodium borohydride at 0 °C so as to reduce the chance of polymerization[97] and isomerization. The primary alcohol of acetonide **74** was then protected with a benzyl ether group to create **75**. Hydrolysis of the acetal under standard conditions, 80% aqueous acetic acid, produced diol **76**. The result of the first attempt to alkylate diol **76** was disappointing. It was intended that compound **76** be subjected to sodium hydride to form the dialkoxide anion and subsequent reaction with mesylate **71** would lead to the dialkylated species **77** in sufficient isolated quantity to carry on with the next step of the synthesis. However, this was not the case, the best results gave a yield of only 20-30%. In an attempt to improve the outcome, the reaction conditions were varied including changing the reaction solvent, increasing the equivalents of **71**, application of heat and the *in situ* generation of the alkyl iodide with addition of tetrabutylammonium iodide. The dialkylated compound was even obtained by monoalkylation of the primary and secondary alcohols of compound **76**. But the overall yield of the dialkylated glycerol **77** also suffered due to the addition of protection and deprotection steps.

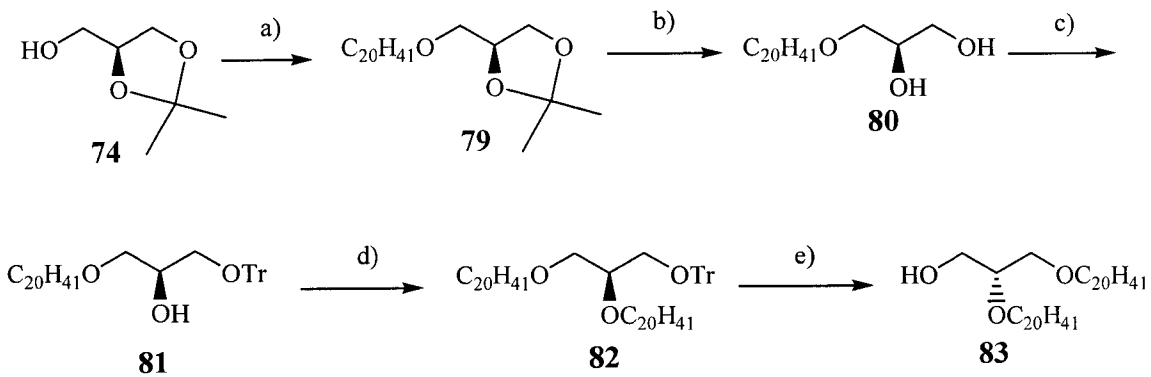


Scheme 3.6: a) $ZnCl_2$, acetone, 40% b) i) $NaIO_4$, CH_2Cl_2, sat. $NaHCO_3$ ii) $NaBH_4$, CH_2Cl_2, MeOH, 0 °C, 56% c) BnBr, NaH, THF, 83% d) 80% aq. AcOH, Δ, 95% e) **72**, KOH, toluene, 69% f) $Pd(OH)_2$, H_2, EtOAc, 95%.

Attention was then directed toward varying the leaving group present on the alkyl chain. Other options were a tosylate, halides such as bromide or iodide or even the use of triflate. The iodide was chosen because it was easily formed in a reaction of inexpensive reagents, it was stable upon storage and it is known to be a very effective leaving group. The only concern was the possibility of triphenylphosphine oxide, the byproduct from generation of **72,** interfering with future reactions. Generally, triphenylphosphine oxide is difficult to remove during purification but in this case 1-iodo-(3*R*/*S*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecane **72** is soluble in hexane, so the white solid impurity is easily removed by filtration. Compound **76** was converted to **77** *via in situ* formation of a dialkoxide species, generated by potassium hydroxide in refluxing toluene, followed by nucleophilic displacement of iodide of **72**. This method generated **77** in the best yield of 69%. However, when the exact conditions were applied to large scale synthesis, the

yield, although not as low as before dropped to 50%. In the last step of the sequence, the primary hydroxyl of **78**, which is required for glycosylation and phosporamidate coupling reactions, was unmasked by deprotection of the benzyl ether over palladium hydroxide in an atmosphere of hydrogen.

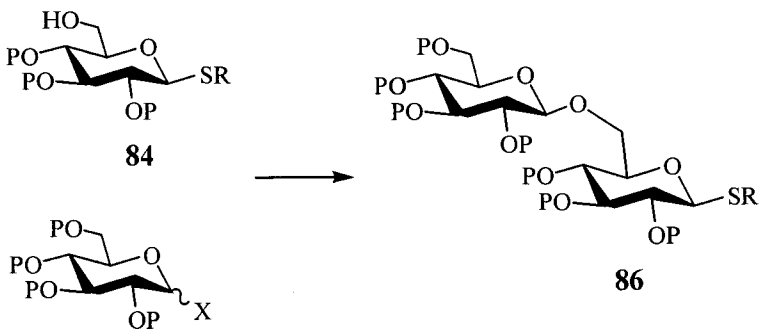
The natural archaeol lipid **83** with the (*R*)-configuration at C2 of glycerol was obtained in five steps from **74** (Scheme 3.7). The primary hydroxyl of compound **74** was alkylated using sodium hydride as a base. Subsequent nucleophilic displacement of the mesylate of **71** produced the monoalkylated acetonide **79** in 80% yield. Hydrolysis of the acetal proceeded as normal producing the diol **80**. The primary hydroxyl was selectively protected with the bulky trityl protecting group which afforded the secondary hydroxyl **81** for alkylation. Alkylation of the 2-OH proceeded in a respectable 74% yield considering the neighbouring sterically hindering trityl group. The deprotection of the trityl group of **82** was done under acidic conditions and resulted in a disappointing yield of 61%. The crude product was difficult to purify due to the trityl methyl alcohol impurity. Compound **83** was obtained in pure form after repetitive medium pressure chromatography.



Scheme 3.7: Synthesis of **83** a) **71**, NaH, Bu_4NI, DMF, Δ, 80% b) 80% aq. AcOH, Δ, 97% c) TrCl, pyridine, CH_2Cl_2, 86% d) **71**, NaH, Bu_4NI, DMF, Δ, 74% e) HBF_4, CH_2Cl_2, MeOH 61%.

3.3 Synthesis of Gentiobiosyl Glycerolipids

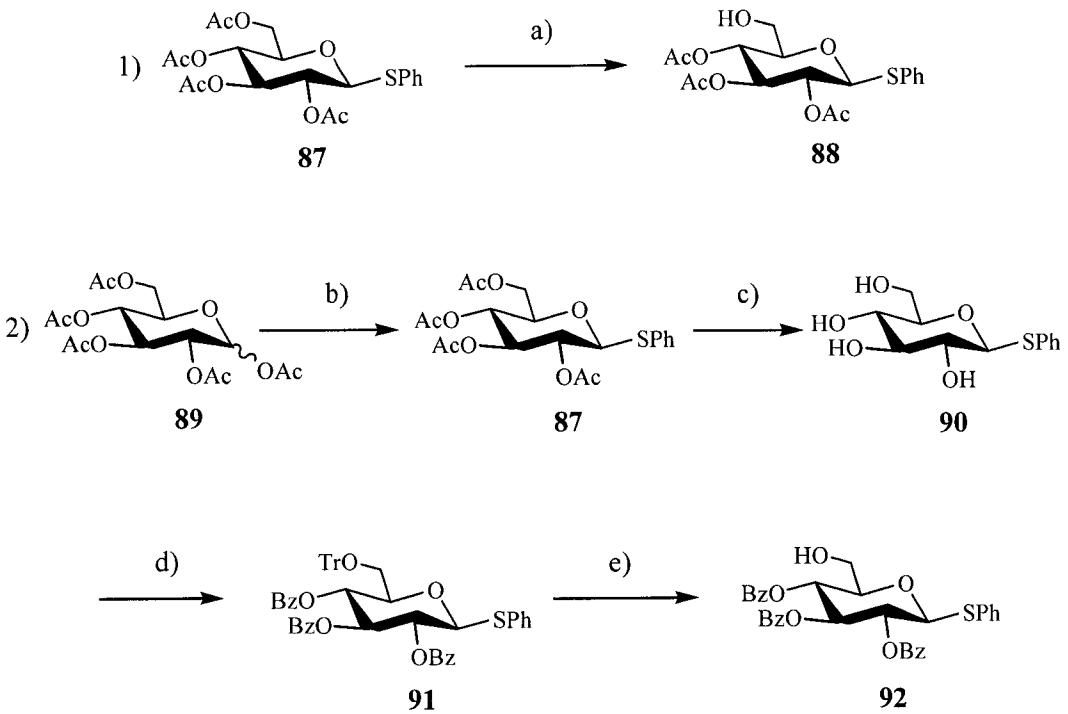
With the synthesis of the hydrophobic core complete, the focus shifted to constructing the polar head groups and coupling to the archaeol lipid. The construction of gentiobiose encompassed the synthesis of a thio donor **84** that can be glycosylated at C6. The 6-OH must be free to undergo glycosylation with a suitable donor **85** such that the conditions used to activate this donor do not disturb the integrity of the sulfide used to couple **86** to **78** and **83** (Figure 3.2). Also, the protecting group strategy must enhance β-selectivity for the construction of **86** and gentiobiosyl lipids **96** and **97**.



P = Acyl protecting group, X = Halide

Figure 3.2: Construction of a gentiobiosyl donor **86**

Recently, a strategy was published for the selective deprotection of a primary 6-OAc using an organotin catalyst (Scheme 3.8).[98,99,100] If this synthetic manipulation is as efficient as claimed then it would be an expedient route to a glucosyl acceptor **88** from the easily obtained peracetylated thioglucosyl precursor **87**. Attempts to employ this transformation resulted in yields that were 20% lower than those reported.[100] In the presence of $((t\text{Bu})_2\text{SnOHCl})_2$, the 6-*O* acetate of **87** was selectively deprotected in 62% yield.

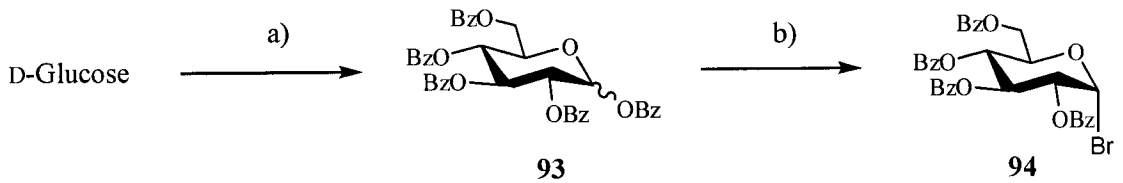


Scheme 3.8: Synthesis of glucosyl acceptors **88** and **92**; a) $((t\text{Bu})_2\text{SnOHCl})_2$, MeOH, THF, 62% b) thiophenol, BF_3OEt_2, CH_2Cl_2, 0 °C, 75% c) NaOMe, MeOH, 85% d) i) TrCl, pyridine ii) BzCl, pyridine 69% e) HBF_4, CH_2Cl_2, MeOH 65%.

However, compound **88** was susceptible to acetyl group migration and its synthetic utility was limited due to the acidic nature of the subsequent glycosylation reaction. Therefore, a different acceptor must be used in the synthesis of the disaccharide donor. The alternative benzoylated glycosyl acceptor **92** was synthesized from peracetylated glucose in four steps. Conversion of **89** into **87** involved functionalization of the anomeric acetate to the respective thiophenyl group using thiophenol and boron trifluoride diethyl etherate. Removal of the acetate groups under standard Zemplen conditions yielded **90**, followed by a one pot regioselective tritylation of the 6-hydroxyl and benzoylation of the remaining hydroxyls produced **91** in 69% yield. Removal of the trityl group afforded the glucosyl acceptor **92** that was used in the subsequent glycosylation step. The trityl group was deprotected without acyl group migration. This

was verified by the chemical shift of H-4 which is expected to be further downfield due to the electron withdrawing nature of the acyl protecting group.

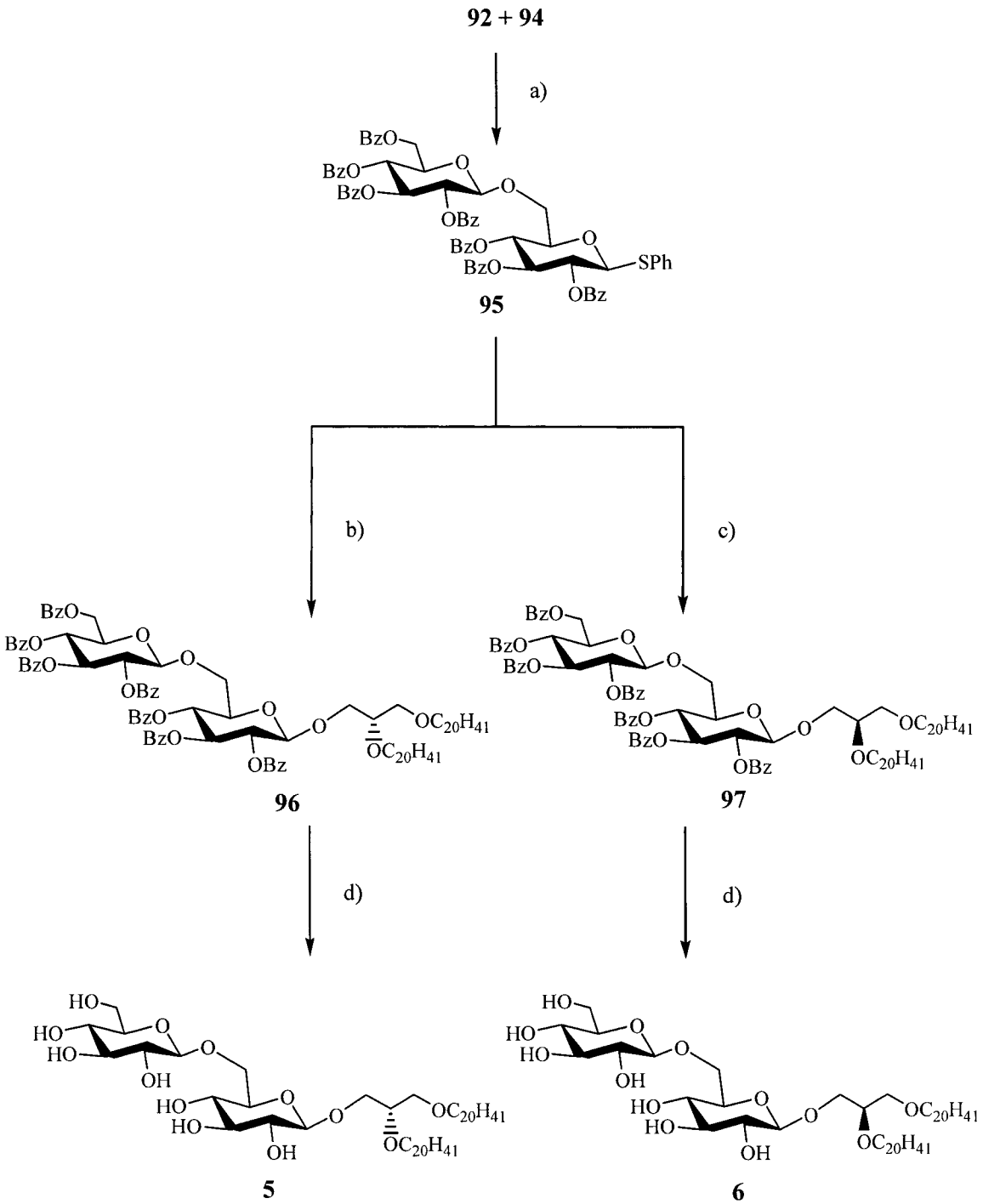
The glycosyl donor was synthesized from D-glucose (Scheme 3.9). The benzoyl protecting group at the C-2 position provided the neighbouring group participation required to favour the formation of the *trans*-β-1,2 linkage. The perbenzoyl glucose was synthesized using D-glucose in the presence of benzoyl chloride and pyridine. The thermodynamic α-bromide **94** was then produced by reaction of **93** with hydrogen bromide.



93 **94**

Scheme 3.9: Synthesis of the glucosyl donor **94**; a) BzCl, pyridine, 0 °C, 70% b) HBr, CH_2Cl_2, 0 °C, quantitative.

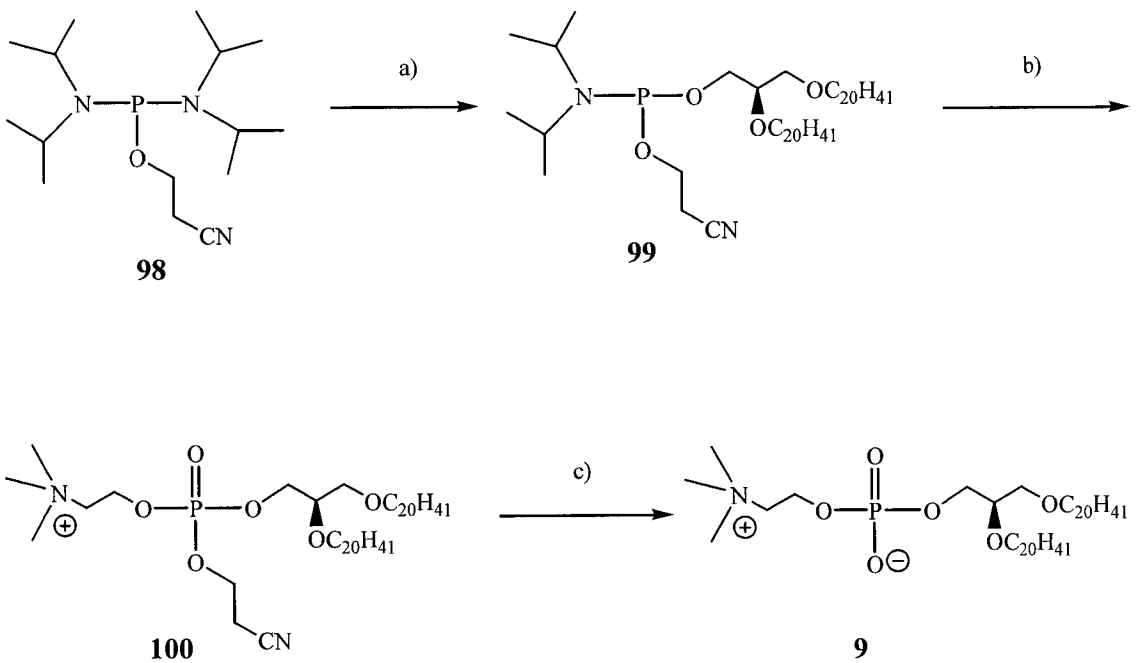
Acceptor **92** was glycosylated with glucosyl bromide **94** using silver triflate as a promoter without 2,6 lutidine to disfavour formation of an orthoester (Scheme 3.10). None of the α product was detected and the gentiobiosyl donor **95** was isolated in 78% yield. Glycosidation of **95** with **78** and **83** promoted by NIS and triflic acid proceed quickly to create **96** in a 16:1 β/α ratio and **97** in a 15:1 β/α ratio. The β anomer was easily purified and was isolated in 80 and 75% yield, respectively. Deprotection, under Zemplen conditions with gentle heating, afforded the final deprotected gentiobiosyl lipids, **5** and **6**. Both **5** and **6** were characterized by 1D and 2D NMR and HRMS experiments.



Scheme 3.10: Synthesis of gentiobiosyl glycerolipids, **5** and **6**; a) AgOTf, CH_2Cl_2, 3 Å MS, 78% b) **83**, NIS, TfOH, CH_2Cl_2, 3 Å MS, 75% c) **78**, NIS, TfOH, CH_2Cl_2, 3 Å MS, 81% d) NaOMe, MeOH, Δ, 81% from **96** and 86% from **97**.

3.4 Synthesis of Archaetidylcholine

Archaetidylcholine is a lipid found in the methanogen, *Methanopyrus kandleri*. All archaesome studies performed on the polar extracts of archaebacteria to date are devoid of this specific lipid. Its synthesis was required to determine its adjuvanticity. The most efficient route began with the commercially available phosphoradiamidate **98** followed by two tetrazole mediated coupling reactions with the proper reagent (Scheme 3.11).



Scheme 3.11: Synthesis of **9** a) **78**, tetrazole, CH_2Cl_2, MeCN, 76% b) i) choline tosylate, tetrazole, CH_2Cl_2, MeCN ii) *t*-BHP, 77% c) cat. NaOMe, MeOH, 83%.

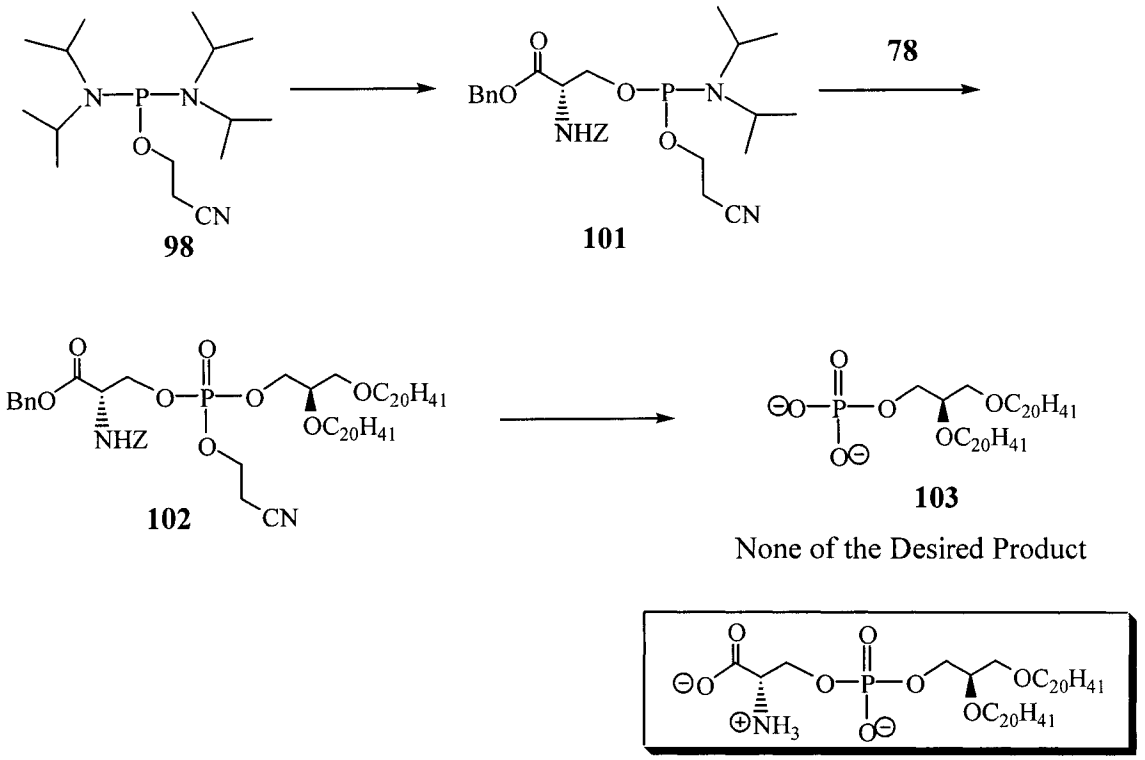
The initial phosphoramidate coupling reaction between **98** and choline chloride did not yield any appreciable amounts of the choline phosphoramidate. Typically, the first coupling reactions were difficult to moniter by TLC even with the use of a small percentage of triethylamine in the eluent. Reasons for the lack of product are unknown. Perhaps the chlorine counter-ion is able to form hydrochloric acid in the reaction mixture

thereby destroying **98** or the choline phoshoramidate. However, changing the choline counter ion and the order of coupling solved this problem. The choline tosylate was chosen as an alternative to choline chloride. This reagent could be synthesized from methyl tosylate ester and dimethylaminoethanol or obtained from commercial sources. The first coupling reaction between compound **98** and **78** proceeded smoothly to produce **99** in 76% yield. The next coupling step was carried out in analogous fashion except after one hour the intermediate phosphite was oxidized *in situ* using *t*-butyl hydrogen peroxide (Scheme 3.11). The phosphotriester **100** was then purified using column chromatography and the isolated yield was 77%. The isolated product was stable for many months when stored at –20 °C. The deprotection of the cyanoethyl group to produce the phoshodiester was catalysed by sodium methoxide. After workup and purification it was found that the compound was in a trimethylammonium tosylate salt form. This compound, which was soluble in MeOH, was converted into its zwitterionic form **9** *via* passage through a reverse phase sep-pak cartridge.

3.5 Synthesis of (*R*)- and (*S*)-Archaetidylserine

The synthesis of compounds **7** and **8** were completed in a similar manner to that used to construct **9**. Compound **101** was constructed from *N*-Z-serine benzyl ester and **98** after coupling under standard activation conditions. Compound **78** was then coupled to the protected serine phosphoramidate followed by phosphorus oxidation to yield the phosphotriester **102** (Scheme 3.12). The deprotection strategy for **102** involved base catalyzed removal of the cyanoethyl group and then hydrogenation of the *N*-benzyloxycarbonyl and the benzyl ester to produce the fully deprotected

archaetidylserine. However, when the phosphotriester was subjected to basic conditions the serine under went β-elimination to give the archaetidyl phosphoric acid **103**.

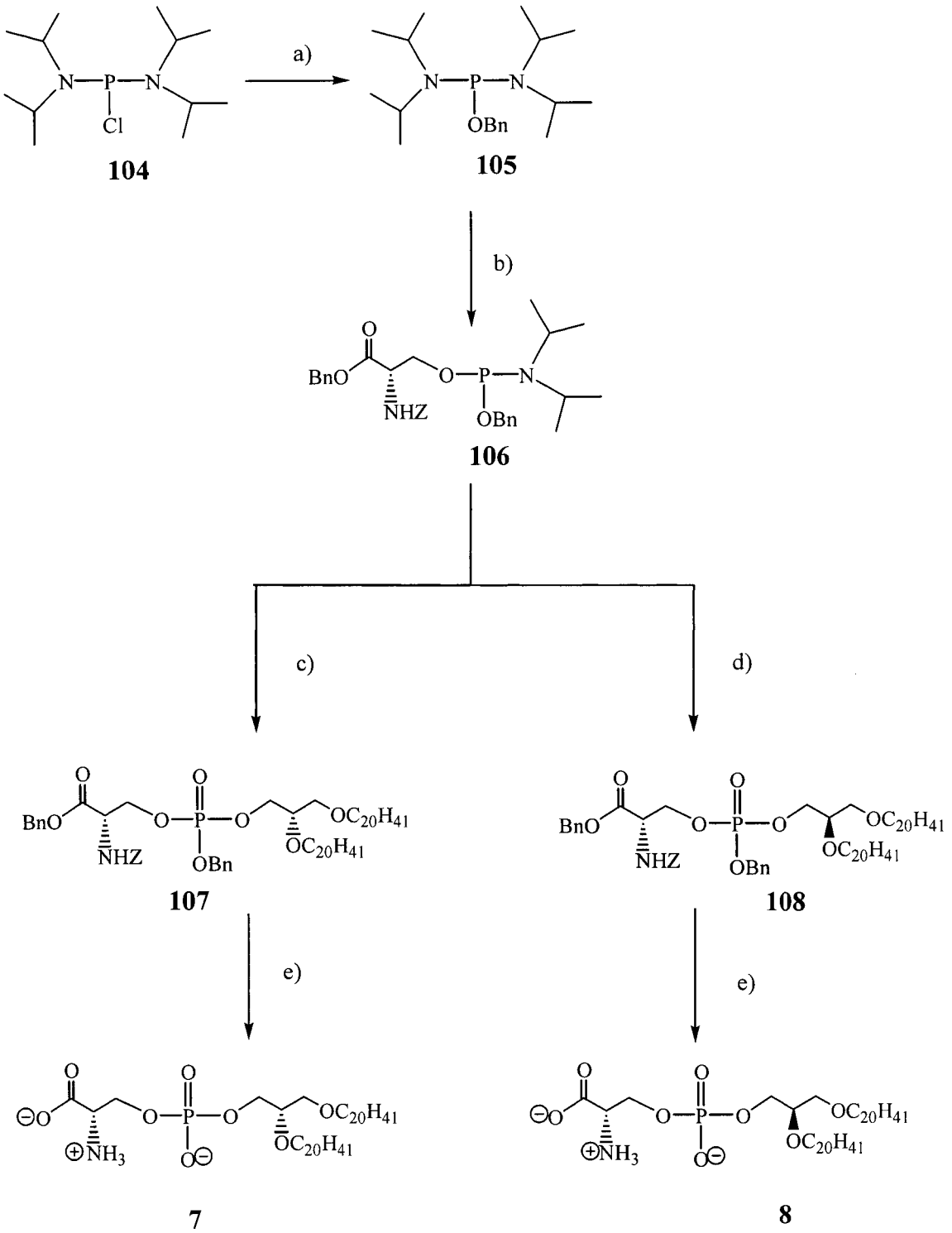


Scheme 3.12: Base catalysed elimination of serine.

In light of these findings implementation of a neutral deprotection strategy was undertaken to obtain target compounds **7** and **8**. Precedent for this type of transformation has been reported. The phosphorylating reagent benzyloxybis(diisopropylamino)phosphine **105** was employed in the synthesis of biologically important compounds containing phosphomono- and diesters.[101] The synthesis of the phosphorodiamidate was completed following the literature protocol. However, one interesting note to mention, is that reagent **104** obtained from Aldrich varied in purity and this had a direct effect on the utility of **105** as a phosphorylating reagent. The last batch of **104** was obtained as a fresh loose white powder whereas

samples used before were a yellowish solid. The resulting reaction of **104** with benzyl alcohol in the presence of triethylamine gave a clear liquid after filtration. The benzyloxybis(diisopropylamino)phosphine **105** was stored in the freezer at -20 °C and was stable for one month.

The benzyloxy serine phosphoramidate **106** was synthesized from **105** and *N*-Z-serine benzyl ester and coupling was catalyzed by tetrazole in 75% yield (Scheme 3.13). This compound served as the common precursor used to synthesize **7** and **8**. Tetrazole mediated coupling of **106** to the primary alcohols of **83** and **78** and subsequent *in situ* oxidation to the triester produced the protected form of compounds **7** and **8** in 73 and 70% yield (Scheme 3.13). These compounds were chromatographically stable and were stable for at least 6 months at -20 °C. Compounds **107** and **108** were deprotected in one step using palladium hydroxide under an atmosphere of H_2. The compounds were then purified using several methods including desalting *via* size exclusion chromatography and Iatrobeads column chromatography. The spectral data obtained for the synthetic compounds exactly matched that found for the natural compound isolated from *Methanobrevibacter arboriphilus*.[102] The presence of the carboxyl functional group was verified by IR spectroscopy, TLC was ninhydrin positive and the molecular ion peak from HRMS matched that calculated for compounds **7** and **8**.



Scheme 3.13: Synthesis of **7** and **8**; a) BnOH, triethylamine, THF, 0 °C b) *N*-Z-serine benzyl ester, tetrazole, CH_2Cl_2, MeCN, 81% c) i) **83**, tetrazole, CH_2Cl_2, MeCN ii) *t*-BHP, 73% d) **78**, tetrazole, CH_2Cl_2, MeCN ii) *t*-BHP, 70% e) $Pd(OH)_2$, H_2, cyclohexane, MeOH, 74 and 79% for **7** and **8**.

Chapter 4

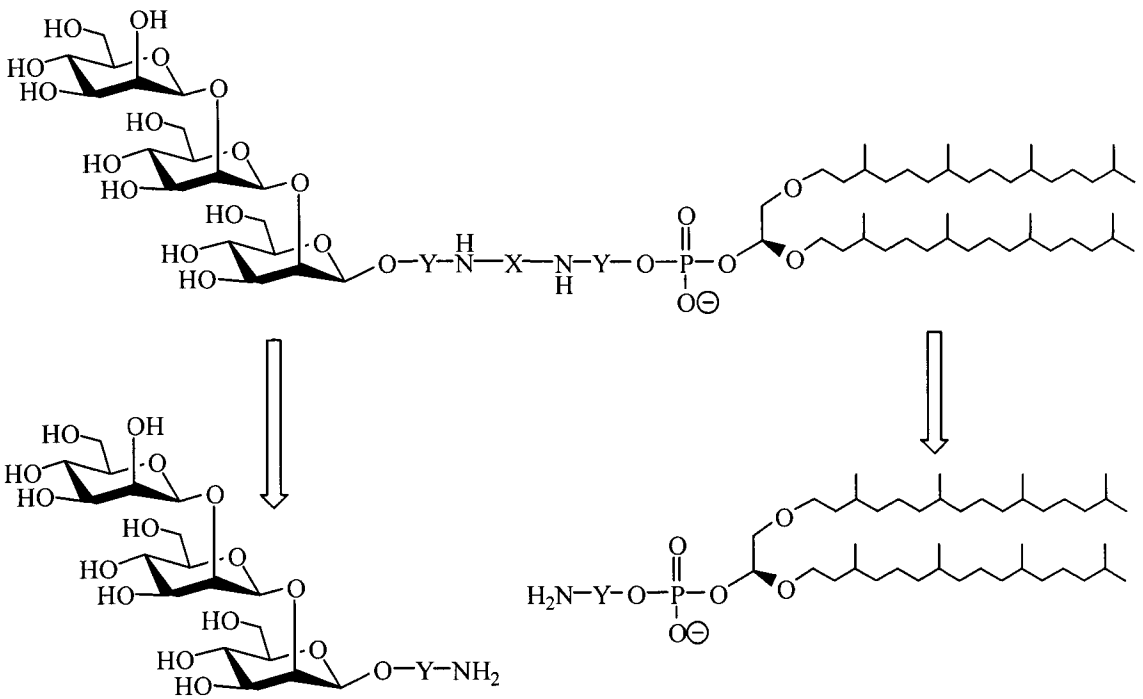
The Synthesis of an Archaeal Neoglycophospholipid

4.1 Scope of the Project

One of the many mechanisms that APC's use to uptake antigen is by cell surface expression of carbohydrate binding proteins, called lectins, that recognize carbohydrate oligomers rich in α-mannose.[103,104] Scientists have used these biological mechanisms for developing liposomal delivery of antigen to APC's to elicit cell-mediated and humoral immunity. One such experiment has incorporated a branched α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranoside, attached to dipalmitoylphosphatidyl- ethanolamine *via* a Lemieux linker ($-O(CH_2)_8CO_2CH_3$), within a liposomal formulation.[105] They found that mannosylated liposomes were taken up more efficiently by dendritic cells than liposomes composed of neutral and negative lipids thereby supporting a mannose receptor mediated endocytosis pathway.

The carbohydrate β-mannan cell wall epitotes of *Candida albicans* has received extensive attention in our laboratory as a possible vaccine target against mucosal infections. From previous research, the optimal synthetic carbohydrate epitotes that are required for high affinity binding by β-mannan specific IgM and IgG monoclonal antibody, is the repeating β-(1→2) mannosyl disaccharide and trisaccharide. These β-mannan oligomers, which have unique helical structures in solution,[106] are recognized by macrophages that display a 32 kDa β-(1→2) mannose binding protein.[107,108,109] Because our group has become increasingly interested in developing a vaccine for candidiasis, an

epitope specific adjuvant may be useful rather than relying on traditional adjuvants. Therefore, it is possible that liposomes displaying the synthetic β-mannan trisaccharide antigen may stimulate an immune response such that it could be used as an adjuvant for immunizations involving synthetic β-mannan protein conjugate vaccines.



Y = Linker X = Bridging atom(s)

Figure 4.1: The proposed structure of the neoglycophospholipid containing the β-(1→2) mannosyl trisaccharide and the archaeal lipid.

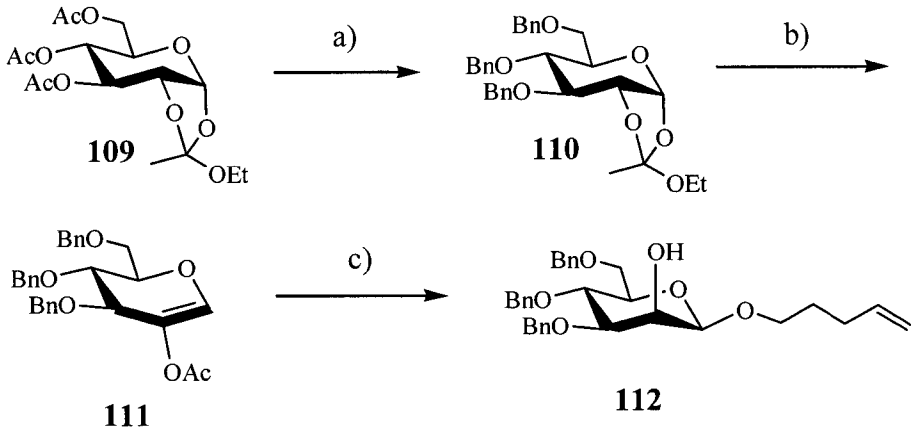
The synthesis of the corresponding neoglycophospholipid was envisioned to test this theory (Figure 4.1). The proposed synthetic structure contains the β-(1→2) trimannoside and the phosphoarchaeol lipid bridged by X through the amino functional groups of linker Y. The synthesis of the trisaccharide with a functionalized aglycone for furthur elaboration is well established. The aglycone typically used is the Lemieux linker ($-O(CH_2)_8CO_2CH_3$) and allyl or pentenyl alcohol. The latter choices can be easily

converted to 3-(2-aminoethylthio)propyl or 5-(2-aminoethylthio)pentyl *via* a photoaddition reaction with 2-aminoethanethiol.

Because archaeal lipids act as strong immunostimulatory molecules with strong *in vivo* stability these structures were used in the glycolipid synthesis. The same 2-cyanoethoxy-*N*,*N*-(diisopropylamino)phosphorodiamidate chemistry implemented for the construction of archaetidylcholine **9** will be used for the synthesis of the amino phosphoarchaeal lipid. However, instead of choline being used in the second coupling step, a small molecular weight, bifunctional molecule is preferentially desired. The molecule is bifunctional in the sense that it must meet two criteria: 1) it must contain a hydroxyl group that can be used in the initial phosphoramidate coupling and 2) it must be functionalized such that the lipid component can be coupled to the trisaccharide antigen. Commercially available molecules that may be used include 2-aminoethanol or 2-aminoethoxyethanol. Coupling of many carbohydrate antigens to carrier proteins has led to the development of strategies to bridge two primary amines. Such methods include the use of diethyl squarate,[64] adipic acid chemistry[110,111] or thiourea linkages.[112] However, the squarate linker is not believed to be biologically inert and antibodies may be raised toward it,[113,114] so its use should be limited. The thiourea linkage can be synthesized by reaction between an isothiocyanate and a primary amine. The isothiocyanate is constructed by reaction of a primary amine with reagents such as thiophosgene or 1,1'-thiocarbonyldi-2(1*H*)-pyridone. The 1,1'-thiocarbonyldi-2(1*H*)-pyridone has recently been used to synthesize neomycin B conjugates[115,116,117] and is a very mild reagent.[118]

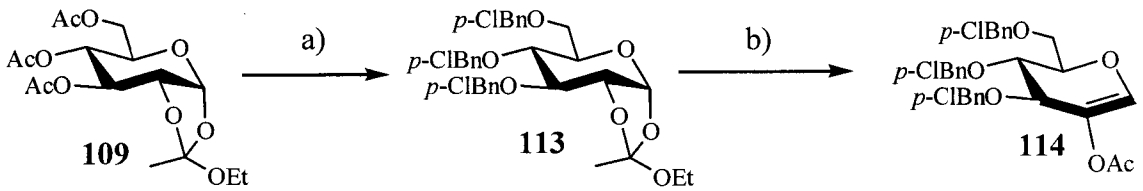
4.2 Synthesis of β-Mannan Neoglycolipid 123

The synthesis of the trisaccharide portion of the target neoglycolipid was completed using the ulosyl bromide glycosylation strategy used by Nitz[66] and developed by Lichtenthaler[73] to produce the desired *cis*-1,2-β linkages. This is a convenient route to constructing the β-(1→2) mannan structures because the resulting 2-keto group of a glycosid-2-ulose can be directly converted to the secondary hydroxyl used in subsequent glycosylation reactions without an oxidation step. The synthesis started by constructing a suitable glycosyl acceptor. Starting from peracetylated glucose, compound **109** was synthesized by reacting the intermediate glucosyl bromide with ethanol, tetrabutylammonium bromide and 2,6-lutidine (Scheme 4.1). In a one pot reaction, the acetyl protecting groups were exchanged for benzyl ethers in good yield. Thermal fragmentation of **110** using pyridine as a base proceeded in 69% yield. The resulting 2-acetoxyglucal **111** was converted to the ulosyl bromide using standard conditions and 4-penten-1-ol was glycosylated to afford the β-glucosid-2-ulose. The ulose was then reduced to the manno configuration using sodium borohydride at 0 °C to yield the mannosyl acceptor **112**. The anomeric configuration of the acceptor was confirmed by measurement of the heteronuclear H1-C1 one bond coupling constant of the anomeric carbon using heteronuclear correlation spectroscopy, HMQC ($^1J_{C1\text{-}H1}$ = 156.9 Hz).



Scheme 4.1: Synthesis of acceptor **112** a) BnBr, KOH, THF, 50 °C, 73% b) pyridine, bromobenzene, 150 °C, 69% c) i) NBS, EtOH, CH_2Cl_2, 3 Å MS, 0 °C ii) 4-penten-1-ol, AgOTf, TTBP, CH_2Cl_2, 3 Å MS, -40 °C to -5 °C iii) $NaBH_4$, CH_2Cl_2, MeOH, 0 °C, 61% over three steps.

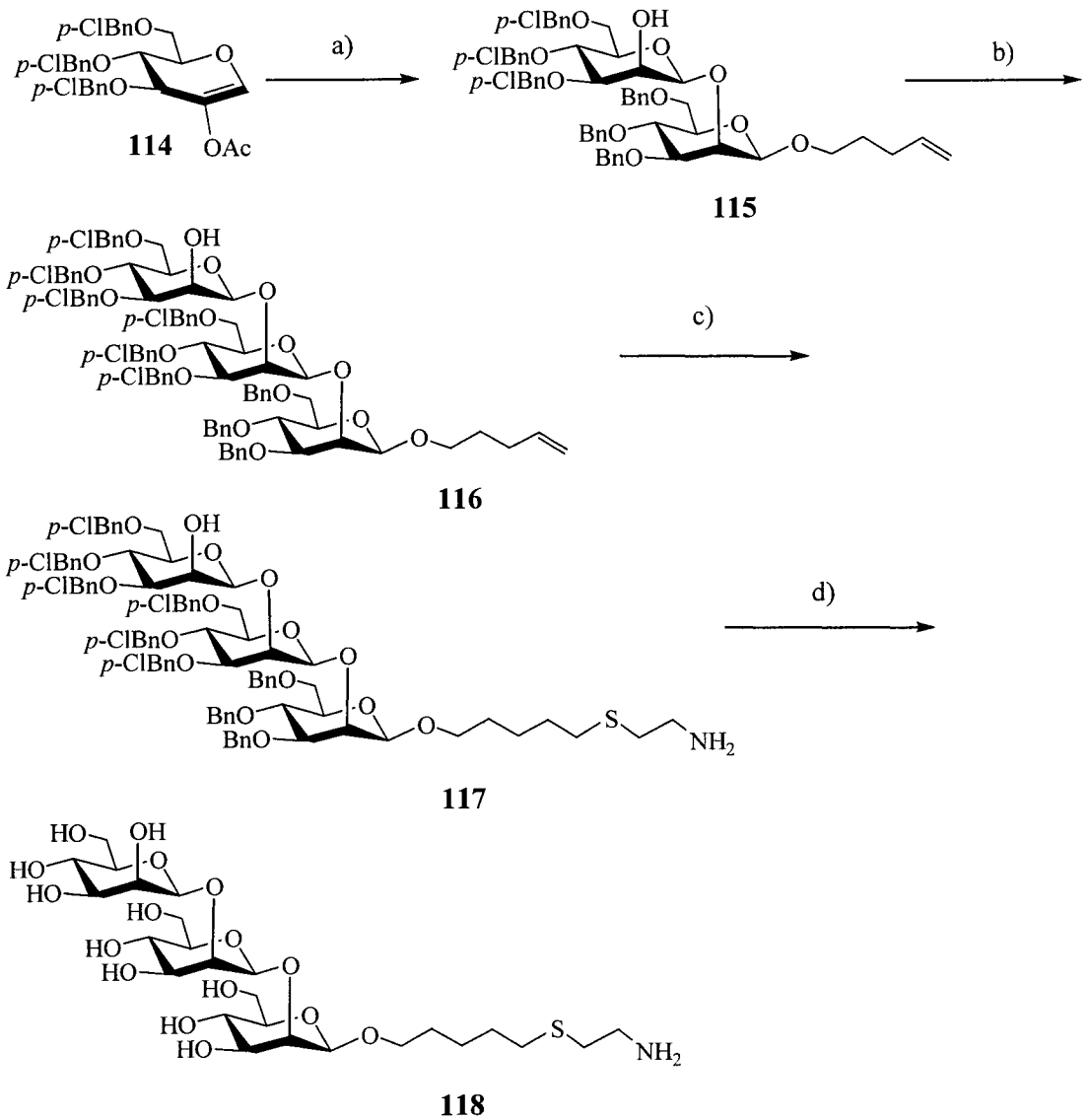
The use of benzyl protecting groups on the ulosyl bromide causes problems during the glycosylation reactions[114] so the *p*-chlorobenzyl compound **114** was used as a replacement to produce an improved ulosyl bromide donor. Its synthesis is analogous to the procedure used for the synthesis of **111** (Scheme 4.2).



Scheme 4.2: Synthesis of glucal **114** a) *p*-ClBnCl, KOH, THF, 50 °C, 76% b) pyridine, bromobenzene, 150 °C, 79%.

Glycosylation of acceptor **112** and reduction of the intermediate glycosid-2-ulose proceeded in a yield of 74% to give the disaccharide **115** (Scheme 4.3). After purification, there was no detectable presence of the β-gluco-anomer. The trisaccharide **116** was synthesized in analogous fashion, with a small drop in overall yield. Presumably this decrease in yield occurs during the glycosylation of the disaccharide with the glycosyl donor and not during the reduction using L-selectride. This reaction to

synthesize the trisaccharide is amenable to larger scale synthesis which proves the utility of this general glycosylation strategy. The anomeric configurations of the di- and trisaccharides were again confirmed by measurement of $^{1}J_{C1,H1}$ coupling constants employing HMQC experiments.

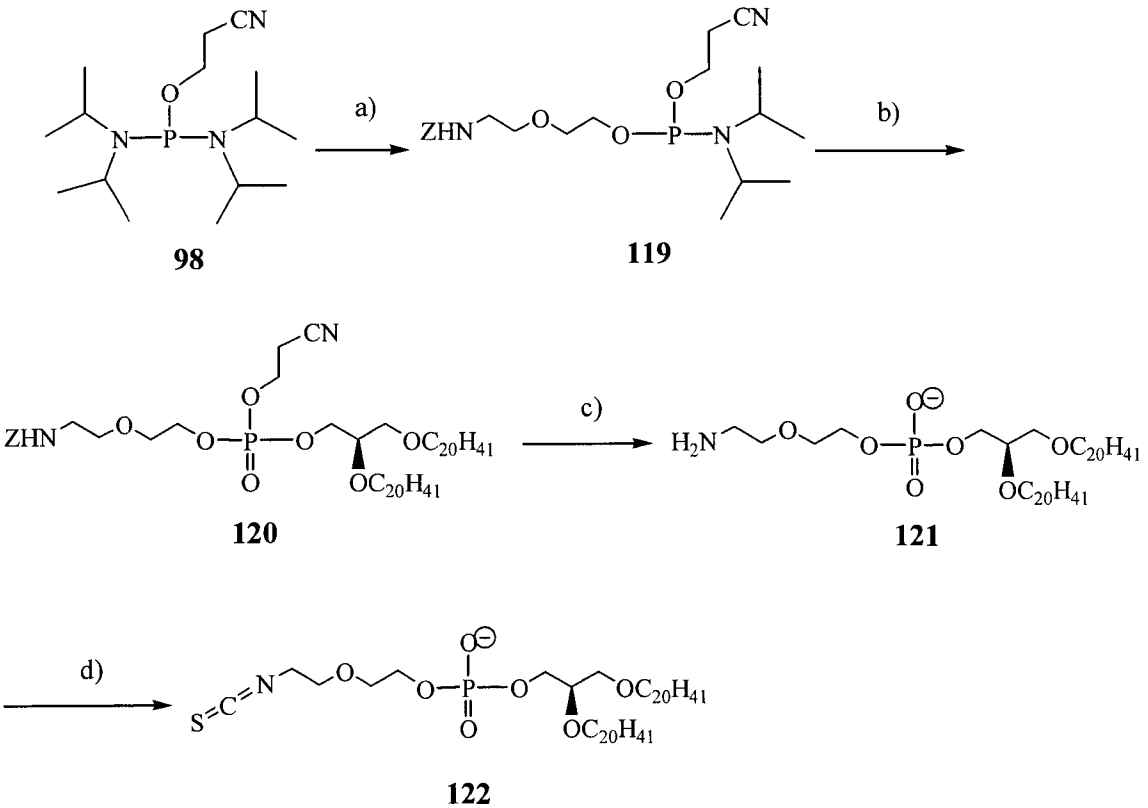


Scheme 4.3: Synthesis of trisaccharide **118** a) i) NBS, EtOH, CH_2Cl_2, 3 Å MS, 0 °C ii) **112**, AgOTf, TTBP, CH_2Cl_2, 3 Å MS, -40 °C to -5 °C iii) L-selectride, THF, - 78 °C, 74% over glycosylation and reduction steps b) i) **114**, NBS, EtOH, CH_2Cl_2, 3 Å MS, 0 °C ii) AgOTf, TTBP, CH_3CN, 3 Å MS, -40 °C to -5 °C iii) L-selectride, THF, - 78 °C, 66% over glycosylation and reduction steps c) 2-Aminoethylthiol hydrochloride, MeOH, CH_2Cl_2, UV light (365 nm), 82% d) Na, NH_3, THF, - 78 °C, 57%.

With the protected trisaccharide in hand, elaboration of the pentenyl aglycon was completed using the conventional method. Elongation of the aglycon was completed using the UV reaction with 2-aminoethanethiol hydrochloride to yield the (2-aminoethylthio)pentyl analogue **117**. Global deprotection of trisaccharide **117** by Birch reduction using anhydrous ammonia and sodium metal gave the desired trisaccharide as an acetic acid salt. This crude compound was then passed through a hydroxide exchange resin column to give the free amine **118**, in a moderate, albeit unoptimized, 57% yield.

Attention was now focused on synthesizing the hydrophobic component of the target glycolipid. Commercially available phosphoamidate **98** was coupled with 2-(2-(*N*-benzyloxycarbonyl)aminoethoxy)ethanol under standard conditions (Scheme 4.4). This compound proved difficult to purify, the crude product was passed through a silica gel column packed with triethylamine, but the resulting product was not completely pure and degradation on silica gel led to a decrease in yields. The next coupling step proceeded in the same fashion, the phosphoramidate **119** and primary alcohol of **78** were dissolved in dichloromethane followed by addition of tetrazole dissolved in acetonitrile. The formation of an insoluble salt in the reaction mixture signaled that the reaction was complete. The intermediate phosphite was transformed into the corresponding phosphate by oxidation using *tert*-butyl hydrogen peroxide. The phosphotriester **120** was isolated in 81% yield after column chromatography. One pot deprotection of phosphate **120** was done using a catalytic amount of sodium methoxide followed by addition of palladium hydroxide and hydrogenation unger an atmosphere of H_2 gas. Earlier attempts at this one-pot reaction produced a diamine compound, presumably from the reduction of the cyano protecting group and the benzyloxycarbonyl protecting group. Longer reaction

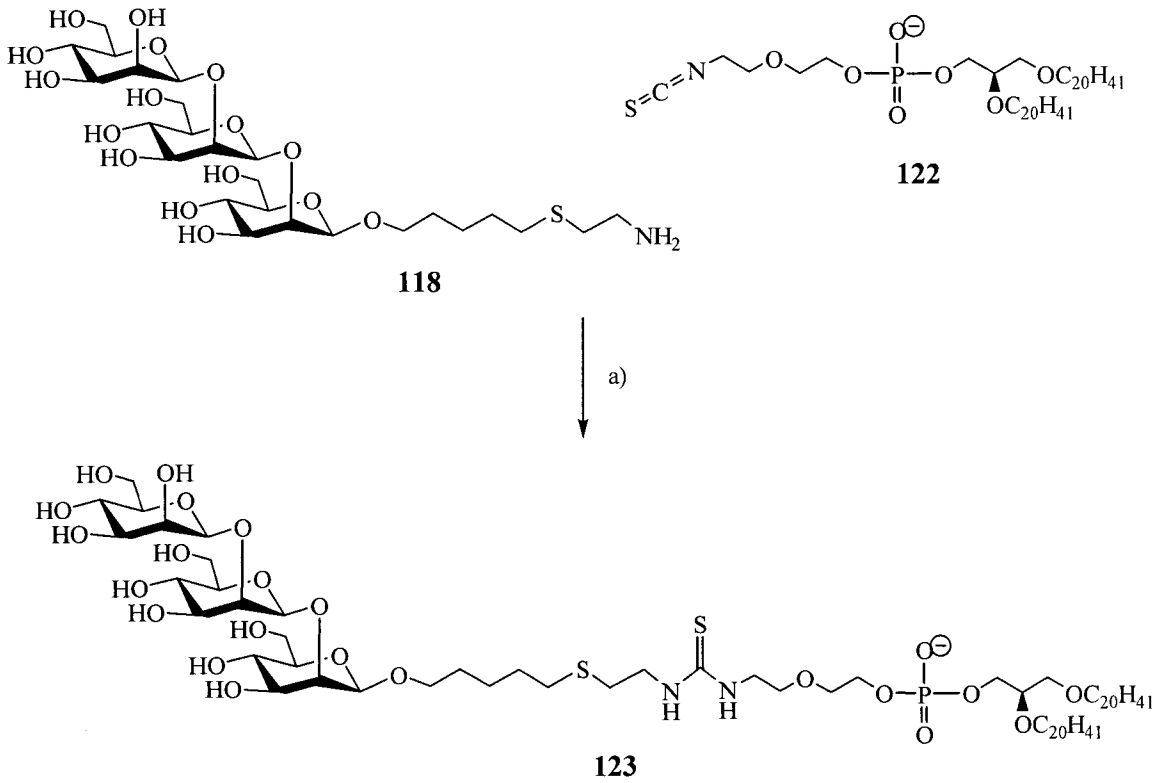
time and a larger volume of sodium methoxide ensured that the phosphotriester **120** had been completely converted to the phosphodiester **121**.



Scheme 4.4: Synthesis of isothiocyanate **122** a) 2-(2-(*N*-benzyloxycarbonyl)aminoethoxy)ethanol, tetrazole, CH_2Cl_2, CH_3CN, 75% b) i) **78**, tetrazole, CH_2Cl_2, CH_3CN ii) *t*-BHP, 81% c) i) cat. NaOMe, MeOH, cyclohexane ii) $Pd(OH)_2$, H_2, MeOH, cyclohexane 69% d) 1,1'-thiocarbonyldi-2(1*H*)-pyridone, DMAP, CH_2Cl_2, 74%.

With two synthetic precursors in hand, both of which contained primary amines, a decision had to be made about which compound would undergo the functional group conversion to the isothiocyanate. As trisaccharide **118** was deemed more synthetically valuable and phosphodiester **121** and 1,1'-thiocarbonyldi-2(1*H*)-pyridone had similar solubility properties, the amine of **121** was converted to the isothiocyanate. The primary amine of **121** underwent functional group conversion to the desired isothiocyanate **122** under very mild conditions, by reaction with 1,1'-thiocarbonyldi-2(1*H*)-pyridone and

DMAP. The reaction was monitered by TLC and appeared to be quantitative, however the purified yield was only 74%. Compound **122** is likely not stable to silica gel chromatography and in the presence of the solvent, methanol, used to elute the compound.



Scheme 4.5: Coupling of **118** and **122** *via* formation of a thiourea linkage. a) pyridine, DMAP, DMF, CH_2Cl_2, 72%.

The largest concern in the final step of the reaction sequence was the inherent polarity difference of **118** and **122**. While the carbohydrate antigen is soluble in water and methanol, the hydrophobic component **122** is soluble in toluene, dichloromethane and chloroform. This could have posed a problem, however, the coupling reaction between **118** and **122** proceeded smoothly using pyridine and DMAP in a combination of dichloromethane and dimethylformamide (Scheme 4.5). The purified glycolipid **123**,

isolated as a DMAP salt, was obtained in 72% yield. The presence of the alkyl chains and trisaccharide were established by ^{1}H NMR and 2D NMR experiments, whereas the phosphate group and the thiocarbonyl of the thiourea linkage were confirmed by ^{31}P and ^{13}C NMR, respectively. A molecular ion peak corresponding to the molecular weight of the neoglycolipid was found using negative mode electrospray high resolution mass spectroscopy.

Chapter 5

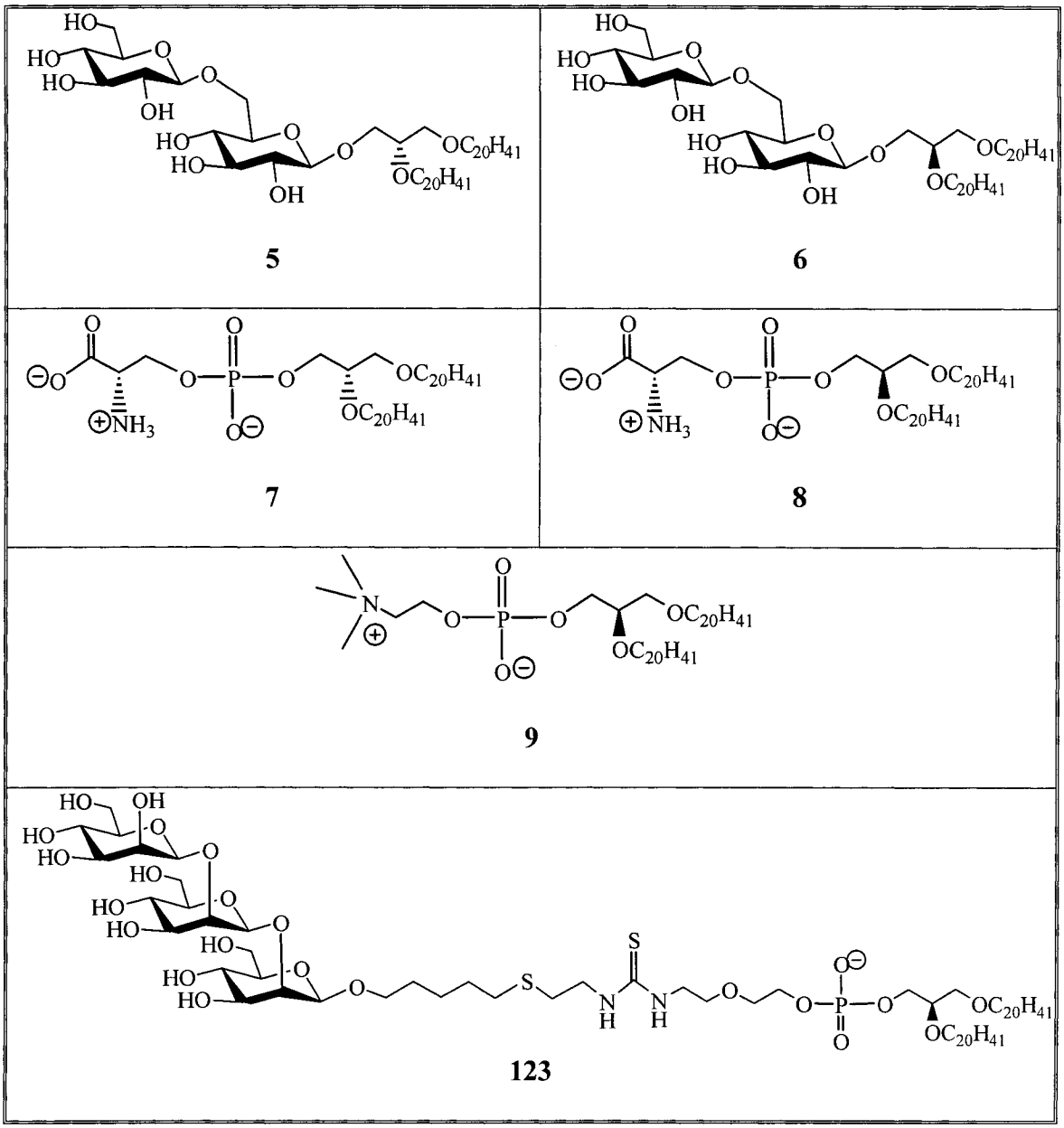
Immunological Studies of the Synthetic Lipids

5.1 Immunostimulatory Properties of Synthetic Lipids, 5-9 and 123.

The adjuvant properties of compounds of synthetic compounds **5-9** and **123** (Table 5.1) were assessed by quantifying the humoral and adaptive immune response determined by the presence of IgM antibody and subsequent class switch to the IgG isotype. The antibody response was measured for the protein, ovalbumin, which was encapsulated within the liposome aqueous core.

Groups of 4 to 7 BALB/c mice were immunized by combined subcutaneous (s.c.) and intraperitoneal (i.p.) routes with different liposome formulations according to those listed in Table 5.2. The sera of the mice were assayed for IgM and IgG antibodies specific for the protein antigen. This was done by an indirect titration ELISA assay. The protein antigen was coated in a 96 well plate and incubated with different dilutions of sera. The ovalbumin specific antibody present within the sera bound to the immolibilized protein antigen and this antibody-protein complex was then quantified by binding to commercially available secondary antibody that is in the form of an antibody-enzyme conjugate. The enzyme mediates the conversion of a substrate, added to each well, into a coloured species. The intensity of colour or absorbance is proportional to the antibody-antigen complex that is bound in the well. This is an indirect approach in the sense that the absorbance is not measured directly from the binding between the primary serum antibody and protein antigen. High absorbance readings at high serum dilutions represents an effective immune response. Generally an OD between

Table 5.1: Synthetic Archaeal Lipids.



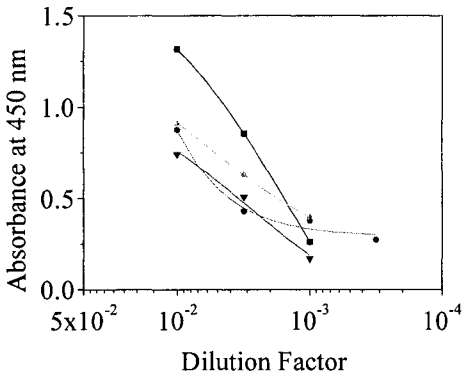
0.100 to 0.200 above background is used as the cutoff for determining a titer of an immune response. Furthermore, the humoral response can be quantified and cell mediated immunity can be verified if there is an IgM to IgG class switch. All figures in this chapter contain a line graph which shows the entire progression of the sera titration

starting from low dilution (1:100) to higher dilution (max 1:1,000,000). The table to the left of the graph shows a comparison of pre- and post-immunization sera at the same dilution factor. If the prebleed A_{450} is less than 0.100 then it was concluded that the mouse has not been pre-exposed to the antigen.

Table 5.2: Liposome Formulations for Adjuvanticity Experiments.

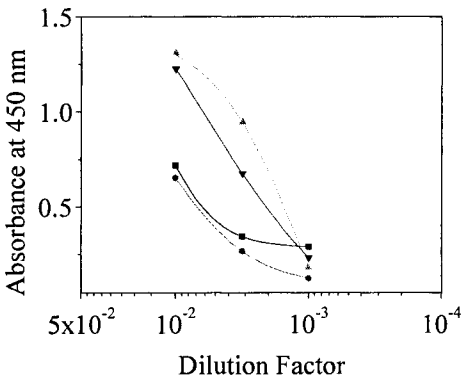
Formulation	Archaea Component	Other Components	Antigen
I	NA	Freund's Adjuvant	Ovalbumin
II	NA	No Adjuvant	Ovalbumin
III	NA	DSPC, PC, Cholesterol	Ovalbumin
IV	**5**	DSPC, PC, Cholesterol	Ovalbumin
V	**6**	DSPC, PC, Cholesterol	Ovalbumin
VI	**6** and **8**	DSPC, Cholesterol	Ovalbumin
VII	**8** and **9**	DSPC, Cholesterol	Ovalbumin
VIII	**7**	DSPC, Cholesterol	Ovalbumin
IX	**8**	DSPC, Cholesterol	Ovalbumin
X	**123**	DSPC, Cholesterol	Ovalbumin

In the interest of having a thorough investigation, three control experiments were undertaken. These control studies included ovalbumin without adjuvant, ovalbumin with Freund's adjuvant and ovalbumin encapsulated by liposomes constructed from conventional ester lipids. Figure 5.1 outlines the immune response obtained from the three control immunizations. One would expect to obtain a better response from the Freund's adjuvant formulation, however both the IgM responses to the OVA without adjuvant and OVA with Freund's adjuvant are very similar (Figure 5.1, A and B). The



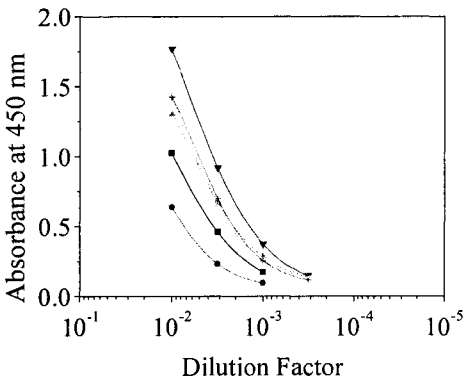
A)

Mouse	OD of Pre-immunization Sera at 1:1,000	OD of Post-immunization Sera at 1:1,000
■	0.075	0.263
♦	0.208	0.379
▲	0.204	0.396
▼	0.140	0.171



B)

Mouse	OD of Pre-immunization Sera at 1:1,000	OD of Post-immunization Sera at 1:1,000
■	0.101	0.291
♦	0.136	0.127
▲	0.099	0.183
▼	0.098	0.233



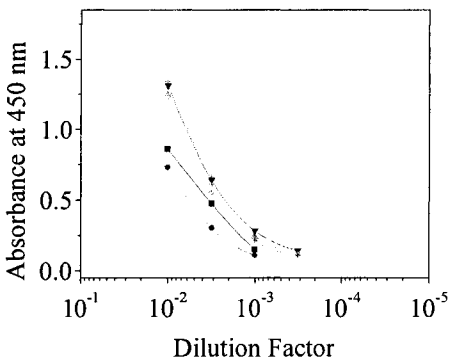
C)

Mouse	OD of Pre-immunization Sera at 1:100	OD of Post-immunization Sera at 1:100
■	0.894	1.026
♦	0.621	0.640
▲	1.061	1.305
▼	1.489	1.770
◊	1.293	1.428
+	1.449	1.426
	1.549	1.703

Figure 5.1: A) IgM titration against OVA injected with Freund's adjuvant (Formulation *I*). B) IgM titration against OVA injected without adjuvant (Formualtion *II*). C) IgM titration against OVA encapsulated in conventional liposomes (Formulation *III*).

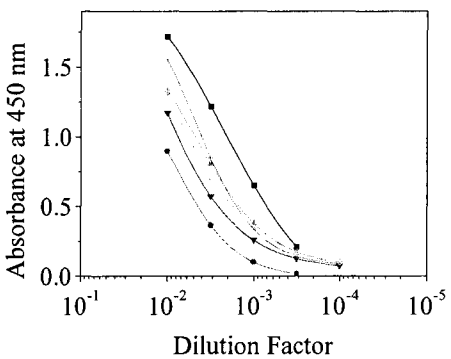
minimum OD was reached at a dilution just below 1:1,000. The response to OVA encapsulated in conventional liposomes was difficult to ascertain. The OD of the prebleed antibody titers were very high and there was effectively no antibody response to this formulation.

The first synthetic compounds to be tested for their immunostimulatory capability were **5** and **6**, the two gentiobiosyl glycerolipids. No attempts were made to formulate liposomes strictly from these lipids. The two compounds instead were mixed with conventional phospholipids and liposomes were formed after hydration of the lipid film



A)

Mouse	OD of Pre-immunization Sera at 1:100	OD of Post-immunization Sera at 1:100
■	0.809	0.862
◆	1.259	0.734
▲	1.162	1.249
▼	0.758	1.313
●	1.106	1.232
+	1.133	1.342
	1.525	1.705



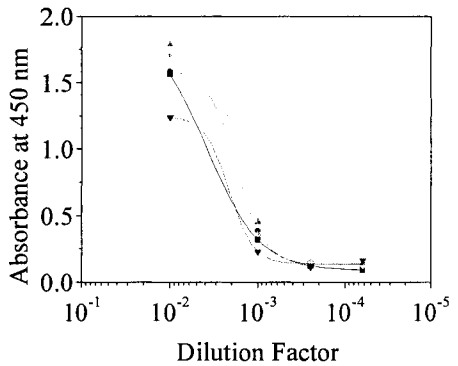
B)

Mouse	OD of Pre-immunization Sera at 1:100	OD of Post-immunization Sera at 1:100
■	1.730	1.715
◆	0.883	0.898
▲	1.169	1.334
▼	1.110	1.173
●	0.967	1.556
+	1.126	1.320
	1.030	1.446

Figure 5.2: A) IgM titration against OVA encapsulated in a mixture of **5** and conventional phospholipids (Formulation *IV*) B) IgM titration against OVA encapsulated in a mixture of **6** and conventional phospholipids (Formulation *V*)

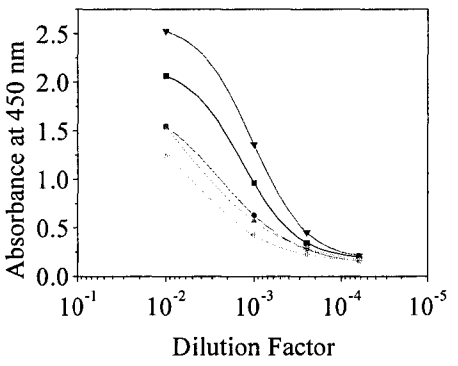
with a buffer solution. In general, there was no observation of adjuvanicity toward the encapsulated antigen in these liposomal formulations. Not only are the pre-immunization antibody titers very high but the immune response observed is much less pronounced than the control immunization without adjuvant. Elevated pre-immunization titers in Figure 5.1C, 5.2A, and 5.2B are attributed to the age of the mice and not to the lack of blocking (controls did not develop a colorimeric reaction) during the ELISA protocol.

In an attempt to learn more about how the variation of lipid composition affects adjuvanticity toward the encapsulated antigen, separate liposome formulations consisting of different types of Archaea lipids were used. Formulations composed of compounds **6** and **9** mixed with **8** (Formulations *VI* and *VII*) and formulations consisting only of compounds **7** and **8** (Formulations *VIII* and *IX*) were used. First attempts involved formulating liposomes from these components excluding conventional ester phospholipids. However, these formulations did not form lamellar structures as determined by microscopy, instead the hydrated lipid formed aggregates. Even though it appears Sprott *et al.* had little difficulty forming liposomes from Archaea lipids, literature reports have suggested that total polar lipid extracts from specific species form liposomes only when there was a 25 mol% inclusion of conventional phospholipids.[119] The lipid hydration was repeated as before with 35-40 mol% DSPC. With this conventional lipid within the lipid formulations *VI-IX*, hydration proceeded smoothly and the lipid suspension was much easier to extrude. The immune response to the OVA entrapped within the liposome mixtures *VI* and *VII* was only of the IgM isotype with no class switch to IgG. In addition, the IgM immune response when compared to the prebleed titers was not very potent (Figure 5.3).



A)

Mouse	OD of Pre-immunization Sera at 1:1,000	OD of Post-immunization Sera at 1:1,000
■	0.242	0.321
♦	0.342	0.389
▲	0.258	0.455
▼	0.498	0.231
●	0.188	0.364



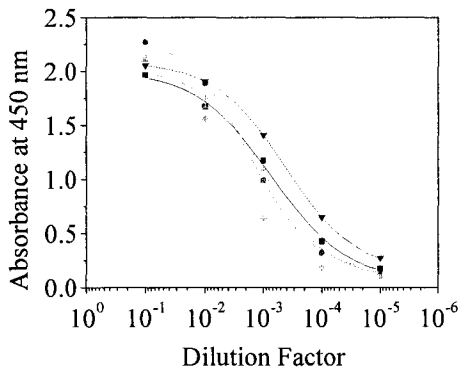
B)

Mouse	OD of Pre-immunization Sera at 1:1,000	OD of Post-immunization Sera at 1:1,000
■	0.362	0.957
♦	0.999	0.632
▲	0.406	0.571
▼	0.972	1.359
●	0.373	0.428

Figure 5.3: A) IgM titration against OVA encapsulated in liposomes containing a mixture of **6, 8** and conventional phospholipids. (Formulation *VI*) B) IgM titration against OVA encapsulated in liposomes containing a mixture of **8, 9** and conventional phospholipids. (Formulation *VII*)

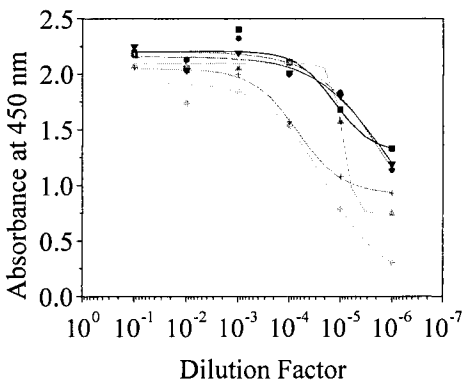
The next set of liposome immunizations consisted of an Archaea component composed entirely of archaetidylserine, **7** or **8**. In similar fashion as before, separate formulations containing compounds **7** or **8** were mixed with DSPC and cholesterol. The liposome mixture was formed during hydration with PBS buffer. The immune response from both of these formulations was very potent. The IgM response (Figure 5.4 A and Figure 5.5 A for **7** and **8**) titred for both formulations out to a serum dilution of 1:10,000

to 1:100,000 whereas the IgG class response was much greater (Figure 5.4 B), the 1:1,000 post immunization sera was substantially higher than that for the 1:1,000 pre-immunization sera. Furthermore, the titre endpoint had not been reached for 5 of the 6 mice at a dilution of 1:1,000,000. Likewise, ELISA results for the IgG antibody response for compound **8** were similar (Figure 5.5 B).



Mouse	OD of Pre-immunization Sera at 1:1,000	OD of Post-immunization Sera at 1:1,000
■	0.101	1.176
◆	0.126	0.996
▲	0.305	0.987
▼	0.155	1.412
◇	0.289	0.640
+	0.103	1.091

A)



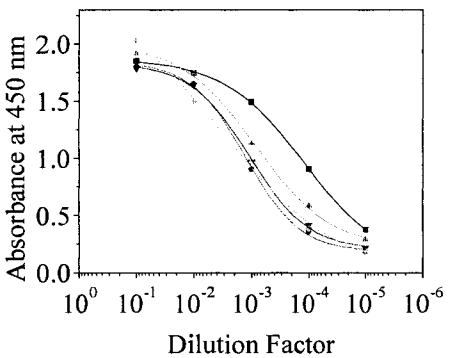
Mouse	OD of Pre-immunization Sera at 1:1,000	OD of Post-immunization Sera at 1:1,000
■	0.060	2.404
◆	0.064	2.323
▲	0.069	2.056
▼	0.060	2.191
◇	0.080	1.845
+	0.063	2.000

B)

Figure 5.4: A) IgM titration against OVA encapsulated in a mixture of **7** and conventional phospholipids. (Formulation *VIII*) B) IgG titration against OVA encapsulated in a mixture of **7** and conventional phospholipids. (Formulation *VIII*)

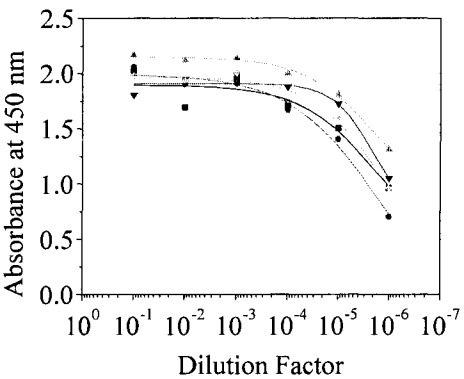
This implies that both glycerol epimers **7** and **8** elicit the same immune response, which means that the stereochemistry of glycerol has no affect on the adjuvanticity

towards the encapsulated ovalbumin. It is also worth noting the difference in immune response between formulation *IX* and formulations *VI* and *VII*. With the inclusion of compounds **6** and **9** with **8** (formulations *VI* and *VII*) the immune response dropped drastically with only a very weak IgM response (previous study, Figure 5.3). Whereas, when the archaeol lipid component of the liposome formulation consisted of 100% archaetidylserine, there was an initial IgM response with a strong class switch to the IgG phenotype.



A)

Mouse	OD of Pre-immunization Sera at 1:1,000	OD of Post-immunization Sera at 1:1,000
■	0.333	1.495
◆	0.121	0.907
▲	0.119	1.136
▼	0.241	0.972
✳	0.238	0.967

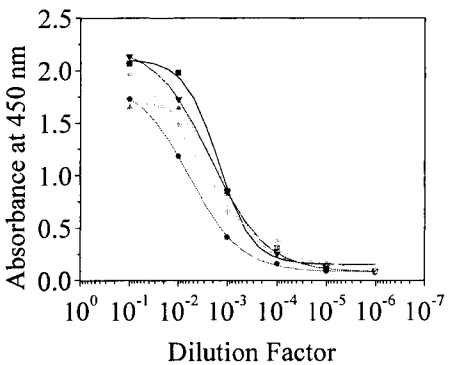


B)

Mouse	OD of Pre-immunization Sera at 1:1,000	OD of Post-immunization Sera at 1:1,000
■	0.085	1.951
◆	0.073	1.910
▲	0.086	2.143
▼	0.079	1.989
✳	0.086	2.008

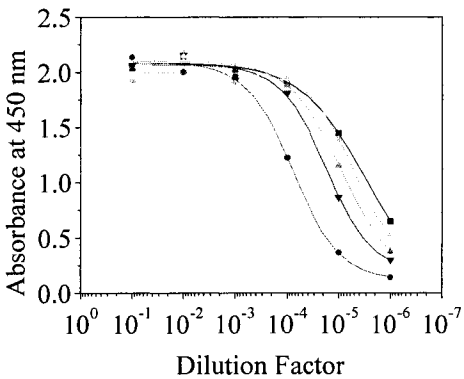
Figure 5.5: A) IgM titration against OVA encapsulated in a mixture of **8** and conventional phospholipids. (Formulation *IX*) B) IgG titration against OVA encapsulated in a mixture of **8** and conventional phospholipids. (Formulation *IX*)

Compound **123** in 30-35 mol% and distearoylphosphocholine in 65-70 mol% were formulated into liposomes that encapsulated ovalbumin and passed through a 100 nm polycarbonate membrane. The murine antisera were tested by ELISA for the response to ovalbumin and to the β-mannan trisaccharide found in the neoglycolipid, **123**. First, the immune response toward the ovalbumin was quite impressive. Not only is the IgM reponse to ovalbumin encapsulated in liposomes containing **123** far superior, but so is the IgG response. This is quite remarkable, considering the very weak IgM immune



A)

Mouse	OD of Pre-immunization Sera at 1:1,000	OD of Post-immunization Sera at 1:1,000
■	0.282	0.853
♦	0.236	0.417
▲	0.119	0.822
▼	0.144	0.848
●	0.187	0.657

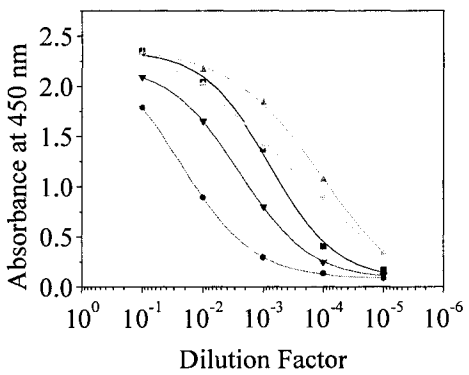


B)

Mouse	OD of Pre-immunization Sera at 1:1,000	OD of Post-immunization Sera at 1:1,000
■	0.075	2.027
♦	0.071	1.966
▲	0.069	2.043
▼	0.064	1.930
●	0.068	1.910

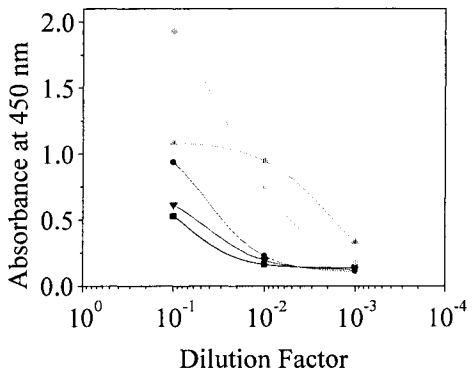
Figure 5.6: A) IgM titration against OVA encapsulated in liposomes containing **123**. (Formulation *X*) B) IgG titration against OVA encapsulated in liposomes containing **123**. (Formulation *X*)

response obtained for formulations containing **5** and **6** and for conventional liposomes. The IgG response was still detectable at dilutions of 1:1,000,000 (Figure 5.6 B). This data suggests that when the β-mannan glycolipid **123** is incorporated into a liposome formulation consisting predominantly of ester lipids, it increases the immune response to encapsulated antigen to a far superior degree when compared to the control conventional liposomes.



Mouse	OD of Pre-immunization Sera at 1:1,000	OD of Post-immunization Sera at 1:1,000
■	0.148	1.377
♦	0.205	0.297
▲	0.099	1.842
▼	0.086	0.800
●	0.084	1.406

A)



Mouse	OD of Pre-immunization Sera at 1:1,000	OD of Post-immunization Sera at 1:1,000
■	0.061	0.136
♦	0.091	0.109
▲	0.115	0.338
▼	0.058	0.124
●	0.054	0.178

B)

Figure 5.7: A) IgM titration against β-mannan trisaccharide/BSA conjugate. (Formulation *X*) B) IgG titration against β-mannan trisaccharide/BSA conjugate. (Formulation *X*)

The last set of ELISA results represent the IgM and IgG response to the trisaccharide component of **123** (Figure 5.7 A and B). The ELISA plates were coated with a β-mannan/BSA conjugate instead of ovalbumin. The results indicate that after one booster immunization there was definitely an IgM antibody reponse. In Figure 5.7 A, the table represents pre- and post-immunization sera and 4 of the 5 mice all show a substantial increased OD at a dilution of 1:1,000 and is sustained to 1:10,000. However, there was no class switch to an IgG isotype as illustrated in Figure 5.7 B.

The above study was undertaken to determine the immunostimulatory capability of the synthetic compounds **5-9** and **123**. The outcome from immunization of formulations *IV*, *V*, *VIII* and *IX* are not surprising. Our results presented here confirm the observation that archaeosomes rich in serine stimulate a strong immune response to encapsulated antigen whereas those with gentiobiosyl polar head groups have little immunostimulatory capability. However, incorporation of archaetidylserine does not ensure an immunological reaction. The impressive immune response of formulation *IX*, that contained 65 mol% archaetidylserine, was drastically diminished when part of the archaetidylserine component was replaced with **6** (Formulation *VI*) and **9** (Formulation *VII*). This observation may be due to membrane physiology. In natural membranes, serine head groups are thought to be oriented inward toward the cytosol. In the case of formulation *IX* there is 65 mol% archaetidylserine **8** included in the lipid composition and with such a high concentration, one may assume that some of the serine head groups will be displayed on the outer surface of the liposome. Therefore, this will enhance receptor-mediated phagocytosis of these formulations. However, in the case of formulations *VI* and *VII* that contain only 30 mol% archaetidylserine, the serine polar head group could be

limited to only the inner membrane which mimics natural non-apoptotic membranes. Because these serine head groups are not displayed on the outer surface there will be no receptor-mediated endocytosis and the cascade of immunological reactions, responsible for the humoral response of formulation *IX*, will not occur.

Structural characteristics of the synthetic archaeol lipid that are not present in the natural lipids, such as (*S*) stereochemistry at C-2 of the glycerol moiety and the racemic C-3 methyl group of the phytanyl alkyl chain, appear to have no detrimental effect on the immunological outcome. Also, reports of the archaeosome delivery system suggest that the vesicles high in the caldarchaeal type lipid are most effective at stimulating a potent immune response. In fact this was not the case in our study, the archaeal lipid is a sufficient structural component to stimulate a substantial humoral response in the case of serine and the β-mannan trisaccharide as polar head groups. In the case of the serine head group, the archaeal lipid is essential for adjuvanticity. Even though it was not in the scope of this study, it has been documented in the literature that liposomes containing phosphatidylserine are poor adjuvants.[120]

The carbohydrate receptor-mediated recognition of liposomes was very successful in the case of **123** however liposomes containing gentiobiosyl glycerolipids **5** and **6** had little adjuvant effect. This was the most potent liposome formulation of all immunizations undertaken in this study. The archaea glycolipid **123** makes up only 35 mol% of the entire lipid component. No other liposome formulation, containing such a small quantity of the archaea component, afforded such a strong humoral response to ovalbumin. Also, the encapsulated antigen dose for this formulation was the smallest when compared to all other formulations, which had between 17 and 25 μg. The initial

immunization provided an ovalbumin dose of 10 μg whereas the booster immunization had 8 μg of antigen per dose. Whether or not this increased adjuvanticity is due to the recognition process of the β-mannan trisaccharide remains to be seen. However, it is encouraging that after one booster immunization there was a moderate IgM response to the trisaccharide antigen.

Conclusion

The chemical synthesis of natural and non-natural archaeal lipids has been described including one containing a β-mannan trisaccharide antigen. Liposomes consisting of the two synthetic serine based lipids **7** and **8** and the β-mannose conjugate **123** were the only archaeal lipid component to have immunostimulatory capability superior to Freund's adjuvant and liposomes consisting of conventional ester phospholipids. We have identified that there is T-cell involvement with Ig class switch however further immunological profiling would give us a better idea of the extent of a cell-mediated (Th1) immune response. Our preliminary results suggest that liposome formulations *VIII*, *IX* and *X*, which incorporate the lipids **7**, **8** or **123** appear to be the most useful as adjuvants and warrant further attention. These experiments were carried out using a model antigen, ovalbumin, therefore to further validate this adjuvant system a biologically relevent antigen, such as a synthetic saccharide conjugate, should be tested. Future experiments would include using liposome formulations *VIII*, *IX* or *X* in immunizations involving glycoconjugates developed for a *C. albicans* vaccine[64,121] and comparing the response to vaccines formulated with conventional adjuvants such as Freund's or Alum. As before murine sera would then be evaluated for IgM and IgG antibodies against the carbohydrate antigen.

Chapter 6

Experimental Section

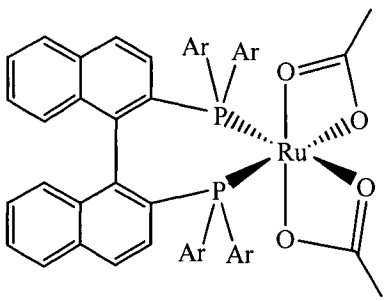
6.1 General Experimental:

All chemicals were of analytical grade and were used without further purification with the exception of *N*-bromosuccinimide (recrystallized from 100% acetone). All solvents were dried according to literature protocols and used without further purification. All molecular sieves, stir bars and round bottom flasks were dried under a flame and then cooled *in vacuo*. The IR-200 (OH^-) resin used in the preparation of **123** was made using IR-200 (Cl^-). The resin was washed 2M NaOH (300 mL), distilled water (300 mL or until neutral pH), and then MeOH (200 mL). This resin would last for about 2 weeks and was used for only one application. Analytical thin layer chromatography (TLC) was completed on silica gel 60-F_{254}. Plates were visualized using UV and/or 5% sulphuric acid in EtOH, molybdate or ninhydrin stain. Medium pressure chromatography was preformed using silica gel (230-400 mesh) or Iatrobeads at a flow rate 5-10 mL/min. Sephadex LH-20 was pretreated with a 50:50 mixture of dichloromethane:methanol for 8 hrs in a sealed container. HPLC was performed using a Waters 600 Delta system on both normal and reversed-phase columns with UV absorbance detector. Compounds were eluted from normal phase column using 9% MeOH in dichloromethane and a combination of MeOH in water from reversed phase column.

^1H NMR was recorded at 400, 500 and 600 MHz and ^{13}C NMR and ^{31}P spectra recorded at 125 MHz and 202.3 MHz, respectively. Spectra were calibrated to residual solvent signals for $CDCl_3$ (δ_H 7.24 ppm, δ_C 77.0 ppm), CD_3OD (δ_H 3.30 ppm, δ_C 49.00

ppm) and D_2O (reference to 0.1% external acetone δ_H 2.225 ppm, δ_C 31.07 ppm). The tentative assignment of resonances for all compounds was made with the help of two dimensional homonuclear and heteronuclear chemical shift correlation experiments. Electro-spray mass spectroscopy and microanalyses were preformed by the analytical services of this department. Optical rotations were measured using a Perkin Elmer 241 polarimeter for samples in 10 cm cell at 22 ± 2 °C. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

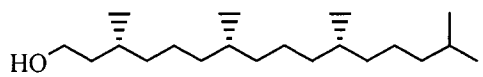
[(S)-2,2'-Bis(diphenylphosphino)-1,1'-binapthyl]diacetatoruthenium(II) ***65***



The procedure used was analogous to that described in *Inorganic Chemistry*, 1988, 27, 3, 567. Working in a glove box, $[RuCl_2(cod)]_n$ (0.45 g, 1.60 mmol) and *S*-BINAP (1.0789 g, 1.72 mmol) was added to a Schlenk flask. The flask was then placed under argon and andydrous toluene (45 mL) and triethylamine (0.957 mL, 6.88 mmol) were added. The solution was then heated at reflux for 14 h. The resulting solution was cooled to room temperature and concentrated under reduced pressure. The brown solid was dissolved in dry dichloromethane (40 mL) and insoluble impurities were removed by filtration through Celite 545. Anhydrous sodium acetate (0.8 g) was added to the dichloromethane solution followed by *tert*-butyl alcohol (100 mL). Under argon, the solution was heated at reflux for 10 h. After cooling to room temperature, the solution was concentrated and extracted with diethyl ether (2 x 30 mL). The diethyl ether solution containing the crude

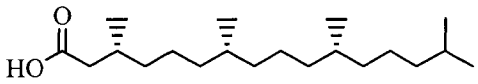
catalyst was concentrated and extracted with 100% ethanol (2 x 30 mL). Evaporation of the ethanol yielded a orange-brown solid. The solid was recrystallized from of toluene and hexanes. The recrystallized product was used for the catalytic hydrogenation reactions. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (m, 5H, Ar), 7.45 (m, 15H, Ar), 7.20 (m, 4H, Ar), 7.08 (m, 5H, Ar), 6.88 (m, 3H, Ar), 6.62 (m, 2H, Ar), 6.50 (m, 6H, Ar), 1.79 (s, 6H, OCOC$\underline{H_3}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.02, 138.11, 134.65, 134.61, 134.56, 133.65, 133.60, 133.58, 133.55, 133.13, 129.57, 129.56, 128.05, 128.01, 127.96, 127.86, 127.83, 127.81, 127.77, 127.72, 127.65, 126.90, 126.87, 126.85, 126.80, 126.03, 125.15, 23.49; ^{31}P NMR (162 MHz, $CDCl_3$) δ 65.56.

*(3*R/S*,7*R*,11*R*)-3,7,11,15-Tetramethylhexadecan-1-ol* ***66***



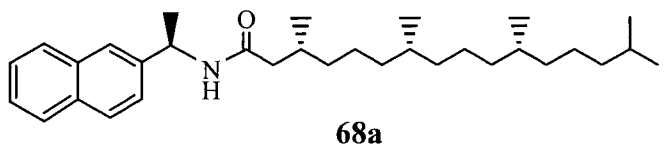
The *E/Z* isomer mixture of phytol was partially separated on silica gel column (8:1 hexane:diethyl ether). *E*-phytol (0.956, 3.23 mmol) was mixed with methanol (10 mL) and **65** was added. The reaction solution was then bubbled with H_2 gas and then placed under an atmosphere of H_2 gas (1600 psi). After 4 d, the reaction was co-concentrated with toluene and purified by column chromatography (8:1 hexane:diethyl ether) to yield a clear liquid (0.925 g, 96%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.73-3.63 (m, 2H, HO-C$\underline{H_2}$-alkyl), 1.65-1.48 (m, 3H, HO-CH_2-$\underline{\text{alkyl}}$), 1.43-1.02 (m, 22H, HO-CH_2-$\underline{\text{alkyl}}$), 0.91-0.83 (m, 15H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 61.28, 40.07, 39.99, 39.37, 37.49, 37.45, 37.39, 37.33, 37.29, 32.79, 29.54, 27.97, 24.80, 24.48, 24.46, 24.37, 22.71, 22.62, 19.75, 19.69, 19.62; HRMS calcd. for $C_{21}H_{44}O_3S$: 298.3236; found (M-H^+) 297.3173.

*(3*R/S*,7*R*,11*R*)-3,7,11,15-Tetramethylhexadecanoic acid* ***67***

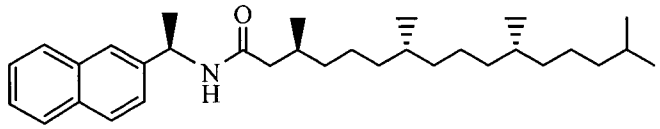


To a solution of **66** (416 mg, 1.39 mmol) in acetic acid/acetone (1:2 ratio, 30 mL) was added chromium trioxide (293 mg, 2.93 mmol, 2.1 eq) in water slowly over 15 minutes. The reaction was stirred for 3 hours and then diluted with water. Sodium metabisulfate was added to the solution to destroy the chromium trioxide. The mixture was extracted with diethyl ether (10 mL). The organic layer was dried over sodium sulphate and concentrated under reduced pressure. The resulting liquid was purified on silica gel and eluted with 4:1 hexane:diethyl ether to give **67** as a clear liquid. (309 mg, 71%) ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.34 (ddd, 1H, HO_2C-CHa, J = 2.9, 5.9, 15.0 Hz), 2.12 (ddd, 1H, HO_2C-CHb, J = 3.2, 8.2, 14.9 Hz), 1.94 (sextet, 1H, HO_2C-CH_2-CHa), 1.51 (septet, 1H, HO_2C-CH_2-CHb), 1.4-1.0 (m, 22H, O-CH_2-alkyl), 0.96-0.94 (m, 5H, CH_3), 0.86-0.84 (m, 10H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.83, 41.65, 41.58, 39.35, 37.42, 37.37, 37.34, 37.29, 37.27, 37.03, 37.00, 36.96, 32.77, 32.75, 32.72, 30.16, 30.14, 27.95, 24.78, 24.77, 24.44, 24.43, 24.30, 22.68, 22.59, 19.72, 19.70, 19.65, 19.63; HRMS calcd. for $C_{20}H_{40}O_2$: 312.3028; found 312.3030.

(3R,7R,11R)-3,7,11,15-Tetramethyl-N-[(1'R)-1'-(naphthyl)ethyl]hexadecanamide ***68a*** *and (3S,7R,11R)-3,7,11,15-Tetramethyl-N-[(1'R)-1'-(naphthyl) ethyl]hexadecanamide* ***68b***



68a

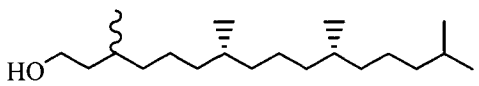


68b

To a solution of compound **67** (85 mg, 0.27 mmol) in diethyl ether under an argon atmosphere was added dimethylformamide (12 μL) and oxalyl chloride (37 μL, 0.30 mmol). The reaction was stirred for 45 min and then concentrated under reduced pressure. After drying *in vacuo*, the crude acid chloride was dissolved in chloroform (1.0 mL) under argon, (*R*)-(+)-1-(1-naphthyl)ethylamine in a solution of chloroform (0.5 mL) and triethylamine (29 μL) was added slowly to the reaction mixture. After 1 h the solvent was removed and the yellow paste was dissolved in hexanes. The insoluble material was removed by filtration through Celite 545. The filtrate was concentrated and the crude product was purified by normal phase HPLC (12:1 to 7:1 hexane:ethyl acetate) to obtain pure **68a** and **68b**. **68a** (96 mg, 76%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, 1H, Ar , J = 8.7 Hz), 7.87 (d, 1H, Ar , J = 8.7 Hz), 7.80 (d, 1H, Ar , J = 8.2 Hz), 7.56-7.43 (m, 4H, Ar), 5.97 (quintet, 1H, Nap-C<u>H</u>(CH_3)-NH), 5.65 (d, 1H, Nap-CH(CH_3)-N<u>H</u>), 2.17 (ddd, 1H, HO_2C-C<u>Ha</u>, J = 2.4, 5.7, 13.5 Hz), 2.02-1.94 (m, 1H), 1.90 (ddd, 1H, HO_2C-C<u>Hb</u>, J = 2.3, 8.1, 13.5 Hz), 1.68 (d, 1H, Nap-CH(C<u>H$_3$</u>)-NH, J = 6.9 Hz), 1.54 (septet, 1H), 1.40-1.02 (m, 22H, O-CH_2-<u>alkyl</u>), 0.90-0.84 (m, 15H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.39, 138.27, 133.93, 131.19, 128.70, 128.38, 126.52, 125.89, 125.10, 123.62, 122.54, 44.71, 44.64, 44.39, 39.38, 38.16, 37.46, 37.42, 37.40, 37.31, 37.17, 37.14, 32.78, 31.24, 30.86, 29.70, 27.98, 24.82, 24.80, 24.48, 24.41, 24.40, 22.72, 22.63, 20.52, 19.76, 19.72, 19.69, 19.66; HRMS calcd. for $C_{32}H_{51}NO$: 465.3971; found 465.3963. **68b** (6 mg, 4.8 %): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, 1H, Ar , J = 8.69 Hz), 7.87 (d, 1H, Ar , J = 8.43 Hz), 7.81 (d, 1H, Ar , J = 7.90 Hz), 7.56-7.43 (m, 4H, Ar), 5.97 (pentet, 1H, Nap-C<u>H</u>(CH_3)-NH), 5.59 (d, 1H, Nap-CH(CH_3)-N<u>H</u>), 2.17 (ddd, 1H, HO_2C-C<u>Ha</u>, J = 2.77, 5.83, 13.57 Hz), 2.02-1.94 (m, 1H), 1.90 (ddd, 1H, HO_2C-C<u>Hb</u>, J =

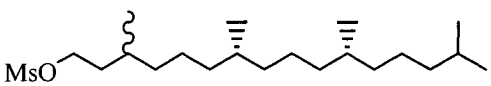
2.48, 8.32, 13.57 Hz), 1.69 (d, 1H, Nap-CH(C<u>H</u>$_3$)-NH, J = 6.87 Hz), 1.59-1.50 (m, 1H), 1.40-1.02 (m, 22H, O-CH$_2$-<u>alkyl</u>), 0.90-0.84 (m, 15H, CH$_3$); HRMS calcd. for $C_{32}H_{51}NO$: 465.3971; found 465.3964.

(3R/S,7R,11R)-3,7,11,15-Tetramethylhexadecan-1-ol (C3 Racemic) ***69***



To the E/Z isomer mixture of phytol (6.39 g, 21.6 mmol) in ethanol was added Raney Ni (2 mL, water solution). The reaction solution was bubbled with H_2 gas and then placed under an atmosphere of H_2 (3 psi). After 24 h, the solution was co-concentrated with toluene and purified by column chromatography (8:1 hexane:diethyl ether) to yield a clear liquid (6.31 g, 98%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.73-3.63 (m, 2H, HO-C<u>H</u>$_2$-alkyl), 1.65-1.48 (m, 3H, HO-CH$_2$-<u>alkyl</u>), 1.43-1.02 (m, 22H, HO-CH$_2$-<u>alkyl</u>), 0.91-0.83 (m, 15H, CH$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 61.28, 40.07, 39.99, 39.37, 37.49, 37.45, 37.39, 37.33, 37.31, 37.29, 32.79, 29.54, 29.52, 27.97, 24.80, 24.46, 24.37, 22.71, 22.62, 19.75, 19.69; HRMS HRMS calcd. for $C_{20}H_{41}O$: 376.3011; found (M-H$^+$) 374.2854. HRMS calcd. for $C_{21}H_{44}O_3S$: 298.3236; found (M-H) 297.3163.

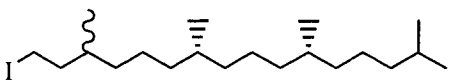
*1-Methanesulfonic acid-(3*R/S*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl ester 71*



To a 0 °C solution of **69** (16.25 g, 54.4 mmol) in pyridine (75 mL) and dichloromethane (75 mL) was added methanesulfonyl chloride (4.63 ml, 59.8 mmol) slowly over 30 min. The reaction mixture was stirred for an additional 45 min and the reaction was complete. The solution was concentrated and redissolved in dichloromethane, washed with 2M HCl (2 x 50 mL), sodium bicarbonate (20 mL, saturated), water (50 mL), dried over sodium

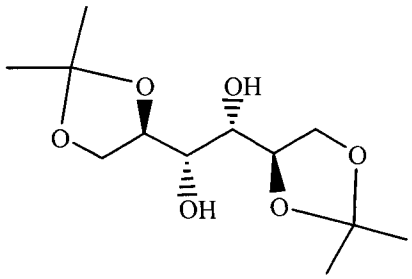
sulphate and concentrated. The product was used without further purification (18.5 g, 90%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.21 (m, 2H, MsO-C<u>H</u>$_2$-alkyl), 2.97 (s, 3H, $CH_{3(mesylate)}$), 1.76 (m, 1H), 1.62-1.44 (m, 3H, O-CH_2-<u>alkyl</u>), 1.40-0.98 (m, 20H, O-CH_2-<u>alkyl</u>), 0.92-0.80 (m, 15H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 68.55, 39.35, 37.41, 37.34, 37.29, 37.26, 37.20, 37.17, 37.09, 37.06, 36.03, 35.97, 32.76, 32.74, 32.72, 29.38, 29.37, 27.94, 24.77, 24.76, 24.45, 24.43, 24.21, 22.68, 22.59, 19.72, 19.65, 19.58, 19.28, 19.22; HRMS calcd. for $C_{21}H_{44}O_3S$: 376.3011; found (M-2H) 374.2854.

*1-Iodo-(3*R/S*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecane* ***72***



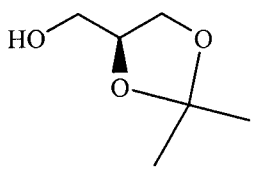
To a 0 °C solution of **69** (2.18 g, 7.3 mmol) in distilled dichloromethane (30 ml) was added imidazole (0.597 g, 8.76 mmol) and triphenylphosphine (2.30 g, 8.76 mmol). Iodine (2.23 g, 8.76 mmol) was added slowly over 30 minutes. After 2 h the reaction solution was diluted with cold hexane and filtered through a Celite plug. The filtrate was concentrated and the crude product was purified on a silica gel column and eluted with 100% hexanes. The purified product was a colourless liquid (2.68 g, 90%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.25 (m, 1H, I-C<u>H</u>$_2$), 3.17 (m, 1H, I-C<u>H</u>$_2$), 1.88 (m, 1H, I-CH_2-C<u>H</u>$_2$), 1.53 (m, 2H), 1.22 (m, 19H), 0.86 (m, 15H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 41.03, 40.97, 39.37, 37.43, 37.38, 37.32, 37.29, 37.24, 37.19, 36.60, 36.54, 33. 90, 33.87, 32.77, 32.74, 27.29, 24.80, 24.47, 24.20, 22.71, 22.62, 19.76, 19.70, 19.64, 18.78, 18.71, 5.31; HRMS calcd. for $C_{20}H_{41}I$: 408.4440; found 408.2260.

*1,2:5,6-Di-O-isopropylidene-*D*-mannitol* ***73***



D-Mannitol (5.0 g, 27.45 mmol) was added to a solution of freshly fused zinc chloride (7.85 g, 57.64 mmol) in acetone (48 mL, 27.45 mmol). After 18 h, water (100 mL) was added followed by sodium carbonate until the solution was basic. The aqueous mixture was then concentrated, diluted with dichloromethane and filtered. The organic layer was then washed with water, dried over sodium sulphate, and concentrated to a white powder. This crude product was chromatographed (1:1 hexanes/ethyl acetate) to give a white solid (2.89 g, 40%); $[\alpha]_D$ - 2.7 (*c* 1.0, DMF); ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.18 (dd, 2H, CH_2-C<u>H</u>-CH(OH), *J* = 6.2 Hz), 4.12 (dd, 2H, C<u>H</u>$_2$-CH-CH(OH), *J* = 6.2, 8.6 Hz), 3.97 (dd, 2H, C<u>H</u>$_2$-CH-CH(OH), *J* = 5.7, 8.6 Hz), 3.75 (ddd, 2H, CH_2-CH-C<u>H</u>(OH), *J* = 2.0, 8.2 Hz), 2.58 (d, 1H, CH_2-CH-CH(O<u>H</u>), *J* = 6.6 Hz), 1.40 (s, 3H, CH_3), 1.34 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 109.31, 76.26, 71.21, 66.74, 26.78, 25.27; ES (Positive) HRMS calcd. for $C_{12}H_{22}O_6Na$ (M + Na^+): 285.1308; found 285.1306; Anal. calcd. for $C_{12}H_{22}O_6$: C, 54.95; H, 8.45; found: C, 54.83; H, 8.61.

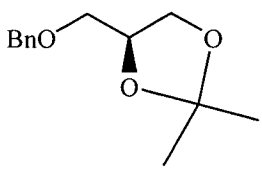
S-*1,2-O-Isopropylidene glycerol* ***74***



The reaction was initiated by slow addition of sodium periodate (2.54 g, 12.1 mmol) to a sodium bicarbonate (0.6 mL, saturated) buffered solution of **73** (1.62 g, 6.18 mmol) in

dichloromethane (16 mL). After 4 h, sodium sulphate was added to the reaction mixture, the solution was filtered and the filter cake successively washed with dichloromethane (4 x 25 mL). The dichloromethane filtrate was then diluted with an equal volume of methanol (100 mL) and the solution was cooled to 0 °C. Sodium borohydride (0.36 g, 6.18 mmol, 1 eq) was added and the reaction was finished after 1 h. The solution was concentrated and diluted with dichloromethane. The organic layer was washed with citric acid (15 mL, 2% w/v), water (10 mL), dried with sodium sulphate, and concentrated under reduced pressure. The crude product was chromatographed (2:1 hexanes/ethyl acetate) to give a clear syrup (1.43 g, 56%) $[\alpha]_D$ + 10 (*c* 1.2, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$) δ 4.23 (dddd, 1H, $CH_{(glycerol)}$, J = 3.7, 5.3, 6.6, 6.6 Hz) 4.12 (dd, 1H, $CH_{2a(glycerol)}$, $J_{CH,CH2a}$ = 6.7 Hz, J_{gem} = 8.2 Hz), 3.78 (dd, 1H, $CH_{2a`(glycerol)}$, $J_{CH,CH2a`}$ = 6.6 Hz, J_{gem} = 8.2 Hz) 3.72 (dd, 1H, $CH_{2b(glycerol)}$, $J_{CH,CH2b}$ = 3.8 Hz, J_{gem} = 11.7 Hz); 3.58 (dd, 1H, $CH_{2b`(glycerol)}$, $J_{CH,CH2b`}$ = 5.3 Hz, J_{gem} = 11.7 Hz), 1.43 (s, 3H, CH_3), 1.36 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 109.40, 76.14, 65.68, 62.99, 26.67, 25.24; ES (Positive) HRMS calcd. for $C_6H_{12}O_3Na$ ($M + Na^+$): 155.0678; found 155.0678.

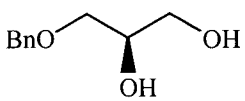
R-*3-O-Benzyl-1,2-O-isopropylidene glycerol* ***75***



Compound **74** (1.08 g, 8.2 mmol) was dissolved in THF (30 mL) and sodium hydride (0.82 mg, 24.5 mmol, 60% oil immersion) was added. After stirring for 30 minutes, benzyl bromide (2.91 mL, 24.5 mmol) was added. The brown solution was then heated at reflux at 66 °C for 4 h. After the addition of methanol (2 mL), the reaction solution

was then concentrated, diluted with dichloromethane (20 mL) and washed with water (30 mL). After drying with sodium sulphate and concentrating the solution, column chromatography (4:1 hexane:ethyl acetate) gave a clear syrup (1.51 g, 83%); $[\alpha]_D$ + 18.4 (*c* 4.4, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.39-7.34 (m, 5H, Ar), 4.58 (app. dd, 2H, $C\underline{H}_2Ph$, J_{gem} = 12.1 Hz), 4.31 (dddd, 1H, $CH_{(glycerol)}$, J = 5.6, 6.3, 6.3, 5.6 Hz), 4.06 (dd, 1H, $CH_{2a(glycerol)}$, $J_{CH,CH2a}$ = 6.4 Hz, J_{gem} = 8.2 Hz), 3.75 (dd, 1H, $CH_{2a'(glycerol)}$, $J_{CH,CH2a'}$ = 6.3 Hz, J_{gem} = 8.2 Hz), 3.57 (dd, 1H, $CH_{2b(glycerol)}$, $J_{CH,CH2b}$ = 5.7 Hz, J_{gem} = 9.9 Hz), 3.48 (dd, 1H, $CH_{2b'(glycerol)}$, $J_{CH,CH2b'}$ = 5.6 Hz, J_{gem} = 9.9 Hz), 1.43 (s, 3H, CH_3), 1.37 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.99, 128.39, 127.72, 127.71, 109.39, 74.76, 73.52, 71.12, 66.90, 26.79, 25.41; ES (Positive) HRMS calcd. for $C_{13}H_{18}O_3Na$ (M + Na^+): 245.1148; found 245.1148.

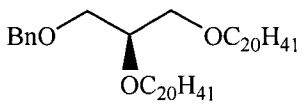
R-*3-O-Benzyl glycerol* ***76***



A solution of **75** (1.35 g) in 80% acetic acid (20 mL, aqueous solution) was heated at 80 °C for 4 hours. The reaction mixture was concentrated and diluted with dichloromethane. The organic layer was washed with sodium bicarbonate (30 mL) and water (30 mL), dried over sodium sulphate and concentrated. The resulting yellow syrup was purified by chromatography (1:1 hexanes:ethyl acetate) to yield a clear syrup (1.05 g, 95%); $[\alpha]_D$ + 4.4 (*c* 6.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.47-7.38 (m, 5H, Ar), 4.51 (s, 2H, $C\underline{H}_2Ph$), 3.89 (dddd, 1H, $CH_{(glycerol)}$, J = 3.8, 4.0, 5.7, 6.3 Hz), 3.69 (dd, 1H, $CH_{2a(glycerol)}$, $J_{CH,CH2a}$ = 3.8 Hz, J_{gem} = 11.4 Hz), 3.62 (dd, 1H, $CH_{2a'(glycerol)}$, $J_{CH,CH2a'}$ = 5.7 Hz, J_{gem} = 11.4 Hz), 3.57 (dd, 1H, $CH_{2b(glycerol)}$, $J_{CH,CH2b}$ = 4.0 Hz, J_{gem} = 9.7 Hz), 3.48 (dd, 1H,

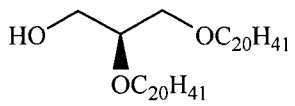
$CH_{2b'(glycerol)}$, $J_{CH,CH2b'}$ = 6.2 Hz, J_{gem} = 9.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.66, 128.50, 127.90, 127.79, 73.57, 71.76, 70.67, 64.04; ES (Positive) HRMS calcd. for $C_{10}H_{14}O_3Na$ ($M + Na^+$): 205.0835; found 205.0835; Anal. calcd. for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74; found: C, 65.76; H, 7.76

R-*3-O-Benzyl-1,2-di-O-[(3*R/S*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl] glycerol 77*



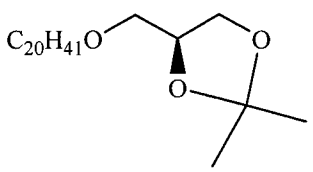
Compounds **76** (36 mg, 0.2 mmol) and **72** (0.329 g, 0.81 mmol) were dissolved in toluene (20 mL). Powdered KOH (0.68 g, 1.2 mmol) was added to the reaction and it was heated to 125 °C. The reaction mixture was heated at reflux for 4 d with azeotropic removal of water using a Dean-Stark trap. The mixture was concentrated, dichloromethane was added and the solution was washed with water (30 mL). After drying over sodium sulphate and concentration, the crude product was subjected to column chromatography (50:1 hexanes:ethyl acetate) to yield **77** as a clear syrup (104.6 mg, 69%); ^1H NMR (600 MHz, $CDCl_3$) δ 7.33-7.23 (m, 5H, Ar), 4.54 (s, 2H, $C\underline{H_2}Ph$), 3.65-3.41 (m, 9H, $CH_{(glycerol)}$, $CH_{2(glycerol)}$, 2 x O-$C\underline{H_2}$-alkyl), 1.64-1.00 (m, 48H, O-CH_2-alkyl), 0.88-0.81 (m, 30H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.44, 128.27, 127.54, 127.46, 77.96, 73.35, 70.83, 70.35, 69.96, 68.87, 39.36, 37.50, 37.45, 37.39, 37.28, 37.18, 37.10, 36.73, 36.65, 32.78, 29.89, 29.82, 27.95, 24.78, 24.47, 24.36, 22.69, 22.60, 19.73, 19.67, 19.62; ES (Positive) HRMS calcd. for $C_{50}H_{94}O_3Na$ ($M + Na^+$): 765.7095; found 765.7099; Anal. calcd. for $C_{50}H_{94}O_3$: C, 80.80; H, 12.75; found: C, 80.62; H, 12.94

S-*1,2-Di-O-[(3*R/S*,7*R*,11*R*)-3,7,11,15-Tetramethylhexadecyl] glycerol **78***



Hydrogenation of **77** (95 mg, 0.13 mmol) in ethyl acetate (3 mL) proceeded over palladium hydroxide (20 mg) under H_2 atmosphere. The reaction was complete after 3 h and the solution was filtered through a 0.45 μm filter and concentrated. The crude product was then purified by column chromatography (30:1 hexanes:ethyl acetate) to give a clear syrup (79 mg, 94%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.74-3.42 (m, 9H, $CH_{(glycerol)}$, $CH_{2(glycerol)}$, 2 x O-C<u>H</u>$_2$-alkyl), 2.14 (br. s, 1H, O<u>H</u>), 1.66-1.44 (m, 6H, O-CH_2-<u>alkyl</u>), 1.42-0.98 (m, 42H, O-CH_2-<u>alkyl</u>), 0.88-0.80 (m, 30H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 78.27, 70.97, 70.12, 68.61, 63.10, 39.34, 37.46, 37.42, 37.37, 37.32, 37.30, 37.26, 37.13, 37.05, 36.65, 36.57, 32.76, 32.75, 29.86, 29.83, 29.81, 27.94, 24.77, 24.45, 24.44, 24.33, 22.68, 22.59, 19.72, 19.65, 19.60, 19.59; ES (Positive) HRMS calcd. for $C_{43}H_{88}O_3Na$ ($M + Na^+$): 675.6625; found 675.6620; Anal. calcd. for $C_{43}H_{88}O_3$: C, 79.07; H, 13.58; found: C, 78.89; H, 13.63.

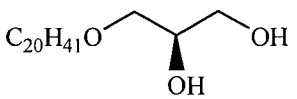
R-*3-O-[(3*R/S*,7*R*,11*R*)-3,7,11,15-Tetramethylhexadecyl]-1,2-O-isopropylidene glycerol* ***79***



Compound **74** (0.119 g, 0.876 mmol) was dissolved in DMF (5 mL) and sodium hydride (60 mg, 1.752 mmol, 60% oil immersion) was added. After stirring for 15 min, a solution of **71** (0.445 g, 1.183 mmol, 1.3 eq) and tetrabutylammonium iodide (30 mg) was added. The brown solution was then heated to 60 °C for 3 h. Excess sodium hydride was destroyed with the addition of methanol (0.5 mL) and the reaction solution was

concentrated, diluted in dichloromethane (20 mL) and washed with water (30 mL). After drying with sodium sulphate and concentration under reduced pressure, column chromatography (30:1 hexanes:ethyl acetate) gave a clear syrup (0.29 g, 80%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.26 (quintet, 1H, CH_2-C<u>H</u>-CH_2), 4.05 (dd, 1H, $CH_{2a(glycerol)}$, J = 6.4, 8.2 Hz), 3.73 (dd, 1H, $CH_{2a`(glycerol)}$, $J$ = 6.5, 8.2 Hz,), 3.55-3.39 (m, 4H, $CH_{2b(glycerol)}$, $CH_{2b`(glycerol)}$, O-C<u>H</u>$_2$-alkyl), 1.67-1.59 (m, 1H, O-CH_2-<u>alkyl</u>), 1.52 (septet, 2H, O-CH_2-<u>alkyl</u>), 1.42 (s, 3H, CH_3 acetal), 1.41-1.01 (m, 24H, O-CH_2-<u>alkyl</u>, CH_3 acetal), 0.89-0.83 (m, 15H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 109.31, 74.76, 71.84, 70.19, 66. 97, 39.37, 37.39, 36.60, 36.53, 32.77, 29.87, 27.97, 26.77, 25.42, 24.79, 24.47, 24.35, 22.70, 22.61, 19.68; ES (Positive) HRMS calcd. for $C_{26}H_{52}O_3Na$ ($M + Na^+$): 435.3808; found 435.3808; Anal. calcd. for $C_{26}H_{52}O_3$: C, 75.67; H, 12.70; found: C, 75.54; H, 12.96.

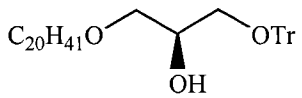
R-*3-O-[(3*R/S,*7*R,*11*R)*-3,7,11,15-Tetramethylhexadecyl]glycerol* **80**



A solution of **79** (0.266 g, 0.64 mmol) in 80% acetic acid (20 mL, aqueous solution) was heated at 90 °C for 4 h. The reaction mixture was concentrated and diluted with dichloromethane. The organic layer was washed with sodium bicarbonate (30 mL) and water (30 mL), dried over sodium sulphate and concentrated. The crude product was chromatographed (6:1 hexanes:ethyl acetate) to yield a clear syrup (0.231 g, 97%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.86 (dddd, 1H, C<u>H</u>$_{(glycerol)}$, J = 3.9, 5.2 Hz), 3.71 (dd, 1H, $CH_{2a(glycerol)}$, $J_{CH,CH2a}$ = 3.9 Hz, J_{gem} = 11.4 Hz), 3.64 (dd, 1H, $CH_{2a`(glycerol)}$, $J_{CH,CH2a`}$ = 5.2 Hz, J_{gem} = 11.4 Hz), 3.55-3.47 (m, 4H, $CH_{2b(glycerol)}$, $CH_{2b`(glycerol)}$, O-C<u>H</u>$_2$-alkyl), 2.42 (s, 2H, OH), 1.67-1.58 (m, 1H, O-CH_2-<u>alkyl</u>), 1.52 (septet, 2H, O-CH_2-<u>alkyl</u>), 1.44-1.02 (m,

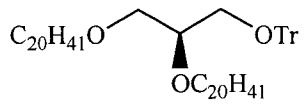
21H, O-CH_2-alkyl), 0.89-0.81 (m, 15H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 72.52, 70.47, 70.16, 64.27, 39.37, 37.49, 37.46, 37.44, 37.43, 37.40, 37.39, 37.34, 37.31, 37.29, 36.64, 36.56, 32.79, 32.77, 29.92, 27.97, 24.80, 24.79, 24.48, 24.46, 24.36, 22.71, 22.61, 19.75, 19.69, 19.63; ES (Positive) HRMS calcd. for $C_{23}H_{49}O_3$ ($M + H^+$): 373.3676; found 373.3678.

S-*3-O-[(3*R/S,*7*R,*11*R)*-3,7,11,15-Tetramethylhexadecyl]-1-O-trityl glycerol* ***81***



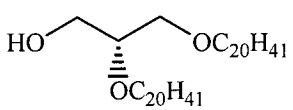
Compound **80** (0.231 g, 0.62 mmol) was dissolved in mixture of pyridine (5 mL) and dichloromethane (5 mL). Trityl chloride (0.259 g, 0.93 mmol) was added and after 2 h the reaction was complete. The solution was concentrated and diluted with dichloromethane. The organic layer was washed with 1M HCl (20 mL), water (20 mL), dried over sodium sulphate and concentrated. Column chromatography (20:1 hexanes:ethyl acetate) yield a clear liquid (328 mg, 86%); ^1H NMR (500 MHz, $CDCl_3$) δ 7.47-7.41 (m, 4H, Ar), 7.35-7.21 (m, 11H, Ar), 3.96 (sextet, 1H, $CH_{(glycerol)}$), 3.58-3.45 (m, 4H, $CH_{2(glycerol)}$, O-$C\underline{H}_2$-alkyl), 3.24-3.17 (m, 2H, $CH_{2(glycerol)}$), 2.20 (d, 1H, CH-O<u>H</u>, *J* = 4.7 Hz), 1.66-1.44 (m, 3H, O-CH_2-alkyl), 1.42-1.02 (m, 21H, O-CH_2-alkyl) 0.90-0.83 (m, 15H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.89, 128.84, 128.68, 127.92, 127.81, 127.24, 127.03, 72.11, 69.95, 69.87, 64.68, 39.38, 37.54, 37.46, 37.41, 37.31, 36.71, 36.63, 29.92, 27.98, 24.49, 24.38, 22.72, 22.63, 19.76, 19.70, 19.66; ES (Positive) HRMS calcd. for $C_{42}H_{62}O_3Na$ ($M + Na^+$): 637.4591; found 637.4590; Anal. calcd. for $C_{42}H_{62}O_3$: C, 82.03; H, 10.16; found: C, 82.26; H, 9.96.

S-*2,3-Di-O-[(3*R/S,*7*R,*11*R)*-3,7,11,15-tetramethylhexadecyl]-1-O-trityl glycerol* ***82***



Compound **81** (0.101 g, 0.165 mmol) was dissolved in DMF (5 mL) and sodium hydride (55 mg, 1.65 mmol, 60% oil immersion) was added. After stirring for 15 min, a solution of **71** (0.109 g, 0.288 mmol, 1.75 eq) and tetrabutylammonium iodide (25 mg) was added. The brown solution was then heated to 75 °C for 24 h. Excess sodium hydride was destroyed by the addition of methanol (0.5 mL) and the reaction solution was concentrated, diluted in dichloromethane (20 mL) and washed with water (30 mL). After drying with sodium sulphate, filtering and concentrating the solution, column chromatography (100:1 hexanes:ethyl acetate) gave a clear syrup (109 mg, 74%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49-7.19 (m, 15H, Ar), 3.64-3.38 (m, 7H, $CH_{2(glycerol)}$, CH $_{(glycerol)}$, 2 x O-CH_2-alkyl), 3.22-3.14 (m, 2H, $CH_{2(glycerol)}$), 1.68-1.00 (m, 48H, O-CH_2-alkyl), 0.92-0.81 (m, 30H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.16, 144.08, 144.06, 128.85, 128.81, 128.74, 128.62, 127.82, 127.79, 127.76, 127.68, 126.85, 78.37, 75.34, 71.26, 69.91, 68.98, 63.72, 39.37, 37.57, 37.48, 37.42, 36.77, 32.81, 29.87, 27.96, 24.80, 24.49, 24.38, 22.71, 22.62, 19.75, 19.69, 19.63; ES (Positive) HRMS calcd. for $C_{62}H_{102}O_3Na$ (M + Na^+): 917.7721; found 917.7726; Anal. calcd. for $C_{62}H_{102}O_3$: C, 83.16; H, 11.48; found: C, 82.90; H, 11.58.

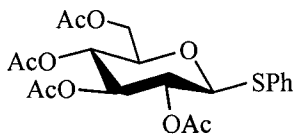
R-*2,3-Di-O-[(3*R/S*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl]glycerol* ***83***



To a solution of **82** (0.368 g, 0.41 mmol) in dichloromethane (5 mL) was added hydrofluoroboric acid (2 mL). The reaction was stirred vigorously for 1 h and methanol

(2 mL) was added. The solution was neutralized with sodium bicarbonate and the organic layer was washed with water (20 mL), dried over sodium sulphate and concentrated. After chromatographic purification (50:1 hexanes:ethyl acetate to 30:1 hexanes:ethyl acetate) the product was obtained as a clear oil (164 mg, 61%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.74-3.42 (m, 9H, $CH_{(glycerol)}$, 2 x $CH_{2(glycerol)}$, 2 x O-C<u>H</u>$_2$-alkyl), 2.14 (br. s, 1H, OH), 1.66-1.44 (m, 6H, O-CH_2-<u>alkyl</u>), 1.42-0.98 (m, 42H, O-CH_2-<u>alkyl</u>), 0.88-0.80 (m, 30H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 78.29, 70.97, 70.16, 68.65, 63.13, 39.37, 37.50, 37.48, 37.46, 37.41, 37.39, 37.36, 37.33, 37.29, 37.12, 37.09, 36.69, 36.61, 32.87, 32.86, 32.79, 32.78, 27.97, 24.80, 24.79, 24.49, 24.48, 24.37, 22.71, 22.62, 19.75, 19.70, 19.68, 19.63, 19.62; ES (Positive) HRMS calcd. for $C_{43}H_{88}O_3Na$ ($M + Na^+$): 675.6625; found 675.6626.

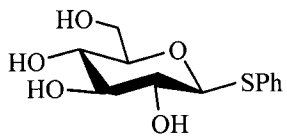
Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside ***87***



Thiophenol (16.29 ml, 158.7 mmol) was added to a 0 °C solution of peracetylated D-glucose **89** (20.64 g, 52.9 mmol) dissolved in distilled dichloromethane (100 mL). Boron trifluoride etherate (20.77 mL, 163.9 mmol) was added dropwise over 20 min. The solution was warmed to room temperature and left for 12 h. The solution was neutralized with triethylamine, concentrated, and diluted with dichloromethane. The organic layer was washed with water (2 x 50 mL), sodium bicarbonate (2 x 50 mL), and then water (50 mL), dried over sodium sulphate, and concentrated under reduced pressure. The syrup was recrystallized (100% hexanes) to afford the product as white crystals (16.24 g, 75%); $[\alpha]_D$ - 16.9 (*c* 2.5, $CHCl_3$): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51-7.48 (m, 2H, Ar), 7.33-

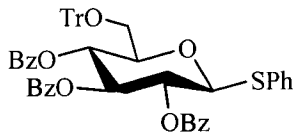
7.30 (m, 3H, Ar), 5.22 (app. t, 1H, H-3, $J_{2,3} = J_{3,4} = 9.3$ Hz), 5.04 (dd, 1H, H-4, $J_{3,4} = 9.5$ Hz, $J_{4,5} = 10.0$ Hz), 4.97 (dd, 1H, H-2, $J_{1,2} = 10.0$ Hz, $J_{2,3} = 9.3$ Hz), 4.71 (d, 1H, H-1, $J_{1,2} = 10.0$ Hz), 4.22 (dd, 1H, H-6b, $J_{5,6} = 5.1$ Hz, $J_{gem} = 12.3$ Hz), 4.18 (dd, 1H, H-6a, $J_{5,6} = 2.6$ Hz, $J_{gem} = 12.3$ Hz), 3.72 (ddd, 1H, H-5, $J_{4,5} = 10.0$ Hz, $J_{5,6a} = 2.6$ Hz, $J_{5,6b} = 5.1$ Hz), 2.08 (s, 3H, $O_2CC\underline{H}_3$), 2.07 (s, 3H, $O_2CC\underline{H}_3$), 2.01 (s, 3H, $O_2CC\underline{H}_3$), 1.98 (s, 3H, $O_2CC\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.51, 170.12, 169.35, 169.20, 133.11, 131.63, 128.91, 85.72, 75.80, 73.96, 69.95, 68.23, 62.14, 20.72, 20.69, 20.56, 20.55; ES (positive) HRMS calcd. for $C_{20}H_{24}O_9SNa$ ($M + Na^+$): 463.1033; found 463.1031.

Phenyl 1-thio-β-D-glucopyranoside ***90***



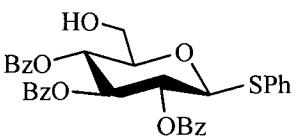
Distilled methanol was added to a flask containing **87** (3.58 g, 8.13 mmol) where upon Na metal (25 mg) was added. After 2 hours, the reaction mixture was neutralized with H+ ion exchange resin, filtered and concentrated. The product was dried under vacuum to give a white powder. This was then recrystalized (100% ethyl acetate) to give white crystals (1.88 g, 85%): $[\alpha]_D$ - 12.9 (*c* 1.3, DMF): ^1H NMR (600 MHz, D_2O) δ 7.59-7.57 (m, 2H, Ar), 7.44-7.37 (m, 3H, Ar), 4.79 (d, 1H, H-1, $J_{1,2} = 9.8$ Hz), 3.89 (dd, 1H, H-6a, $J_{5,6} = 2.3$ Hz, $J_{gem} = 12.4$ Hz), 3.71 (dd, 1H, H-6b, $J_{5,6} = 5.8$ Hz, $J_{gem} = 12.4$ Hz), 3.52 (app. t, 1H, H-3, $J_{2,3} = J_{3,4} = 9.3$ Hz), 3.48 (ddd, 1H, H-5, $J_{4,5} = 9.3$ Hz, $J_{5,6a} = 2.3$ Hz, $J_{5,6b} = 5.8$ Hz), 3.41 (app. t, 1H, H-4, $J_{3,4} = J_{4,5} = 9.3$ Hz), 3.35 (dd, 1H, H-2, $J_{1,2} = 9.8$ Hz, $J_{2,3} = 9.3$ Hz); ^{13}C NMR (125 MHz, D_2O) δ 132.85, 132.50, 130.19, 128.97, 88.77, 80.77, 78.10, 72.60, 70.24, 61.68; ES (positive) HRMS calcd. for $C_{12}H_{16}O_5SNa$ ($M + Na^+$): 295.0610; found 295.0613.

Phenyl 2,3,4-tri-O-benzoyl-1-thio-6-O-trityl-β-D-glucopyranoside ***91***



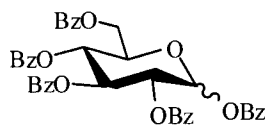
Compund **90** (1.45 g, 5.33 mmol) was dissolved in pyridine (30 mL) and trityl chloride (1.78 g, 6.39 mmol) was added. After 6 h, benzoyl chloride (3.71 mL, 32 mmol) was added and the reaction mixture was stirred for an additional 12 h. The solution was co-concentrated with toluene, diluted with dichloromethane and washed with 1M HCl (2 x 100 mL), water (100 mL), and dried over sodium sulphate. After concentration, the crude product was purified by chromatography (3:1 hexanes:ethyl acetate) to yield a clear glass (3.04 g, 69%): $[\alpha]_D$ + 17.1 (*c* 4.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.01-7.98 (m, 2H, Ar), 7.82-7.79 (m, 2H, Ar), 7.72-7.68 (m, 2H, Ar), 7.67-7.64 (m, 2H, Ar), 7.56-7.38 (m, 11H, Ar), 7.36-7.24 (m, 7H, Ar), 7.22-7.16 (m, 6H, Ar), 7.15-7.11 (m, 3H, Ar), 5.81 (app. t, 1H, H-3, $J_{2,3} = J_{3,4} = 9.7$ Hz), 5.55 (app. t, 1H, H-2, $J_{1,2} = J_{2,3} = 9.7$ Hz), 5.54 (app. t, 1H, H-4, $J_{3,4} = J_{4,5} = 9.7$ Hz), 5.06 (d, 1H, H-1, $J_{1,2} = 9.7$ Hz), 3.90 (ddd, 1H, H-5, $J_{4,5} = 9.7$ Hz, $J_{5,6} = 3.8$ Hz), 3.36 (dd, 2H, H-6a, H-6b, $J = 3.8$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.82, 165.13, 164.80, 143.59, 133.25, 133.09, 133.03, 132.33, 130.18, 129.88, 129.76, 129.67, 129.33, 129.00, 128.61, 128.37, 128.18, 127.75, 126.93, 86.25, 78.23, 74.57, 70.75, 69.17, 62.57; ES (positive) HRMS calcd. for $C_{52}H_{42}O_8SNa$ ($M + Na^+$): 849.2492; found 849.2496.

Phenyl 2,3,4-tri-O-benzoyl-1-thio-β-D-glucopyranoside ***92***



To a solution of **91** (2.75 g, 3.33 mmol) in dichloromethane (10 mL) was added hydrofluoroboric acid (1 mL). The reaction mixture was stirred vigorously for 1 hour and methanol (2 mL) was added. The solution was neutralized with sodium bicarbonate and the organic layer was washed with water (20 mL), dried over sodium sulphate and concentrated. After chromatographic purification, the product was obtained as a white powder (1.27 g, 65%): $[\alpha]_D$ + 19.6 (*c* 2.2, $CHCl_3$): ^1H NMR (500 MHz, $CDCl_3$) δ 7.99-7.96 (m, 2H, Ar), 7.95-7.92 (m, 2H, Ar), 7.82-7.80 (m, 2H, Ar), 7.55-7.50 (m, 4H, Ar), 7.44-7.36 (m, 6H, Ar), 7.35-7.32 (m, 2H, Ar), 7.29-7.25 (m, 2H, Ar), 5.94 (app. t, 1H, H-3, $J_{2,3}$ = 9.5 Hz), 5.49 (dd, 1H, H-2, $J_{1,2}$ = 10.0 Hz, $J_{2,3}$ = 9.5 Hz), 5.48 (app. t, 1H, H-4, $J_{3,4} = J_{4,5}$ = 9.5 Hz), 5.06 (d, 1H, H-1, $J_{1,2}$ = 10.0 Hz), 3.85 (m, 2H, H-5, H-6a, $J_{4,5}$ = 9.5 Hz, $J_{5,6b}$ = 4.9 Hz), 3.75 (dd, 1H, H-6b, $J_{5,6}$ = 4.9 Hz, J_{gem} = 12.7 Hz), 2.49 (br. s, 1H, 6-OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.90, 165.79, 165.02, 133.66, 133.32, 133.23, 133.11, 131.74, 129.91, 129.86, 129.72, 129.19, 129.06, 128.81, 128.55, 128.49, 128.44, 128.39, 128.28, 86.21, 78.88, 76.75, 74.03, 70.62, 69.30, 61.63; ES (positive) HRMS calcd. for $C_{20}H_{24}O_9SNa$ ($M + Na^+$): 607.1397; found 463.1393; Anal. Calcd. for $C_{20}H_{24}O_9S$: C, 67.79; H, 4.83; S, 5.48; found: C, 67.45; H, 4.71; S, 5.48.

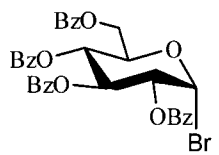
1,2,3,4,6-O-Benzoyl-α,β-D-glucopyranoside ***93***



D-Glucose (25.75 g, 142.9 mmol) was dissolved in 300 mL of pyridine. The solution was cooled to 0 °C and benzoyl chloride (124.4 mL, 1.07 mol) was added over 30 min. After 12 hours, the reaction mixture was co-concentrated under vacuum with toluene. The resulting oil was dissolved in dichloromethane and washed with 1M HCl (2 x 100 mL),

water (100 mL), and dried over sodium sulphate. After concentration, the crude product was purified by column chromatography (95:5 dichloromethane:methanol) to yield the product as a clear glass (70.58 g, 70%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19-8.17 (m, 2H, Ar), 8.06-8.01 (m, 5H, Ar), 7.97-7.85 (m, 11H, Ar), 7.69-7.65 (m, 1H, Ar), 7.69-7.65 (m, 27H, Ar), 6.87 (d, 1H, H-1α, $J_{1,2}$ = 3.7 Hz), 6.33 (app. t, 1H, H-3α, $J_{2,3} = J_{3,4}$ = 9.9 Hz), 6.31 (d, 1H, H-1β, $J_{1,2}$ = 8.0 Hz), 6.04 (app. t, 1H, H-3β, $J_{2,3} = J_{3,4}$ = 9.4 Hz), 5.87 (app. t, 1H, H-4α, $J_{3,4} = J_{4,5}$ = 9.7 Hz), 5.86 (dd, 1H, H-2β, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.4 Hz), 5.84 (app. t, 1H, H-4β, $J_{4,5}$ = 9.5 Hz), 4.68-4.61 (m, 3H, H-5α, H-6α, H-6β), 4.55-4.48 (m, 2H, H-6α, H-6β), 4.42 (ddd, 1H, H-5β, J = 3.0, 4.8, 9.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.09, 166.07,165.89, 165.66, 165.33, 165.12, 164.58, 164.38, 133.90, 133.81, 133.51, 133.47, 133.43, 133.33, 133.11, 133.07, 130.19, 130.02, 129.88, 129.85, 129.83, 129.82, 129.78, 129.73, 129.54, 128.99, 128.84, 128.78, 128.71, 128.69, 129.57, 128.51, 128.43, 128.40, 128.37, 128.33, 92.71, 90.05, 73.20, 72.84, 70.87, 70.51, 70.48, 70.45, 69.10, 68.86, 62.70, 62.47; ES (positive) HRMS calcd. for $C_{41}H_{32}O_{11}Na$ ($M + Na^+$): 723.1836; found 723.1832.

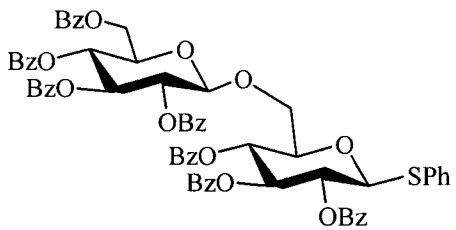
2,3,4,6-Tetra-O-benzoyl-α-D-glucopyranosyl bromide ***94***



Freshly distilled dichloromethane (30 mL) was added to **93** (4.34 g, 6.58 mmol) and cooled to 0 °C. Hydrogen bromide (33% solution in acetic acid, 25 mL) was added to the cooled solution and after 2 h the reaction mixture was co-concentrated with toluene (3 x 50 mL) to a yellow syrup. The syrup was dissolved in 50 mL of dichloromethane and

washed with saturated sodium bicarbonate (2 x 30 mL). After drying over sodium sulphate the solution was concentrated to give a white powder which was used without further purification (3.86 g, 94%): $[\alpha]_D$ + 119.9 (*c* 2.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.10-7.80 (m, 8H, Ar), 7.60-7.15 (m, 12H, Ar), 6.87 (d, 1H, H-1, $J_{1,2}$ = 3.4 Hz), 6.33 (app. t, 1H, H-3, $J_{2,3} = J_{3,4}$ = 9.8 Hz), 5.83 (app. t, 1H, H-4, $J_{3,4}$ = 9.8 Hz), 5.34 (dd, 1H, H-2, $J_{1,2}$ = 3.4 Hz, $J_{2,3}$ = 9.8 Hz), 4.77-4.62 (m, 2H, H-5, H-6a), 4.52 (dd, 1H, H-6b, $J_{5,6}$ = 4.8 Hz, J_{gem} = 12.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.00, 165.55, 165.28, 165.08, 133.77, 133.61, 133.32, 133.23, 130.07, 129.92, 129.82, 129.73, 129.47, 128.82, 128.54, 128.45, 128.34, 86.87, 72.73, 71.48, 70.64, 68.04, 61.96; ES (positive) HRMS calcd. for $C_{34}H_{27}BrO_9Na$ ($M + Na^+$): 681.0730; found 681.0732.

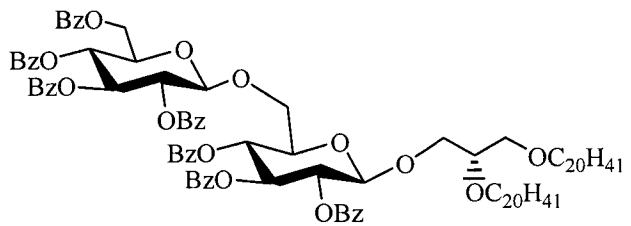
*Phenyl 2,3,4-tri-O-benzoyl-6-O-(2,3,4,6-tetra-O-benzoyl-*β*-D-glucopyranosyl)-1-thio-*β*-D-glucopyranoside* ***95***



Distilled dichloromethane (6 mL) was added to a round bottom flask containing **92** (0.717 g, 1.22 mmol), silver triflate (0.504 g, 1.96 mmol) and 3 Å molecular sieves under an argon atmosphere. This solution was stirred at –40 °C for 1 h. Compound **94** (0.970 g, 1.47 mmol) was dissolved in dichloromethane (3 mL) and added dropwise to the solution of the glycosyl acceptor. After 2 h, the solution was filtered and diluted with dichloromethane. The organic layer was washed with sodium bicarbonate (2 x 20 mL), water (30 mL), dried over sodium sulphate and concentrated. Chromatographic purification (4:1 hexanes:ethyl acetate) gave the glycosyl donor as a white powder (1.11

g, 78%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06-8.02 (m, 2H, Ar), 7.94-7.88 (m, 6H, Ar), 7.84-7.80 (m, 4H, Ar), 7.76-7.72 (m, 2H, Ar), 7.56-7.21 (m, 26H, Ar), 5.82 (app. t, 1H, H-3`, $J_{2,3} = J_{3,4} = 9.5$ Hz), 5.79 (app. t, 1H, H-3, $J_{2,3} = J_{3,4} = 9.5$ Hz), 5.58 (app. t, 1H, H-4`, $J_{3,4} = J_{4,5} = 9.5$ Hz), 5.48 (dd, 1H, H-2`, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 9.5$ Hz), 5.34 (app. t, 1H, H-2, $J_{1,2} = J_{2,3} = 9.5$ Hz), 5.26 (app. t, 1H, H-4, $J_{3,4} = J_{4,5} = 9.5$ Hz), 4.95 (d, 1H, H-1`, $J_{1,2} = 7.8$ Hz), 4.91 (d, 1H, H-1, $J_{1,2} = 9.9$ Hz), 4.59 (dd, 1H, H-6a`, $J_{5,6} = 3.3$ Hz, $J_{gem} = 12.3$ Hz), 4.40 (dd, 1H, H-6b`, $J_{5,6} = 5.3$ Hz, $J_{gem} = 12.3$ Hz), 4.06-4.00 (m, 2H, H-5` and H-5), 3.99-3.92 (m, 2H, H-6a and H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.04, 165.75, 165.61, 165.28, 165.17, 165.12, 164.96, 133.43, 133.40, 133.25, 133.16, 133.13, 133.02, 131.76, 129.85, 129.80, 129.73, 129.68, 129.56, 129.28, 129.24, 129.13, 128.87, 128.82, 128.67, 128.48, 128.44, 128.39, 128.35, 128.31, 128.27, 128.20, 100.99 ($^1J_{C1`-H1`} = 162.3$ Hz), 85.86, 78.52, 77.26, 74.07, 72.94, 72.29, 71.88, 70.54, 69.66, 69.55, 68.21, 62.89; ES (Positive) MS calcd. for $C_{67}H_{54}O_{17}SNa$ ($M + Na^+$): 1185.3; found 1185.3.

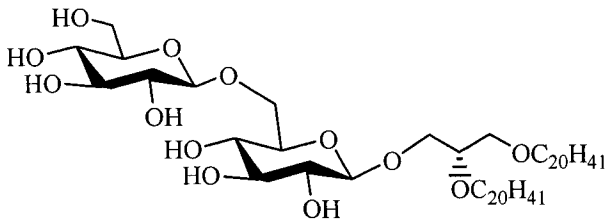
(2S)-2,3-Bis[(3R/S,7R,11R)-3,7,11,15-tetramethylhexadecyloxy]propan-1-yl 6-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-2,3,4-tri-O-benzoyl-β-D-glucopyranoside ***96***



The procedure used was analogous to that described for **97**. Glycosyl donor **95** (106 mg, 0.091 mmol), **83** (46 mg, 0.069 mmol), *N*-iodosuccinimide (32 mg, 0.14 mmol), triflic acid (9 μL), 3 Å molecular sieves and dichloromethane (3 mL). Column chromatography (8:1 hexanes:ethyl acetate) gave the glycolipid **96** (89 mg, 75%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02-7.96 (m, 4H, Ar), 7.95-7.91 (m, 2H, Ar), 7.90-7.83 (m, 4H, Ar), 7.82-7.76

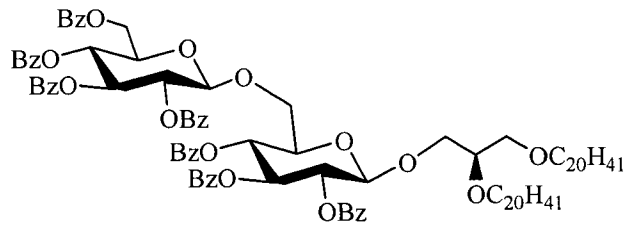
(m, 4H, Ar), 7.56-7.52 (m, 1H, Ar), 7.51-7.46 (m, 4H, Ar), 7.44-7.39 (m, 4H, Ar), 7.38-7.30 (m, 10H, Ar), 7.29-7.24 (m, 2H, Ar); 5.87 (app. t, 1H, H-3`, $J_{2,3} = J_{3,4} = 9.8$ Hz), 5.78 (app. t, 1H, H-3, $J_{2,3} = J_{3,4} = 9.6$ Hz), 5.60 (app. t, 1H, H-4`, $J_{3,4} = J_{4,5} = 9.8$ Hz), 5.51 (dd, 1H, H-2`, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 9.8$ Hz), 5.37 (dd, 1H, H-2, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 9.6$ Hz), 5.32 (app. t, 1H, H-4, $J_{3,4} = J_{4,5} = 9.6$ Hz), 5.00 (d, 1H, H-1`, $J_{1,2} = 7.8$ Hz), 4.74 (d, 1H, H-1, $J_{1,2} = 7.8$ Hz), 4.58 (dd, 1H, H-6a`, $J_{5,6} = 3.3$ Hz, $J_{gem} = 12.3$ Hz), 4.43 (dd, 1H, H-6b`, $J_{5,6} = 5.1$ Hz, $J_{gem} = 12.3$ Hz), 4.12 (ddd, 1H, H-5`, $J_{4,5} = 9.8$ Hz, $J_{5,6a} = 3.3$ Hz, $J_{5,6b} = 5.1$ Hz), 4.09 (dd, 1H, H-6a, $J_{5,6} = 2.0$ Hz, $J_{gem} = 11.5$ Hz), 3.98 (ddd, 1H, H-5, $J_{4,5} = 9.6$ Hz, $J_{5,6a} = 2.0$ Hz, $J_{5,6b} = 7.3$ Hz), 3.87 (dd, 1H, H-6b, $J_{5,6} = 5.1$ Hz, $J_{gem} = 11.5$ Hz), 3.67 (dd, 1H, $CH_{2(glycerol)}$), 3.50-3.1 (m, 8H, 3 x $CH_{2(glycerol)}$, CH $_{(glycerol)}$, O-C<u>H</u>$_2$-alkyl), 1.56-1.0 (m, 48H, O-CH_2-<u>alkyl</u>), 0.88-0.82 (m, 30 H, C<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.03, 165.65, 165.32, 165.13, 165.02, 164.90,133.37, 133.18, 133.15, 133.08, 129.82, 129.72, 129.57, 129.43, 129.29, 128.93, 128.84, 128.80, 128.39, 128.36, 128.25, 128.21, 101.27 ($^1J_{C1`-H1`}$ = 163.2 Hz, $^1J_{C1-H1}$ = 163.0 Hz), 73.81, 72.86, 72.29, 71.85, 69.99, 69.82, 69.62, 68.79, 68.76, 68.50, 63.02, 39.37, 37.56, 37.48, 37.44, 37.41, 37.30, 36.65, 32.85, 32.81, 29.95, 29.85, 27.98, 24.81, 24.53, 24.44, 24.41, 22.72, 22.63, 19.75, 19.68, 19.62, 19.60, 19.54; ES (positive) MS calcd. for $C_{104}H_{136}O_{20}Na$ ($M + Na^+$): 1729.1; found 1729.0; Anal. Calcd. for $C_{104}H_{136}O_{20}$: C, 73.21; H, 8.03; found: C, 73.04; H, 7.95.

(2S)-2,3-Bis[(3R/S,7R,11R)-3,7,11,15-tetramethylhexadecyloxy]propan-1-yl 6-O-β-D-glucopyranosyl β-D-glucopyranoside ***5***



The procedure used was analogous to that described for **6**. Compound **96** (84 mg, 0.049 mmol), methanol (5 ml), and sodium metal (40 mg). The crude product was purified by chromatography on Iatrobeads (92:8 dichloromethane:MeOH) and then purified further using normal phase HPLC (0% to 8% MeOH in dichloromethane) to give a colourless paste (39 mg, 81%): ^{1}H NMR (500 MHz, CD_3OD) δ 4.36 (d, 1H, H-1`, $J_{1,2}$ = 7.6 Hz), 4.28 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.14 (dd, 1H, H-6a, $J_{5,6}$ = 2.1 Hz, $J_{gem}$ = 11.5 Hz), 3.89 (dd, 1H, $CH_{(glycerol)}$, $J$ = 4.6, 10.6 Hz), 3.86 (dd, 1H, H-6a`, $J_{5,6}$ = 1.8 Hz, J_{gem} = 11.9 Hz), 3.77 (dd, 1H, H-6b, $J_{5,6}$ = 5.6 Hz, J_{gem} 11.5 Hz), 3.71-3.57 (m, 6H, H-6b`, O-C<u>H</u>$_2$-alkyl, $CH_{2(glycerol)}$), 3.56-3.41 (m, 4H, $CH_{2(glycerol)}$, O-C<u>H</u>$_2$-alkyl, H-5), 3.38-3.25 (m, 5H, H-3, H-3`, H-4, H-4`, H-5`), 3.23-3.18 (m, 2H, H-2`, H-2), 168-1.49 (m, 4H, O-$CH_2$-<u>alkyl</u>), 1.44-1.20 (m, 30H, O-$CH_2$-<u>alkyl</u>), 1.2-1.04 (m, 14H, O-$CH_2$-<u>alkyl</u>), 0.92-0.85 (m, 30H, $CH_3$); $^{13}$C NMR (125 MHz, $CD_3OD$) δ 104.94 ($^{1}J_{C1`-H1`}$ = 163.1 Hz, $^{1}J_{C1-H1}$ = 162.6 Hz), 79.50, 79.37, 78.04, 77.88, 77.01, 75.11, 75.05, 72.23, 72.17, 72.13, 71.63, 71.47, 70.89, 70.84, 70.82, 70.47, 70.43, 69.92, 69.88, 69.86, 69.59, 62.80, 40.58, 38.83, 38.80, 38.70, 38.66, 38.63, 38.60, 38.57, 38.51, 38.43, 38.32, 38.00, 37.99, 37.94, 37.93, 33.98, 33.94, 31.07, 31.05, 31.01, 30.90, 30.87, 29.17, 25.94, 25.56, 23.15, 23.07, 20.35, 20.29, 20.23, 20.15; ES (positive) HRMS calcd. for $C_{55}H_{108}O_{13}Na$ ($M + Na^+$): 999.7687; found 999.7686.

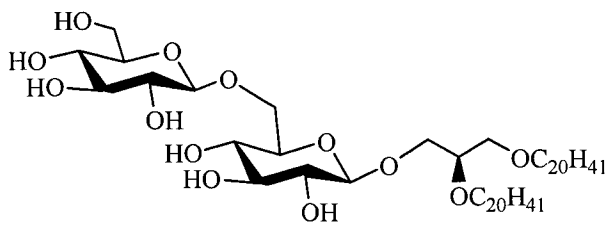
(2R)-2,3-Bis[(3R/S,7R,11R)-3,7,11,15-tetramethylhexadecyloxy]propan-1-yl 6-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-2,3,4-tri-O-benzoyl-β-D-glucopyranoside ***97***



Compound **78** (39 mg, 0.06 mmol) and **95** (84 mg, 0.072 mmol) were dissolved in distilled dichloromethane. Flame-dried 3 Å molecular sieves were added and the solution was stirred for 3 hours under argon. The solution was cooled to –30 °C and after 30 minutes *N*-iodosuccinimide (27 mg, 0.12 mmol) was added followed by triflic acid (7 μL). After 2 h, the reaction was filtered and diluted with dichloromethane (10 mL). The organic layer was washed with sodium carbonate (1M, 20 mL), dried over sodium sulphate and concentrated. Silica gel chromatography (8:1 hexanes:ethyl acetate) afforded the purified product as a white powder (82 mg β-anomer, 81%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00-7.94 (m, 4H, Ar), 7.93-7.90 (m, 2H, Ar), 7.88-7.85 (m, 2H, Ar), 7.84-7.81 (m, 2H, Ar), 7.80-7.74 (m, 4H, Ar), 7.54-7.50 (m, 1H, Ar), 7.49-7.44 (m, 4H, Ar), 7.41-7.37 (m, 4H, Ar), 7.36-7.29 (m, 10H, Ar), 7.27-7.22 (m, 2H, Ar); 5.86 (app. t, 1H, H-3`, $J_{2,3} = J_{3,4} = 9.7$ Hz), 5.77 (app. t, 1H, H-3, $J_{2,3} = J_{3,4} = 9.6$ Hz), 5.59 (app. t, 1H, H-4`, $J_{3,4} = J_{4,5} = 9.7$ Hz), 5.49 (dd, 1H, H-2`, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 9.7$ Hz), 5.35 (dd, 1H, H-2, $J_{1,2} = 7.9$ Hz, $J_{2,3} = 9.9$ Hz), 5.30 (app. t, 1H, H-4, $J_{3,4} = J_{4,5} = 9.6$ Hz), 4.99 (d, 1H, H-1`, $J_{1,2} = 7.8$ Hz), 4.72 (d, 1H, H-1, $J_{1,2} = 7.9$ Hz), 4.57 (dd, 1H, H-6a`, $J_{5,6} = 3.3$ Hz, $J_{gem} = 12.2$ Hz), 4.43 (dd, 1H, H-6b`, $J_{5,6} = 5.2$ Hz, $J_{gem} = 12.2$ Hz), 4.10 (ddd, 1H, H-5`, $J_{4,5} = 9.7$ Hz, $J_{5,6a} = 3.3$ Hz, $J_{5,6b} = 5.2$ Hz), 4.07 (dd, 1H, H-6a, $J_{5,6} = 2.1$ Hz, $J_{gem} = 11.4$ Hz), 3.98 (ddd, 1H, H-5, $J_{4,5} = 9.6$ Hz, $J_{5,6a} = 2.1$ Hz, $J_{5,6b} = 7.4$ Hz), 3.85 (dd, 1H, H-6b, $J_{5,6} = 7.4$ Hz, $J_{gem} = 11.6$ Hz), 3.65 (dd, 1H, $CH_{2(glycerol)}$), 3.46-3.09 (m, 8H, 3 x $CH_{2(glycerol)}$, $CH_{(glycerol)}$, O-CH_2-alkyl), 1.55-0.95 (m, 48H, O-CH_2-alkyl), 0.86-0.80 (m, 30

H, C<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.01, 165.64, 165.31, 165.11, 165.00, 164.88, 133.37, 133.35, 133.17, 133.13, 133.06, 133.03, 129.92, 129.84, 129.82, 129.80, 129.73, 129.71, 129.56, 129.43, 129.28, 128.92, 128,83, 128.79, 128.47, 128.40, 128.37, 128.34, 128.23, 128.19, 101.26 ($^1J_{C1`-H1`}$ = 163.1 Hz, $^1J_{C1-H1}$ = 162.6 Hz), 73.80, 72.85, 72.28, 71.84, 70.46, 70.38, 69.97, 69.81, 69.79, 69.61, 68.78, 68.65, 68.49, 63.00; 39.36, 37.70, 37.60, 37.42, 37.39, 37.28, 37.15, 36.71, 36.69, 36.63, 32.83, 32.79, 29.90, 29.83, 27.96, 24.79, 24.57, 24.51, 22.70, 22.61, 19.74, 19.67, 19.61, 19.59, 19.55, 19.52, 19.49; ES (positive) MS calcd. for $C_{104}H_{136}O_{20}Na$ ($M + Na^+$): 1729.1; found 1729.0; Anal. Calcd. for $C_{104}H_{136}O_{20}$: C, 73.21; H, 8.03; found: C, 72.98; H, 8.10.

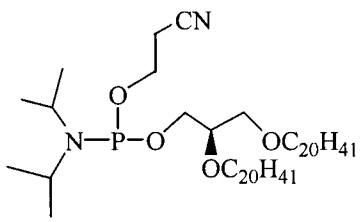
(2R)-2,3-Bis[(3R/S,7R,11R)-3,7,11,15-tetramethylhexadecyloxy]propan-1-yl 6-O-β-D-glucopyranosyl-β-D-glucopyranoside ***6***



Compound **97** (42 mg, 0.025 mmol) was mixed with distilled methanol (3 mL) followed by the addition of sodium metal (25 mg). The reaction was heated at 70 °C for 2 hours. This mixture was neutralized with H+ ion exchange resin, filtered and concentrated. The crude product was purified by chromatography on Iatrobeads (92:8 dichloromethane:MeOH) and then purified further using normal phase HPLC (0% to 8% MeOH in dichloromethane) to give a colourless paste (20 mg, 86%): ^{1}H NMR (600 MHz, CD_3OD) δ 4.36 (d, 1H, H-1`, $J_{1,2}$ = 7.8 Hz), 4.26 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.14 (dd, 1H, H-6a, $J_{5,6}$ = 2.1 Hz, $J_{gem}$ = 11.5 Hz), 3.91 (dd, 1H, $CH_{(glycerol)}$, $J$ = 4.2, 10.1 Hz), 3.86 (dd, 1H, H-6a`, $J_{5,6}$ = 2.1 Hz, J_{gem} = 11.8 Hz), 3.78 (dd, 1H, H-6b, $J_{5,6}$ = 5.4 Hz, J_{gem}

= 11.4 Hz), 3.71-3.57 (m, 6H, H-6b`, O-C$\underline{H_2}$-alkyl, $CH_{2(glycerol)}$), 3.56-3.46 (m, 3H, $CH_{2(glycerol)}$, O-C$\underline{H_2}$-alkyl), 3.43 (ddd, 1H, H-5, $J$ = 2.2, 5.4 Hz), 3.38-3.25 (m, 5H, H-3, H-3`, H-4, H-4`, H-5`), 3.21 (dd, 1H, H-2`, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.1 Hz), 3.18 (dd, 1H, H-2, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.0 Hz), 1.67 (m, 4H, O-CH_2-$\underline{alkyl}$), 1.44-1.20 (m, 30H, O-CH_2-$\underline{alkyl}$), 1.2-1.04 (m, 13H, O-CH_2-$\underline{alkyl}$), 0.92-0.85 (m, 30H, CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 104.92 ($^1J_{C1`-H1`}$ = 159.9 Hz), 104.83 ($^1J_{C1-H1}$ = 158.6 Hz), 79.45, 79.43, 79.29, 78.03, 77.81, 77.03, 75.10, 75.07, 72.44, 72.40, 71.61, 71.44, 70.86, 70.84, 70.25, 69.90, 69.70, 62.80, 40.60, 38.84, 38.80, 38.75, 38.72, 38.62, 38.60, 38.54, 38.45, 38.38, 38.32, 38.24, 38.05, 38.02, 37.99, 37.96, 33.99, 33.95, 31.05, 31.01, 30.90, 30.88, 29.18, 25.95, 25.58, 23.19, 23.11, 20.38, 20.33, 20.27, 20.17; ES (positive) HRMS calcd. for $C_{55}H_{108}O_{13}Na$ ($M + Na^+$): 999.7687; found 999.7685.

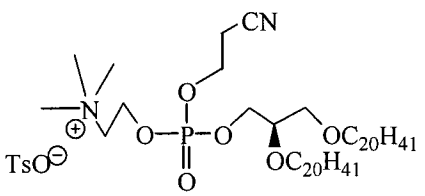
*3-O-[2-Cyanoethyl diisopropyl phosphoramidate]-(2*R*)-1,2-di-O-[(3*R/S*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl] sn-glycerol* ***99***



Compound **98** (90 mg, 0.265 mmol) and **78** (234 mg, 0.36 mmol) were co-concentrated with toluene and dried in vacuo for 3 h. The dried mixture was dissolved into dichloromethane (2 mL) and the reaction was initiated with the addition of tetrazole (350 μL, 0.15 mmol, 3% in acetonitrile). After 45 min a precipitate formed. The reaction was diluted with dichloromethane (2 mL) and washed with sodium bicarbonate (5 mL, 5% solution). The organic layer was dried over sodium sulphate, concentrated and the product was quickly passed through a silica gel column (5% triethylamine in 5:1

hexanes:ethyl acetate). The purified phosphoramidate was obtained as a clear syrup (194 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.90-3.80 (m, 2H, NC-CH_2-CH_2-O), 3.78-3.44 (m, 11H, 2 x O-CH_2-alkyl, $CH_{(isopropyl)}$, 2 x $CH_{2(glycerol)}$, $CH_{(glycerol)}$), 2.66-2.62 (m, 2H, CN-CH_2-CH_2-O), 1.66-1.46 (m, 6H, O-CH_2-alkyl), 1.42-1.20 (m, 26H, O-CH_2-alkyl), 1.20-1.16 (m, 12H, $CH_{3(isopropyl)}$), 1.16-1.00 (m, 16H, O-CH_2-alkyl), 0.89-0.81 (m, 30H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 117.56, 117.53, 78.48, 70.71, 70.02, 68.87, 63.20, 63.16, 63.08, 63.04, 58.56, 58.51, 58.41, 58.37, 43.18, 43.15, 43.08, 39.38, 37.48, 37.41, 37.31, 37.22, 37.14, 36.78, 36.70, 32.81, 29.94, 29.88, 29.84, 27.98, 24.81, 24.65, 24.60, 24.55, 24.50, 24.40, 22.72, 22.62, 20.38, 20.33, 19.75, 19.69, 19.64, 19.45; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 149.75, 149.59; ES (Positive) HRMS calcd. for $C_{52}H_{106}N_2O_4P$ ($M + H^+$): 853.7884; found 853.7884.

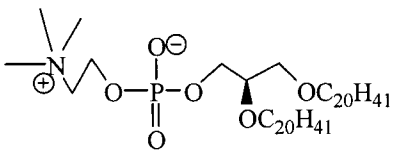
3-O-[2-Cyanoethyl 2-(trimethylammonium)ethyl phosphonato]-(2R)-1,2-di-O-[(3R/S,7R,11R)-3,7,11,15-tetramethylhexadecyl] glycerol ***100***



Choline tosylate salt (136 mg, 0.5 mmol) and **99** (210 mg, 0.25 mmol) were co-concentrated in toluene and dried in vacuo for 3 h. The reagents were dissolved in anhydrous dichloromethane (3 mL) under argon, followed by the addition of tetrazole (500 μL, 0.12 mmol, 3% in acetonitrile). After 1 h, *tert*-butyl hydrogen peroxide (300 μL, 5.0-6.0M in decane) was added to the stirring solution. The reaction solution was diluted with dichloromethane (3 mL) and washed with sodium bicarbonate (10 mL, 5% solution). The organic layer was dried over sodium sulphate and concentrated. The crude product was eluted (3:1 hexanes:ethyl acetate) from a silica gel column to yield a clear syrup (197

mg, 77%). This compound was isolated and characterized as the tosylate salt. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, 2H, $Ar_{(tosylate)}$), 7.17 (d, 2H, $Ar_{(tosylate)}$), 4.64-4.54 (m, 2H, Me_3N-CH_2-$C\underline{H}_2$-O), 4.38-4.20 (m, 3H, NC-CH_2-$C\underline{H}_2$-O, CH_{2a}), 4.16-4.05 (m, 3H, Me_3N-$C\underline{H}_2$-CH_2-O, $CH_{2a'}$), 3.63-3.52 (m, 3H, O-$C\underline{H}_2$-alkyl, CH_{2b}), 3.50-3.42 (m, 4H, O-$C\underline{H}_2$-alkyl, $CH_{(glycerol)}$, $CH_{2b'}$), 3.39 (s, 9H, $\underline{Me}_3N$-CH_2-CH_2-O), 2.79-2.74 (m, 2H, NC-$C\underline{H}_2$-CH_2-O), 2.35 (s, 3H, $CH_{3(tosylate)}$), 1.64-1.46 (m, 6H, O-CH_2-alkyl), 1.42-1.00 (m, 42H, O-CH_2-alkyl), 0.89-0.81 (m, 30H, $C\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.20, 139.70, 128.83, 125.73, 116.79, 83.29, 70.27, 70.24, 69.23, 69.19, 69.17, 69.06, 68.93, 68.32, 68.31, 68.29, 68.28, 68.21, 68.16, 65.47, 65.45, 65.42, 65.37, 62.59, 61.89, 61.85, 54.55, 39.36, 37.63, 37.53, 37.49, 37.46, 37.42, 37.39, 37.29, 37.05, 36.70, 36.60, 32.79, 29.99, 29.96, 29.91, 29.83, 27.96, 24.86, 24.79, 24.48, 24.42, 24.37, 22.71, 22.61, 21.28, 19.74, 19.67, 19.61, 19.50; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ -221.17, -229.20; ES (Positive) HRMS calcd. for $C_{51}H_{104}N_2O_6P$: 871.7626; found 871.7627.

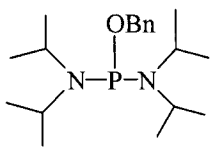
3-O-[2-(Trimethylammonium)ethyl phosphonato]-(2R)-1,2-di-O-[(3R/S,7R,11R)-3,7,11,15-tetramethylhexadecyl] glycerol ***9***



100 (80 mg) was dissolved in MeOH (1 mL) and NaOMe (10 drops). After 2 h, the reaction mixture was concentrated. The crude syrup was dissolved in MeOH/H_2O (0.5 mL each) and flushed through a sep-pak cartridge (50% to 100% MeOH). The product was then further purified using reversed-phase HPLC to give a clear glass (52 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.34-4.29 (m, 2H, Me_3N-CH_2-$C\underline{H}_2$-O), 3.90-3.83 (m, 2H, $CH_{2(glycerol)}$), 3.81-3.78 (m, 2H, Me_3N-$C\underline{H}_2$-CH_2-O), 3.64-3.53 (m, 4H, O-$C\underline{H}_2$-alkyl,

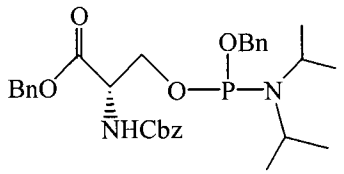
$CH_{(glycerol)}$, CH_{2b}), 3.48-3.42 (m, 3H, O-C$\underline{H_2}$-alkyl, $CH_{2b'}$), 3.39 (s, 9H, $\underline{Me_3}$N-CH_2-CH_2-O), 1.64-1.46 (m, 6H, O-CH_2-$\underline{alkyl}$), 1.42-1.00 (m, 42H, O-CH_2-$\underline{alkyl}$), 0.89-0.81 (m, 30H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 78.17, 71.02, 70.98, 70.08, 68.95, 68.77, 66.12, 65.12, 65.11, 65.09, 59.32, 54.51, 39.37, 37.77, 37.66, 37.61, 37.56, 37.54, 37.45, 37.41, 37.35, 37.30, 37.25, 36.90, 36.82, 32.86, 32.83, 32.80, 30.00, 29.97, 29.87, 27.97, 24.80, 24.58, 24.51, 24.50, 24.40, 22.72, 22.63, 19.75, 19.68, 19.64, 19.61, 19.58; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 2.02; ES (Positive) HRMS calcd. for $C_{48}H_{100}NO_6PNa$ ($M + Na^+$): 840.7180; found 840.7182.

Benzyloxybis(diisopropylamino)phosphine ***105***



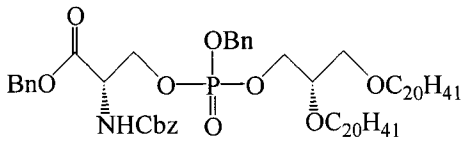
To a solution of tetrahydrofuran and chlorobis(*N*,*N*-diisopropyl)phosphoramidate **104** (2.5 g) at 0 °C, was added a solution of triethylamine and benzyl alcohol in tetrahydrofuran. The reaction was warmed to room temperature. The resulting precipitate was removed by filtration through a plug of Celite 545. The filtrate was evaporated and stored at –20 °C until further use. The yield varied depending on the quality of the chlorobis(*N*,*N*-diisopropyl) phosphoramidate obtained from Aldrich. Spectral data agreed with literature values[101]: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43-7.22 (m, 5H, Ar), 4.73-4.64 (m, 2H, C$\underline{H_2}$Ph), 3.66-3.54 (m, 4H, $CH_{(isopropyl)}$), 1.25-1.17 (m, 24H, $CH_{3(isopropyl)}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 140.48, 128.07, 126.83, 66.27, 66.09, 44.52, 44.42, 24.65, 24.59, 23.87, 23.82; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 124.87.

*N-(Benzyloxycarbonyl)-O-[(benzyloxy)(diisopropylamino) phosphino]-*L*-serine benzyl ester* ***106***



Compound **105** (277 mg, 0.82 mmol) and *N*-Z-serine benzyl ester (351 mg, 1.06 mmol) were concentrated with toluene (3 x 10 mL) and dried in vacuo for 3 h. The syrup was dissolved in dichloromethane (10 mL) under argon and tetrazole (1.07 mL, 0.12 mmol, 3% in acetonitrile) was added. The reaction mixture stirred for 45 min and diluted in dichloromethane. The organic layer was washed with 5% aqueous sodium bicarbonate (15 mL), dried over sodium sulphate and concentrated. The crude product was quickly flushed through a silica gel column and the clear syrup was stored (with 1% triethylamine) at –20 °C until further use (375 mg, 81%). The purified product was a mixture of diastereomers; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38-7.22 (m, 30H, Ar), 5.87 (d, 1H, NH, $J_{NH,CH}$ = 8.7 Hz), 5.66 (d, 1H, NH, $J_{NH,CH}$ = 8.7 Hz), 5.20-5.10 (m, 8H, C$\underline{H}_2$Ph), 4.74-4.58 (m, 4H, C$\underline{H}_2$Ph), 4.56 (app ddt, 2H, $CH_{(serine)}$, J = 2.8, 2.8, 5.7, 8.8 Hz), 4.17 (ddd, 2H, $CH_{2a(serine)}$, J = 2.8, 6.8, 10.0 Hz), 3.94 (ddd, 1H, $CH_{2b(serine)}$, J = 2.9, 7.7, 10.6 Hz), 3.90 (ddd, 1H, $CH_{2b(serine)}$, J = 3.1, 6.1, 9.8 Hz), 3.62-3.54 (m, 4H, $CH_{(isopropyl)}$), 1.18-1.12 (m, 24H, $CH_{3(isopropyl)}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 220.53, 169.98, 156.05, 155.92, 139.25, 139.19, 136.40, 136.32, 135.30, 128.77, 128.70, 128.53, 128.49, 128.30, 128.27, 128.19, 128.09, 128.05, 128.02, 127.40, 127.31, 127.10, 126.92, 67.28, 67.19, 66.97, 66.91, 65.52, 65.45, 65.38, 65.31, 64.23, 64.11, 63.74, 63.62, 55.46, 55.41, 55.32, 55.27, 43.17, 43.08, 24.61, 24.55, 24.49, 24.44; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 150.14, 149.77; ES (Positive) HRMS calcd. for $C_{31}H_{40}N_2O_6P$ ($M + H^+$): 567.2618; found 567.2617.

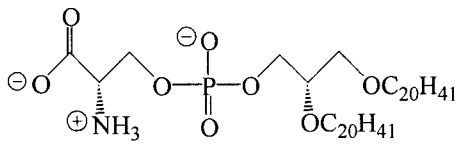
N-Benzyloxycarbonyl-O-[((S)-2,3-di-O-[(3R/S,7R,11R)-3,7,11,15-tetramethylhexadecyl] sn-glycerol)-benyloxy-phoshonato]-L-serine benzyl ester ***107***



Compound **106** (92 mg, 0.16 mmol) and **83** (127 mg, 0.19 mmol) were concentrated with toluene (3 x 10 mL) and dried in vacuo for 3 h. The syrup was dissolved in dichloromethane (3 mL) under argon and tetrazole (350 μL, 0.15 mmol, 3% in acetonitrile) was added. The reaction mixture was stirred for 45 min and *tert*-butyl hydrogen peroxide (300 μL, 5-6M in decane) was added. After 30 min, the reaction was diluted with dichloromethane and the organic layer was washed with 5% aqueous sodium bicarbonate (15 mL), dried over sodium sulphate, filtered and concentrated. The crude product was purified using a silica gel column and the clear syrup was stored at –20 °C. (145 mg, 73%) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.29 (m, 15H, Ar), 5.90 (d, 1H, NH, $J_{NH,CH}$ = 8.2 Hz), 5.85 (d, 1H, NH, $J_{NH,CH}$ = 8.2 Hz), 5.24-5.07 (m, 4H, C$\underline{H}_2$Ph), 5.05-4.96 (m, 2H, C$\underline{H}_2$Ph), 4.62-4.57 (m, 1H, $CH_{(serine)}$), 4.50-4.43 (m, 1H, $CH_{2a(serine)}$), 4.34-4.27 (m, 1H, $CH_{2b(serine)}$), 4.12-4.05 (m, 1H, $CH_{2a(glycerol)}$), 4.02-3.95 (m, 1H, $CH_{2a'(glycerol)}$), 3.59-3.50 (m, 3H, $CH_{(glycerol)}$, O-C$\underline{H}_2$-alkyl), 3.46-3.38 (m, 4H, CH_{2b}, $CH_{2b'}$, O-C$\underline{H}_2$-alkyl), 1.64-1.00 (m, 48H, O-CH_2-$\underline{alkyl}$), 0.89-0.80 (m, 30H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.76, 155.87, 155.86, 136.11, 135.61, 135.56, 135.00, 128.74,128.71, 128.69, 128.68, 128.61, 128.59, 128.50, 128.26, 128.20, 128.16, 128.07, 127.91, 70.16, 69.63, 69.60, 69.54, 69.51, 69.46, 69.09, 68.98, 68.96, 67.69, 67.67, 67.50, 67.45, 67.28, 67.24, 67.16, 54.59, 54.55, 39.37, 37.49, 37.47, 37.43, 37,40, 37.30, 37.06, 37.04, 36.98, 36.96, 36.69, 36.60, 32.81, 29.95, 29.87, 29.79, 27.98, 24.81, 24.80, 24.50, 24.40, 24.38, 22.71,

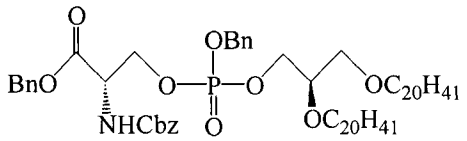
22.62, 19.74, 19.68, 19.64, 19.62, 19.61, 19.58, 19.55; ^{31}P NMR (400 MHz, $CDCl_3$) δ 0.201, 0.128; ES (Positive) HRMS calcd. for $C_{68}H_{112}NO_{10}PNa$ ($M + Na^+$): 1156.7921; found 1156.7925. Anal. Calcd. for $C_{68}H_{112}NO_{10}P$: C, 71.98; H, 9.95; N, 1.23; found: C, 72.16; H, 10.03, N, 1.20.

(2S)-2,3-Di-O-[(3R/S,7R,11R)-3,7,11,15-tetramethylhexadecyl-sn-glycero-1-phospho-L-serine 7



Compound **107** (136 mg, 0.11 mmol) was dissolved into cyclohexane/methanol (1:1, 3 mL) and the flask was flushed with H_2 gas. Palladium hydroxide (20 mg) was added and H_2 balloon attached. The reaction was complete after 10 hours. The reaction mixture was filtered through a 0.45 μm filter and washed with saturated NaCl. The organic solution was then concentrated under reduced pressure. The crude product was passed through a LH-20 Sephadex column using 1:1 mixture of chloroform and methanol. The solvent was evaporated and the crude compound was further purified by Iatrobeads column chromatography (100% chloroform to 80% chloroform/19% MeOH/1% H_2O) to yield the product as a clear glass. The purified product was passed through a 0.45 μm filter and concentrated (73 mg, 74%). Ninhydrin positive; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90-3.80 (m, 2H), 3.65-3.40 (m, 7H), 1.65-1.43 (m, 6H), 1.41-1.00 (m, 42H), 0.88-0.78 (m, 30H); ^{31}P NMR (202.3 MHz, 50% CD_3OD and 50% $CDCl_3$) δ 0.22; IR (cast film, cm^{-1}) 3500-3400 (NH_2), 2954-2868 (sp^3 alkyl), 1633 (COO^- and NH_3^+ overlap), 1462 (alkyl), 1377 ($(CH_3)_2CH$), 1310 (C-O- carboxyl), 1230 and 1058 (P=O, P-O-C), 1090 (C-O-C); ES (Negative) HRMS calcd. for $C_{46}H_{93}NO_8P$: 818.6644; found 818.6636.

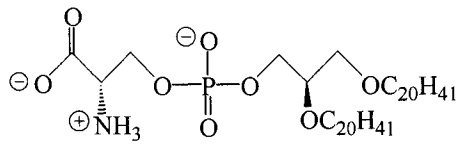
*N-Benzyloxycarbonyl-O-[((2*R*)-1,2-di-O-[(3*R*/*S*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl] sn-glycerol)-benzyloxy-phoshonato]*-L-*serine benzyl ester* ***108***



Compound **106** (75 mg, 0.133 mmol) and **78** (122 mg, 0.186 mmol) were placed in a round bottom flask, concentrated in toluene (3 x 10 mL) and dried in vacuo for 3 hours. The syrup was dissolved in dichloromethane (3 mL) under argon and tetrazole (300 μL, 0.15 mmol, 3% in acetonitrile) was added. The reaction mixture stirred for 45 minutes and *tert*-butyl hydrogen peroxide (300 μL, 5-6M in decane) was added. After 30 minutes, the reaction was diluted with dichloromethane and the organic layer was washed with sodium bicarbonate (15 mL, 5%), dried over sodium sulphate and concentrated. The crude product was purified using a silica gel column and the clear syrup was stored at –20 °C (116 mg, 70 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.29 (m, 15H, Ar), 5.94 (d, 1H, NH, $J_{NH,CH}$ = 7.8 Hz), 5.24-5.08 (m, 4H, C$\underline{H}_2$Ph), 5.05-4.96 (m, 2H, C$\underline{H}_2$Ph), 4.63-4.57 (m, 1H, $CH_{(serine)}$), 4.50-4.42 (m, 1H, $CH_{2a(serine)}$), 4.34-4.28 (m, 1H, $CH_{2b(serine)}$), 4.13-4.06 (m, 1H, $CH_{2a(glycerol)}$), 4.02-3.95 (m, 1H, $CH_{2a'(glycerol)}$), 3.60-3.48 (m, 3H, $CH_{(glycerol)}$, O-C$\underline{H}_2$-alkyl), 3.46-3.37 (m, 4H, $CH_{2b(glycerol)}$, $CH_{2b'(glycerol)}$, O-C$\underline{H}_2$-alkyl), 1.64-1.00 (m, 48H, O-CH_2-$\underline{alkyl}$), 0.89-0.80 (m, 30H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.77, 168.75, 155.91, 155.89, 136.14, 135.58, 135.53, 135.03, 135.00, 128.61, 128.60, 128.50, 128.24, 128.15, 128.06, 127.93, 127.91, 70.16, 69.59, 69.57, 69.54, 69.51, 69.11, 69.02, 69.01, 67.67, 67.47, 67.44, 67.42, 67.25, 67.21, 67.14, 54.63, 54.58, 54.57, 39.38, 37.62, 37.61, 37.57, 37.55, 37.49, 37.48, 37.43, 37.40, 37.30, 37.06, 36.98, 36.96, 36.68, 36.60, 32.10, 29.95, 29.87, 29.79, 29.69, 27.98, 24.80, 24.50, 24.40, 24.38, 22.72, 22.63, 19.75,

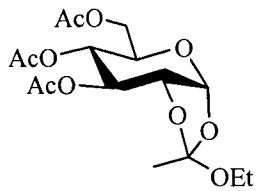
19.68, 19.62, 19.61, 19.58, 19.55, 19.48; ^{31}P NMR (400 MHz, $CDCl_3$) δ 0.062, 0.004; ES (Positive) MS calcd. for $C_{68}H_{112}NO_{10}PNa$ ($M + Na^+$): 1156.7; found 1156.8. Anal. Calcd. for $C_{68}H_{112}NO_{10}P$: C, 71.98; H, 9.95; N, 1.23; found: C, 71.75; H, 10.09, N, 1.23.

(2R)-1,2-Di-O-(3R/S,7R,11R)-3,7,11,15-tetramethylhexadecyl-sn-glycero-3-phospho-L-serine ***8***



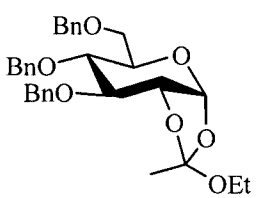
Compound **108** (104 mg, 0.09 mmol) was dissolved into cyclohexane/methanol (1:1, 2 mL) and the flask was flushed with H_2 gas. Palladium hydroxide (15 mg) was added and the reaction was stirred under an H_2 atmosphere. The reaction was complete after 10 h. The reaction mixture was filtered through a 0.45 μm filter and washed with saturated NaCl, and then concentrated under reduced pressure. The crude product was then passed through a LH-20 Sephadex column using 1:1 mixture of chloroform and methanol. The solvent was evaporated and the product further purified by Iatrobeads column chromatography (100%chloroform to 80% chloroform/19% MeOH/1% H_2O) to yield the product as a clear glass. The purified product was passed through a 0.45 μm filter and concentrated (59 mg, 79%). Ninhydrin positive; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90-3.80 (m, 2H), 3.65-3.40 (m, 7H), 1.65-1.43 (m, 6H), 1.41-1.00 (m, 42H), 0.88-0.78 (m, 30H); ^{31}P NMR (202.3 MHz, 50% CD_3OD and 50% $CDCl_3$) δ 1.13; IR (cast film, cm^{-1}) 3500-3400 (NH_2), 2959-2867 (sp^3 alkyl), 1640 (COO^- and NH_3^+ overlap), 1462 (alkyl), 1377 ($(CH_3)_2CH$), 1310 (C-O- carboxyl), 1260 and 1027 (P=O, P-O-C), 1094 (C-O-C); ES (Negative) HRMS calcd. for $C_{46}H_{93}NO_8P$: 818.6644; found 818.6639.

3,4,6-Tri-O-acetyl-1,2-O-(endo/exo-ethoxyethylidene)-α-D-glucopyranoside ***109***



To a stirring solution of freshly prepared 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (10.06 g, 25 mmol) and 2,6-lutidine (100 mL) was added tetrabutylammonium bromide (8.05 g, 25 mmol) followed by ethanol (2.9 mL, 50 mmol). After 10 h, the reaction was diluted with dichloromethane (100 mL) and washed with water (2 x 50 mL), dried over sodium sulphate and concentrated to a yellow syrup. The product was crystallized with 100% ethanol (6.90 g, 75%): $[\alpha]_D$ + 29.6 (*c* 2.1, $CHCl_3$): ^1H NMR (600 MHz, $CDCl_3$) δ 5.71 (d, 1H, H-1, $J_{1,2}$ = 5.2 Hz), 5.19 (app. t, 1H, H-3, $J_{2,3}$ = 2.8 Hz), 4.90 (ddd, 1H, H-4, *J* = 0.9, 2.7, 9.6 Hz), 4.32 (ddd, 1H, H-2, *J* = 0.9, 2.9, 5.2 Hz), 4.23-4.17 (m, 2H, H-6a, H-6b), 3.97-3.93 (m, 1H, H-5), 3.58-3.50 (m, 2H, O-C$\underline{H}_2$-CH_3), 2.11 (s, 3H, O_2C-C$\underline{H}_3$), 2.10 (s, 3H, O_2C-C$\underline{H}_3$), 2.09 (s, 3H, O_2C-C$\underline{H}_3$), 1.72 (s, 3H, exo-C$\underline{H}_3$), 1.59 (s, 1H, endo-C$\underline{H}_3$), 1.18 (t, 3H, J_{vic} = 7.11 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.64, 169.61, 169.14, 121.14, 96.89, 73.08, 70.17, 68.24, 66.94, 63.07, 59.14, 20.76, 20.73, 15.25; ES (Positive) HRMS calcd. for $C_{16}H_{24}O_{10}Na$ ($M + Na^+$): 399.1261; found 399.1264.

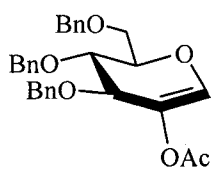
3,4,6-Tri-O-benzyl-1,2-O-(endo/exo-ethoxyethylidene)-α-D-*glucopyranoside* ***110***



Tetrahydrofuran was mixed with **109** (16.23 g, 43.12 mmol) and powdered KOH (24.2 g, 0.431 mol) was added. The solution immediately turned brown and the solution was

heated at 66 °C. Benzyl bromide (20.48 mL, 0.172 mol) was slowly added over 1 h to maintain a steady reflux. The reaction was stirred for 6 h and cooled to room temperature. After concentrating the reaction mixture, it was diluted in dichloromethane (100 mL) and washed with water (2 x 100 mL). The organic layer was then dried over sodium sulphate and concentrated. The crude product was purified by silica gel column chromatography (10:1 toluene:ethyl acetate with 2% triethylamine) to give a yellow syrup (16.47 g, 73%): $[\alpha]_D$ + 30 (*c* 4.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.37-7.17 (m, 20H, Ar), 5.76 (d, 1H, H-1, $J_{1,2}$ = 5.2 Hz), 4.70 (d, 1H, C$\underline{H}_2$Ph, J_{gem} = 11.7 Hz), 4.63-4.57 (m, 3H, C$\underline{H}_2$Ph), 4.50 (d, 1H, C$\underline{H}_2$Ph, J_{gem} = 12.2 Hz), 4.43-4.38 (m, 2H, H-2, C$\underline{H}_2$Ph, $J_{2,3}$ = 4.4 Hz, J_{gem} = 11.7 Hz), 3.87 (app. t, 1H, H-3, $J_{2,3}$ = 4.2 Hz), 3.82-3.78 (m, 1H, H-5), 3.74-3.68 (m, 2H, H-4, H-6a), 3.67-3.63 (m, 1H, H-6b), 3.61-3.50 (m, 2H, O-C$\underline{H}_2$-CH_3), 3.58-3.50 (m, 2H, O-C$\underline{H}_2$-CH_3), 1.69 (s, 3H, exo-C$\underline{H}_3$), 1.55 (s, 1H, endo-C$\underline{H}_3$), 1.20 (t, 3H, J_{vic} = 7.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.10, 137.95, 137.74, 128.40, 128.31, 127.99, 127.83, 127.76, 127.57, 120.96, 97.77, 78.84, 75.80, 74.95, 73.39, 72.92, 72.11, 71.91, 69.17, 58.66, 21.85, 15.32; ES (Positive) HRMS calcd. for $C_{32}H_{38}O_6Na$ ($M + Na^+$): 543.2353; found 543.2350.

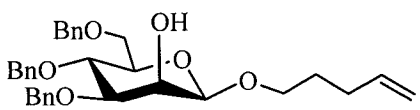
2-O-Acetyl-1,5-anhydro-3,4,6-tri-O-benzyl-D-*arabino-hex-1-enitol* ***111***



Bromobenzene (250 mL) was passed through a plug of alumina oxide into a round bottom flask containing **110** (16.39 g, 31.48 mmol). As soon as the solution began boiling at 156 °C, pyridine (2.11 mL, 26.04 mmol) was added. After 8 h the bromobenzene was removed in vacuo and the brown syrup was purified by

chromatography (20:1 toluene:ethyl acetate) to yield a yellow syrup (10.33 g, 69%): $[\alpha]_D$ + 17.6 (*c* 2.7, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.36-7.25 (m, 15H, Ar), 6.62 (d, 1H, H-1, $J_{1,3}$ = 0.7 Hz), 4.75 (d, 1H, C$\underline{H}_2$Ph, J_{gem} = 11.7 Hz), 4.63 (d, 1H, C$\underline{H}_2$Ph, J_{gem} = 11.7 Hz), 4.60 (d, 1H, C$\underline{H}_2$Ph, J_{gem} = 11.7 Hz), 4.57 (s, 2H, C$\underline{H}_2$Ph), 4.52 (d, 1H, C$\underline{H}_2$Ph, J_{gem} = 11.7 Hz), 4.45 (dt, 1H, H-3, $J_{1,3}$ = 0.7 Hz, $J_{3,4}$ = 5.2 Hz), 4.23 (ddd, 1H, H-5, $J_{4,5}$ = 7.2 Hz, $J_{5,6a}$ = 5.4 Hz, $J_{5,6b}$ = 3.5 Hz), 3.98 (dd, 1H, H-4, $J_{3,4}$ = 5.2 Hz, $J_{4,5}$ = 7.2 Hz), 3.83 (dd, 1H, H-6a, $J_{5,6}$ = 5.4 Hz, J_{gem} = 10.8 Hz), 3.75 (dd, 1H, H-6b, $J_{5,6}$ = 3.52 Hz, J_{gem} = 10.8 Hz), 2.07 (s, 3H, $O_2CC\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.56, 138.25, 138.10, 137.90, 137.80, 129.84, 128.47, 128.44, 128.40, 128.39, 127.87, 127.84, 127.75, 127.74, 127.68, 75.08, 73.90, 73.49, 73.04, 71.92, 67.97, 20.71; ES (Positive) HRMS calcd. for $C_{29}H_{30}O_6Na$ ($M + Na^+$): 497.1934; found 497.1936.

*Pentenyl 3,4,6-tri-O-benzyl-*β*-D-mannopyranoside* ***112***

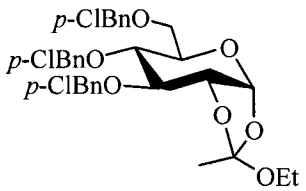


To a solution of **111** (1.05 g, 2.2 mmol) in distilled dichloromethane (5 mL) was added powdered 3 Å molecular sieves. The solution was cooled to 0 °C and stirred for 1 h under argon. Ethanol (0.14 mL, 2.4 mmol) and NBS (0.394 g, 2.2 mmol, recrystallized 100% acetone) were added. The reaction turned yellow within 3 min and the solution was immediately filtered and washed with sodium thiosulphate (25 mL, 5%). The mixture was diluted with 1:1 dichloromethane:ethyl acetate and eluted through a silica gel plug. After co-concentration with toluene, the yellow syrup was dried under vacuum for 3 h. The glycosyl donor, 3,4,6-tri-*O*-benzyl-α-D-*arabino*-hexopyrano-2-ulosyl bromide (1.04 g, 2.0 mmol) in dichloromethane (10 mL), was transferred to a flask

containing 3 Å molecular sieves, dichloromethane (10 mL), 4-penten-1-ol (0.381 mL, 4.1 mmol) and tri-*tert*-butyl pyrimidine (0.630 g, 2.54 mmol). The solution was cooled to –40 °C under argon, and the reaction was initiated with the addition of silver triflate (0.574 g, 22.4 mmol). The reaction mixture was warmed up to –5 °C and filtered, washed with sodium bicarbonate (30 mL, saturated), dried over sodium sulphate, concentrated, and dried *in vacuo*. It was then taken up in dichloromethane (5 mL) and methanol (5 mL) and cooled to 0 °C. Sodium borohydride (84.6 mg, 22.4 mmol) was added and solution was stirred for 1 h. The solution was concentrated and diluted with dichloromethane. The organic layer was washed with citric acid (20 mL, 2%), dried over sodium sulphate, and concentrated. The crude product was purified by silica gel column chromatography (4:1 hexanes:ethyl acetate) to give a white powder (0.696 g, 61% from 2-*O*-acetyl-1,5-anhydro-3,4,6-tri-*O*-benzyl-D-*arabino*-hex-1-enitol): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41-7.15 (m, 15H, Ar), 5.82 (dddd, 1H, CH_2=C<u>H</u>-CH_2-CH_2-CH_2-O, J = 6.4, 6.4, 10.1, 16.9 Hz), 5.06-5.01 (m, 1H, C<u>H$_2$</u>=CH-CH_2-CH_2-CH_2-O), 4.99-4.96 (m, 1H, C<u>H$_2$</u>=CH-CH_2-CH_2-CH_2-O), 4.90 (d, 1H, C<u>H$_2$</u>Ph, J_{gem} = 10.8 Hz), 4.78 (d, 1H, C<u>H$_2$</u>Ph, J_{gem} = 11.8 Hz), 4.68 (d, 1H, C<u>H$_2$</u>Ph, J_{gem} = 11.8 Hz), 4.63 (d, 1H, C<u>H$_2$</u>Ph, J_{gem} = 12.2 Hz), 4.57 (d, 1H, C<u>H$_2$</u>Ph, J_{gem} = 12.2 Hz), 4.55 (d, 1H, C<u>H$_2$</u>Ph, J_{gem} = 10.8 Hz), 4.41 (d, 1H, H-1, $J_{1,2}$ = 0.9 Hz), 4.11 (app. t, 1H, H-2, $J_{2,3}$ = 2.1 Hz), 3.96 (app. dt, 1H, CH_2=CH-CH_2-CH_2-C<u>H$_2$</u>-O, J = 9.5, 6.5, 6.5 Hz), 3.87 (t, 1H, H-4, $J_{4,5}$ = 9.5 Hz), 3.78 (dd, 1H, H-6a, $J_{5,6}$ = 1.9 Hz, J_{gem} = 10.8 Hz), 3.72 (dd, 1H, H-6b, $J_{5,6}$ = 5.4 Hz, J_{gem} = 10.8 Hz), 3.58 (dd, 1H, H-3, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.2 Hz), 3.53 (app. dt, 1H, CH_2=CH-CH_2-CH_2-C<u>H$_2$</u>-O, J = 9.5, 6.5, 6.5 Hz), 3.44 (ddd, 1H, H-5, $J_{4,5}$ = 9.6 Hz, $J_{5,6a}$ = 1.9 Hz, $J_{5,6b}$ = 5.4 Hz), 2.42 (d, 1H, CH(O<u>H</u>), J = 2.3 Hz), 2.14 (m, 2H, CH_2=CH-C<u>H$_2$</u>-CH_2-CH_2-O), 1.74 (m, 2H, CH_2=CH-

CH_2-C<u>H_2</u>-CH_2-O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.35, 138.30, 138.09, 137.91, 128.51, 128.38, 128.35, 128.10, 127.93, 127.86, 127.80, 127.74, 127.57, 114.92, 99.80 ($^1J_{C1,H1}$ = 156.9 Hz), 81.63, 77.37, 77.11, 76.86, 75.37, 75.19, 74.36, 73.54, 71.41, 69.34, 69.13, 68.38, 30.19, 28.74; ES (Positive) HRMS calcd. for $C_{32}H_{38}O_6Na$ (M + Na^+): 541.2560; found 541.2558; Anal. Calcd. for $C_{32}H_{38}O_6$: C, 74.11; H, 7.39; found: C, 73.93; H, 7.75.

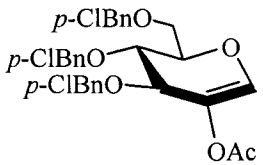
3,4,6-Tri-O-4-chlorobenzyl-1,2-O-(exo-ethoxyethylidene)-α-D-*glucopyranoside* ***113***



The procedure was analogous to that used for the preparation of **110**. Compound **109** (21.88 g, 58.1 mmol), THF (250 mL), KOH (32.62 g, 0.581 mol) and 4-chlorobenzyl chloride (37.45 g, 0.232 mol). Purified by column chromatography (10:1 hexanes:ethyl acetate with 2% triethylamine) to yield a yellow syrup (27.57 g, 76%): $[\alpha]_D$ + 29.8 (*c* 1.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33-7.21 (m, 10H, Ar), 7.11-7.08 (m, 2H, Ar), 5.75 (d, 1H, H-1, $J_{1,2}$ = 5.3 Hz), 4.69 (d, 1H, C<u>H_2</u>Ph, J_{gem} = 11.9 Hz), 4.59-4.53 (m, 3H, C<u>H_2</u>Ph), 4.45 (d, 1H, C<u>H_2</u>Ph, J_{gem} = 12.2 Hz), 4.39 (dd, 1H, H-2, $J_{1,2}$ = 5.3 Hz, $J_{2,3}$ = 3.7 Hz), 4.38 (d, 1H, C<u>H_2</u>Ph, J_{gem} = 11.9 Hz), 3.82 (dd, 1H, H-3, $J_{2,3}$ = 3.7 Hz, $J_{3,4}$ = 4.9 Hz), 3.78 (dt, 1H, H-5, $J_{4,5}$ = 9.4 Hz, $J_{5,6a}$ = 3.3 Hz), 3.68-3.64 (m, 3H, H-4, H-6ab), 3.55 (m, 2H, O-C<u>H_2</u>CH_3), 1.66 (s, 3H, C<u>H_3</u>), 1.20 (t, 3H, J_{vic} = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.48, 136.33, 136.12, 133.75, 133.63, 133.45, 129.19, 129.08, 128.62, 128.53, 128.52, 120.96, 97.76, 79.38, 75.92, 74.95, 72.66, 72.25, 71.12, 70.45, 69.10,

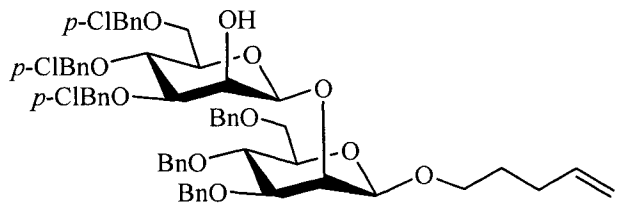
58.74, 21.91, 15.32; ES (Positive) HRMS calcd. for $C_{31}H_{33}O_7Cl_3Na$ (M + Na^+): 645.1184; found 645.1186.

*2-O-Acetyl-1,5-anhydro-3,4,6-tri-O-4-chlorobenzyl-*D*-arabino-hex-1-enitol* ***114***



The procedureused was analogous to that described for **111**. **113** (16.70 g, 26.8 mmol), bromobenzene (300 mL) and pyridine (2.4 mL, 29.4 mmol) were heated at reflux. Purified by column chromatography (12:1 toluene:ethyl acetate) to yield a yellow syrup (12.25 g, 79%): $[\alpha]_D$ + 13 (*c* 1.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.15 (m, 12H, Ar), 6.62 (d, 1H, H-1, $J_{1,3}$ = 0.5 Hz), 4.68 (d, 1H, C$\underline{H}_2$Ph, J_{gem} = 11.9 Hz), 4.58 (d, 1H, C$\underline{H}_2$Ph, J_{gem} = 11.9 Hz), 4.53 (d, 2H, C$\underline{H}_2$Ph, J_{gem} = 11.9 Hz), 4.49 (d, 2H, C$\underline{H}_2$Ph, J_{gem} = 11.9 Hz), 4.43 (dd, 1H, H-3, $J_{1,3}$ = 0.5 Hz, $J_{3,4}$ = 5.3 Hz), 4.19 (ddd, 1H, H-5, $J_{4,5}$ = 7.4 Hz, $J_{5,6a}$ = 5.1 Hz, $J_{5,6b}$ = 3.5 Hz), 3.93 (dd, 1H, H-4, $J_{3,4}$ = 5.3 Hz, $J_{4,5}$ = 7.4 Hz), 3.80 (dd, 1H, H-6a, $J_{5,6}$ = 5.1 Hz, J_{gem} = 10.7 Hz), 3.71 (dd, 1H, H-6b, $J_{5,6}$ = 3.5 Hz, J_{gem} = 10.7 Hz), 2.08 (s, 3H, $O_2CC\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.54, 138.41, 136.50, 136.29, 136.22, 133.67, 133.59, 133.55, 129.69, 128.99, 128.96, 128.86, 128.62, 128.59, 75.41, 73.96, 72.70, 72.25, 71.12, 67.92, 20.71; ES (Positive) HRMS calcd. for $C_{29}H_{27}O_6Cl_3Na$ (M + Na^+): 599.0765; found 599.0761; Anal. Calcd. for $C_{29}H_{27}O_6Cl_3$: C, 60.27; H, 4.71; found: C, 59.92; H, 4.63.

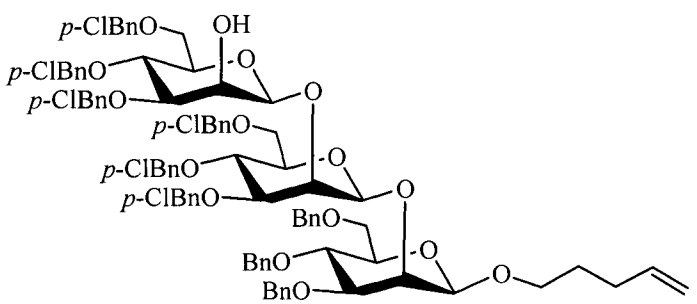
*Pentenyl 2-O-(3,4,6-tri-O-4-chlorobenzyl-*β-D*-mannopyranosyl)-3,4,6-tri-O-benzyl-*β-D*-mannopyranoside* ***115***



The preparation of 3,4,6-tri-*O*-4-chlorobenzyl-α-D-*arabino*-hexopyrano-2-ulosyl bromide was analogous to the procedure used for 3,4,6-tri-*O*-benzyl-α-D-*arabino*-hexopyranos-2-ulosyl bromide in the synthesis of glycosyl acceptor, **112**. The glycosyl donor, 3,4,6-tri-*O*-4-chlorobenzyl-α-D-*arabino*-hexopyrano-2-ulosyl bromide (3.7 g, 6.02 mmol) in dichloromethane (20 mL), was added to a round bottom flask containing 3 Å molecular sieves, dichloromethane (20 mL), **112** (0.97 g, 1.9 mmol) and tri-*tert*-butyl pyrimidine (1.86 g, 7.5 mmol). The solution was cooled to –40 °C under argon, and the reaction was initiated with the addition of silver triflate (1.70 g, 6.6 mmol). The reaction was warmed up to room temperature over 8 h. The solution was filtered, washed with sodium bicarbonate (50 mL, saturated), dried over sodium sulphate, concentrated, and dried in vacuo. The resulting syrup was dissolved in anhydrous THF (40 mL) cooled to –78 °C and L-selectride was added (12 mL, 1M in THF). The solution was warmed to room temperature and methanol (5 mL) was added. The solution was concentrated and diluted with dichloromethane (40 mL) and the organic layer was washed with 1M NaOH (20 mL) and 5% sodium thiosulphate (40 mL), water (50 mL), dried over sodium sulphate and concentrated. The crude product was subjected to column chromatography (3:1 hexanes:ethyl acetate) that yielded a white crystalline solid (1.46 g, 74% 2 steps): $[\alpha]_D$ -35.5 (*c* 1.7, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41-7.08 (m, 27H, Ar), 5.79 (dddd, 1H, CH_2=C<u>H</u>-CH_2-CH_2-CH_2-O, J = 16.8, 10.2, 6.6, 6.6 Hz), 5.03-4.95 (m, 2H, C<u>H$_2$</u>=CH-CH_2-CH_2-CH_2-O), 4.94 (d, 1H, H-1``, $J_{1,2}$ = 0.7 Hz), 4.89 (d, 1H, C<u>H$_2$</u>Ph, J = 11.3 Hz),

4.86 (d, 2H, C$\underline{H_2}$Ph, J = 11.3 Hz), 4.77 (d, 1H, C$\underline{H_2}$Ph, J = 12.1 Hz), 4.62 (d, 1H, C$\underline{H_2}$Ph, J = 12.0 Hz), 4.55 (d, 1H, C$\underline{H_2}$Ph, J = 12.1 Hz), 4.54 (d, 1H, C$\underline{H_2}$Ph, J = 12.0 Hz), 4.50 (d, 1H, C$\underline{H_2}$Ph, J = 11.3 Hz), 4.48-4.41 (m, 4H, 3 x C$\underline{H_2}$Ph, H-2`), 4.38-4.34 (m, 2H, C$\underline{H_2}$Ph, H-1`), 4.31 (dd, 1H, H-2``, $J_{1,2}$ = 0.7 Hz, $J_{2,3}$ = 2.9 Hz), 3.95 (dd, 1H, $CH_2$=$CH_2$-$CH_2$-$CH_2$-C$\underline{H_2}$-O, $J$ = 6.4, 9.4 Hz), 3.90 (app. t, 1H, H-4``, $J_{3,4} = J_{4,5}$ = 9.2 Hz), 3.78 (app. t, 1H, H-4`, $J_{3,4} = J_{4,5}$ = 9.4 Hz), 3.77 (dd, 1H, H-6a`, $J_{5,6}$ = 1.9 Hz, J_{gem} = 10.9 Hz), 3.73 (m, 2H, H-6a``, H-6b`), 3.65 (dd, 1H, H-6b``, $J_{5,6}$ = 5.6 Hz, J_{gem} = 10.6 Hz), 3.56 (dd, 1H, H-3`, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.4 Hz), 3.51 (dd, 1H, H-3``, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.2 Hz), 3.47-3.39 (m, 3H, H-5`, H-5``, CH_2=CH_2-CH_2-CH_2-C$\underline{H_2}$-O), 2.12-2.05 (m, 2H, CH_2=CH_2-C$\underline{H_2}$-CH_2-CH_2-O), 1.70-1.62 (m, 2H, CH_2=CH_2-CH_2-C$\underline{H_2}$-CH_2-O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.27, 138.23, 138.01, 137.77, 136.52, 136.45, 133.50, 133.49, 133.34, 129.17, 129.13, 129.94, 128.57, 128.53, 128.45, 128.34, 128.32, 128.27, 128.09, 128.08, 127.86, 127.62, 127.59, 115.11, 101.01 ($^1J_{C1,H1}$ = 163.6 Hz), 99.30 ($^1J_{C1,H1}$ = 154.0 Hz), 81.51, 80.43, 75.55, 75.01, 74.30, 74.21, 74.09, 73.47, 72.56, 70.95, 70.18, 69.91, 69.76, 69.36, 69.22, 67.55, 28.84; ES (Positive) HRMS calcd. for $C_{59}H_{63}O_{11}Cl_3Na$ ($M + Na^+$): 1075.3333; found 1075.3331; Anal. Calcd. for $C_{59}H_{63}O_{11}Cl_3Na$: C, 67.20; H, 6.02; found: C, 67.39; H, 5.94.

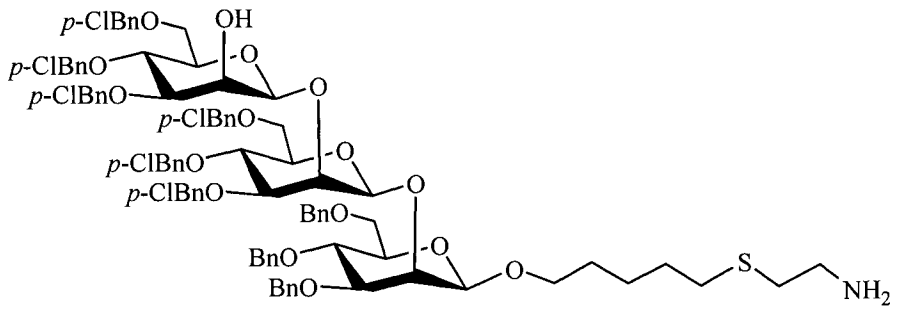
Pentenyl (3,4,6-tri-O-4-chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-O-4-chlorobenzyl-β-D-mannopyranosyl)(1→2)- 3,4,6-tri-O-benzyl-β-D-mannopyranoside ***116***



The procedure was analogous to that used for the preparation of **115**. Glycosyl donor, 3,4,6-tri-*O*-4-chlorobenzyl-α-D-*arabino*-hexopyrano-2-ulosyl bromide (1.60 g, 2.61 mmol), glycosyl acceptor, **115** (0.50 g, 0.474 mmol), tri-*tert*-butyl pyrimidine (0.809 g, 3.3 mmol) and silver triflate (0.737 g, 2.87 mmol) were dissolved in anhydrous acetonitrile (50 mL) containing 3 Å molecular sieves. Reduction of the resulting ulose proceeded with L-selectride (6 mL, 1M in THF) and the crude trisaccharide was purified by silica gel column chromatography (5:1 toluene:ethyl acetate) to yield a colourles syrup (0.495 g, 66%-2 steps): $[\alpha]_D$ - 51.1 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.42-7.46 (m, 2H, Ar), 7.31-7.04 (m, 32H, Ar), 6.99-6.89 (m, 5H, Ar), (dddd, 1H, CH_2=C<u>H</u>-CH_2-CH_2-CH_2-O, *J* = 17.0, 10.3, 6.7, 6.7 Hz), 5.17 (s, 1H, H-1```), 5.12 (s, 1H, H-1``), 5.05-4.97 (m, 2H, C<u>H$_2$</u>=CH-$CH_2$-$CH_2$-$CH_2$-O), 4.91 (d, 1H, C<u>H$_2$</u>Ph, *J* = 10.0 Hz), 4.90 (d, 1H, C<u>H$_2$</u>Ph, *J* = 11.5 Hz), 4.85 (d, 1H, C<u>H$_2$</u>Ph, *J* = 11.2 Hz), 4.77-4.73 (m, 2H, 2 x C<u>H$_2$</u>Ph, *J* = 9.8 Hz and *J* = 11.6 Hz), 4.68 (d, 1H, H-2``, $J_{2,3}$ = 3.3 Hz), 4.65 (d, 1H, C<u>H$_2$</u>Ph, *J* = 12.3 Hz), 4.60 (d, 1H, H-2`, $J_{2,3}$ = 3.3 Hz), 4.50 (d, 1H, C<u>H$_2$</u>Ph, *J* = 11.3 Hz), 4.49 (d, 1H, C<u>H$_2$</u>Ph, *J* = 12.1 Hz), 4.46 (d, 1H, C<u>H$_2$</u>Ph, *J* = 12.1 Hz), 4.43 (d, 1H, C<u>H$_2$</u>Ph, *J* = 10.9 Hz), 4.41 (s, 1H, H-1`), 4.38 (d, 2H, 2 x C<u>H$_2$</u>Ph, *J* = 12.3 Hz), 4.34 (d, 1H, C<u>H$_2$</u>Ph, *J* = 11.4 Hz), 4.33 (d, 1H, C<u>H$_2$</u>Ph, *J* = 10.0 Hz), 4.27 (d, 1H, C<u>H$_2$</u>Ph, *J* = 11.6 Hz), 4.26 (d, 1H, H-2```, $J_{2,3}$ = 3.7 Hz), 4.22 (d, 1H, C<u>H$_2$</u>Ph, *J* = 10.1 Hz), 4.08 (d, 1H, C<u>H$_2$</u>Ph, *J* = 11.9 Hz), 3.97 (dd, 1H, CH_2=CH_2-CH_2-CH_2-C<u>H$_2$</u>-O, *J* = 6.2, 9.3 Hz), 3.90 (app. t, 1H, H-4``, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz), 3.87 (d, 1H, C<u>H$_2$</u>Ph, *J* = 11.9 Hz), 3.82 (app. t, 1H, H-4```, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz), 3.77-3.59 (m, 9H, H-3`, H-4`, H-6a`, H-6b`, H-6a``, H-6b``, H-6a```, H-6b```), 3.56 (dd, 1H, H-3``, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.3 Hz), 3.50-3.38 (m, 6H, H-3```, H-5`, H-5``, H-5```, CH_2=CH_2-CH_2-CH_2-C<u>H$_2$</u>-O), 2.12-2.05 (m, 2H, CH_2=CH_2-

$C\underline{H}_2$-CH_2-CH_2-O), 1.70-1.62 (m, 2H, CH_2=CH_2-CH_2-$C\underline{H}_2$-CH_2-O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.00, 137.88, 137.83, 137.71, 136.88, 136.84, 136.62, 136.54, 136.52, 136.49, 133.49, 138.48, 133.44, 133.38, 133.34, 133.29, 133.02, 129.52, 129.17, 129.07, 129.04, 128.97, 128.93, 128.63, 128.56, 128.52, 128.49, 128.37, 128.35, 128.32, 128.22, 128.09, 115.22, 101.74 ($^1J_{C1,H1}$ = 163.9 Hz), 100.05 ($^1J_{C1,H1}$ = 161.2 Hz), 99.88 ($^1J_{C1,H1}$ = 153.7 Hz), 83.01. 80.66, 80.41, 75.38, 75.27, 75.14, 74.88, 74.69, 74.33, 74.27, 74.21, 74.69, 74.34, 74.27, 74.21, 73.45, 72.66, 72.58, 71.21, 70.07, 69.79, 69.74, 69.63, 69.60, 69.20, 68.83, 68.76, 67.14, 30.19, 28.75; ES (Positive) HRMS calcd. for $C_{86}H_{88}O_{16}Cl_6Na$ (M + Na^+): 1609.4101; found 1609.4100; Anal. Calcd. for $C_{86}H_{88}O_{16}Cl_6$: C, 64.95; H, 5.58; found: C, 64.76; H, 5.50.

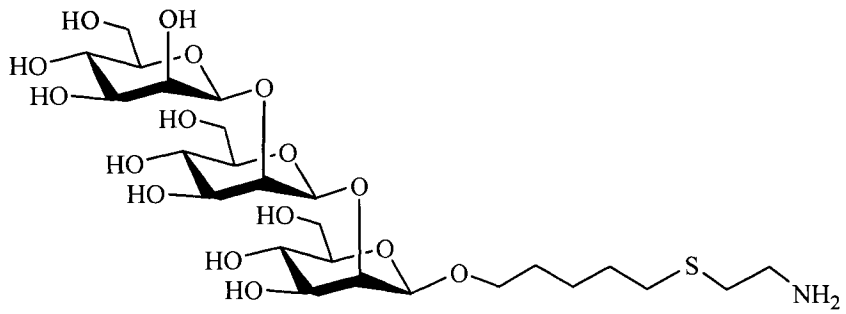
*5-(2-Aminoethylthio)-pentyl (3,4,6-tri-O-4-chlorobenzyl-*β*-D-mannopyranosyl)(1→2)-(3, 4,6-tri-O-4-chlorobenzyl-*β*-D-mannopyranosyl)(1→2)- 3,4,6-tri-O-benzyl-*β*-D-mannopyranoside **117***



In a quartz tube, 2-aminoethylthiol hydrochloride (500 mg, 4.24 mmol) was added to a solution of **116** (106 mg, 0.067 mmol) in methanol (5 mL) and dichloromethane (1 mL). The solution was vigorously stirred until the 2-aminoethylthiol hydrochloride was dissolved and then purged with argon for 2 min. The reaction proceeded under UV light (365 nm) for 14 h. The reaction was concentrated, diluted with dichloromethane (30 mL) and the organic layer was washed with water (30 mL), dried over sodium sulphate and concentrated. Column chromatography (97:3 dichloromethane:methanol) gave a

colourless syrup (91 mg, 82%): $[\alpha]_D$ - 57.7 (*c* 0.76, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.42-7.46 (m, 2H, Ar), 7.31-7.04 (m, 32H, Ar), 6.99-6.89 (m, 5H, Ar), 5.15 (s, 1H, H-1```), 5.13 (s, 1H, H-1``), 4.91 (d, 1H, C$\underline{H}_2$Ph, *J* = 9.9 Hz), 4.90 (d, 1H, C$\underline{H}_2$Ph, *J* = 11.2 Hz), 4.85 (d, 1H, C$\underline{H}_2$Ph, *J* = 11.2 Hz), 4.74 (d, 1H, C$\underline{H}_2$Ph, *J* = 11.2 Hz), 4.73 (d, 1H, C$\underline{H}_2$Ph, *J* = 9.9 Hz), 4.69-4.61 (m, 4H, H-2``, H-2`, 2 x C$\underline{H}_2$Ph), 4.52-4.46 (m, 3H, 3 x C$\underline{H}_2$Ph), 4.45-4.35 (m, 5H, H-1`, 4 x C$\underline{H}_2$Ph), 4.34-4.28 (m, 2H, 2 x C$\underline{H}_2$Ph), 4.26 (d, 1H, H-2```, $J_{2,3}$ = 1.8 Hz), 4.20 (d, 1H, C$\underline{H}_2$Ph, *J* = 10.1 Hz), 4.03 (d, 1H, C$\underline{H}_2$Ph, *J* = 11.9 Hz), 3.97 (dt, 1H, S-CH_2-CH_2-CH_2-CH_2-C$\underline{H}_2$-O, *J* = 6.27, 6.27, 9.35 Hz), 3.93 (app. t, 1H, H-4``, $J_{3,4} = J_{4,5}$ = 9.4 Hz), 3.83 (d, 1H, CH_2Ph, *J* = 12.0 Hz), 3.81 (t, 1H, H-4```, $J_{3,4} = J_{4,5}$ = 9.3 Hz), 3.77-3.56 (m, 9H, H-3`, H-3``, H-4`, H-6a`, H-6b`, H-6a``, H-6b``, H-6a```, H-6b```), 3.50-3.44 (m, 2H, H-5``, H-5```), 3.43-3.38 (m, 3H, H-3```, H-5`, S-CH_2-CH_2-CH_2-CH_2-C$\underline{H}_2$-O), 2.81 (t, 2H, NH_3-C$\underline{H}_2$-CH_2-S), 2.55 (t, 2H, NH_3-CH_2-C$\underline{H}_2$-S), 2.50 (t, 2H, S-C$\underline{H}_2$-CH_2-CH_2-CH_2-CH_2-O), 1.64-1.56 (m, 4H, S-CH_2-C$\underline{H}_2$-CH_2-CH_2-CH_2-O, S-CH_2-CH_2-CH_2-C$\underline{H}_2$-CH_2-O), 1.50-1.37 (m, 2H, S-CH_2-CH_2-C$\underline{H}_2$-CH_2-CH_2-O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.99, 137.85, 137.81, 136.89, 136.87, 136.68, 136.55, 136.53, 136.50, 133.47, 133.43, 133.37, 133.33, 133.28, 132.99, 129.53, 129.19, 129.12, 129.05, 128.94, 128.61, 128.55, 128.52, 128.48, 128.44, 128.39, 128.35, 128.32, 128.21, 128.14, 128.03, 127.77, 127.73, 127.70, 101.80 ($^1J_{C1,H1}$ = 163.5 Hz), 99.95 ($^1J_{C1,H1}$ = 161.7 Hz), 99.93 ($^1J_{C1,H1}$ = 153.3 Hz), 83.08, 80.70, 80.45, 75.39, 75.24, 75.14, 74.89, 74.70, 74.37, 74.33, 74.27, 74.22, 73.47, 72.68, 72.58, 71.33, 70.18, 70.07, 69.81, 69.75, 69.32, 69.16, 68.86, 68.75, 67.11, 41.19, 36.24, 31.75, 29.30, 29.15, 25.16; ES (Positive) HRMS calcd. for $C_{88}H_{96}NO_{16}SCl_6$ ($M + H^+$): 1664.4581; found 1664.4580

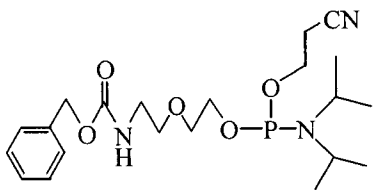
5-(2-Aminoethylthio)-pentyl (β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside ***118***



Ammonia was condensed at –78 °C into a 250 mL 3-neck round bottom flask under argon. Sodium metal (100 mg) was then added to the solution and, once the blue colour persisted, the ammonia was distilled into a second 3-neck round bottom flask. Sodium metal was added again to the distilled ammonia and stirred vigorously with a glass stir rod. Once the blue colour persisted, **117** (96 mg, 0.06 mmol) in anhydrous THF was added. The reaction mixture was stirred for 30 min at –78 °C, methanol (2 mL) was added and the solution was warmed to room temperature. Once the ammonia had evaporated, the mixture was neutralized using acetic acid. The mixture was concentrated and applied to a Sep-pak column and eluted with 10% methanol in water. The crude product was purified by reversed phase HPLC (0 to 20% methanol in water with 2% AcOH) to yield a clear glass (21 mg, 57%). The free amine was obtained from the amino-acetic acid salt by passing the compound through a IR-400 (OH^-) column: ^1H NMR (600 MHz, D_2O) δ 4.95 (s, 1H, H-1```), 4.90 (s, 1H, H-1``), 4.71 (s, 1H, H-1`), 4.35 (d, 1H, H-2``, $J_{1,2}$ = 2.7 Hz), 4.22 (d, 1H, H-2`, $J_{1,2}$ = 2.7 Hz), 4.15 (d, 1H, H-2```, $J_{1,2}$ = 2.8 Hz), 3.95-3.90 (m, 4H, S-CH_2-CH_2-CH_2-CH_2-$C\underline{H}_2$-O, H-6a`, H-6a``, H-6a```), 3.78-3.71 (m, 3H, H-6b`, H-6b``, H-6b```), 3.71-3.67 (m, 1H, H-3``), 3.67-3.55 (m, 5H, H-3, H-3, H-4`, H-4```, S-CH_2-CH_2-CH_2-CH_2-$C\underline{H}_2$-O), 3.51-3.45 (app. t, 1H, H-4``, $J$ = 9.4 Hz), 3.40-3.34 (m, H-5`, H-5``, H-5```), 2.80 (t, 2H, NH_3-$C\underline{H}_2$-CH_2-S) 2.68-2.64 (m,

2H, NH_3-CH_2-$C\underline{H_2}$-S), 2.62 (t, 2H, S-$C\underline{H_2}$-CH_2-CH_2-CH_2-CH_2-O, J = 7.1 Hz), 1.67-1.61 (m, 4H, S-CH_2-CH_2-CH_2-$C\underline{H_2}$-CH_2-O, S-CH_2-$C\underline{H_2}$-CH_2-CH_2-CH_2-O), 1.50-1.44 (m, 2H, S-CH_2-CH_2-$C\underline{H_2}$-CH_2-CH_2-O); ^{13}C NMR (125 MHz, D_2O) δ 102.01 ($^1J_{C1,H1}$ = 163.7 Hz), 101.75 ($^1J_{C1,H1}$ = 161.7 Hz), 100.99 ($^1J_{C1,H1}$ = 158.0 Hz), 79.83, 79.09, 77.25, 77.09, 73.86, 73.20, 72.92, 71.30, 70.77, 68.33, 67.87, 67.62, 61.96, 61.77, 61.66, 58.32, 40.51, 34.16, 31.78, 29.28, 25.52, 17.69; ES (Positive) HRMS calcd. for $C_{25}H_{48}NO_{16}S$ ($M + H^+$): 650.2688; found 650.2682.

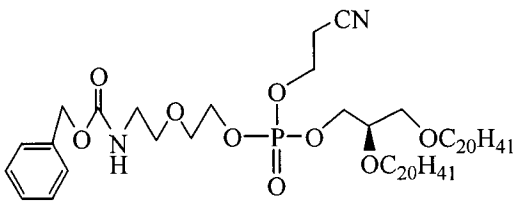
2-(2-(N-Benzyloxycarbonyl)aminoethoxy)ethyl 2-cyanoethyl diisopropyl phosphoramidate ***119***



2-Cyanoethyl tetraisopropyl phosphorodiamidate **98** (118 mg, 0.39 mmol) and 2-*N*-Cbz-aminoethyl ethanol (140 mg, 0.58 mmol) were co-concentrated with toluene and dried in vacuo for 3 h. The dried mixture was then dissolved into anhydrous dichloromethane (3 mL) under argon and the reaction was initiated with the addition of tetrazole (460 μL, 0.19 mmol, 0.5 eq., 3% in acetonitrile). After 45 min a precipitate formed, the reaction was diluted with dichloromethane (3 mL) and washed with sodium bicarbonate (5 mL, 5% solution). The organic layer was dried over sodium sulphate, concentrated and the product was quickly passed through a silica gel column (5% triethylamine in 2:1 ethyl acetate:hexanes). The purified phosphoramidate was obtained as a clear syrup (129 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.28 (m, 5H, Ar), 5.33 (br s, 1H, ZN$\underline{H}$), 5.07 (s, 2H, $C\underline{H_2}$Ph), 3.85-3.66 (m, 4H, 2 x CH_2), 3.65-3.58 (m, 4H, 2 x $CH_{(isopropyl)}$, CH_2), 3.57-3.53 (m, 2H, ZNH-CH_2-$C\underline{H_2}$-O), 3.38-3.33 (m, 2H, ZNH-$C\underline{H_2}$-CH_2-O), 2.57 (t, 2H,

CN-C$\underline{H}_2$-CH_2-O, J = 6.1 Hz), 1.18 (d, 12H, $CH_{3(isopropyl)}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.40, 136.58, 128.48, 128.15, 128.09, 117.62, 71.00, 69.86, 66.66, 62.74, 62.61, 58.46, 58.30, 43.09, 42.99, 40.88, 24.56, 20.25; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 149.62; ES (Positive) MS calcd. for $C_{21}H_{35}O_5P$ ($M + H^+$): 440.2308; found 440.2308.

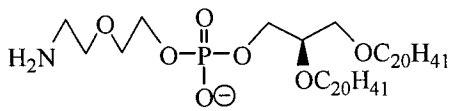
3-O-[2-(2-(N-Benzyloxycarbonyl)aminoethoxy)ethyl 2-cyanoethyl phosphonato]-(2R)-1,2-di-O-[(3R/S,7R,11R)-3,7,11,15-tetramethylhexadecyl] sn-glycerol ***120***



119 (99 mg, 0.22 mmol) and diphytanyl glycerol **78** (176 mg, 0.27 mmol) were co-concentrated in toluene and dried in vacuo for 3 h. The reagents were dissolved in anhydrous dichloromethane (3 mL) under argon, followed by the addition of tetrazole (270 μL, 0.8 mmol, 3% in acetonitrile). After 1 h, *tert*-butyl hydrogen peroxide (100 μL, 5.0-6.0M in decane) was added to the stirring solution. The reaction solution was diluted with dichloromethane (3 mL) and washed with sodium bicarbonate (10 mL, 5% solution). The organic layer was dried over sodium sulphate and concentrated under reduced pressure. The crude product was eluted with 4:1 hexanes:ethyl acetate from a silica gel column to yield a clear syrup (184 mg, 81%) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.29 (m, 5H, Ar), 5.55 (br. s, 1H, ZN$\underline{H}$), 5.15-5.07 (m, 2H, C$\underline{H}_2$Ph), 4.27-4.15 (m, 5H, CN-CH_2-C$\underline{H}_2$-O, O-CH_2-C$\underline{H}_2$-O-P, $CH_{2a(glycerol)}$), 4.12-4.05 (m, 1H, $CH_{2a'(glycerol)}$), 3.67 (t, 2H, O-C$\underline{H}_2$-CH_2-O-P), 3.64-3.35 (m, 11H, 2 x O-C$\underline{H}_2$-alkyl, ZNH-C$\underline{H}_2$-CH_2-O, ZNH-CH_2-C$\underline{H}_2$-O, $CH_{(glycerol)}$, $CH_{2b(glycerol)}$), 2.69-2.63 (m, 2H, CN-C$\underline{H}_2$-CH_2-O), 1.69-1.46 (m, 6H, O-CH_2-$\underline{alkyl}$), 1.42-1.00 (m, 42H, O-CH_2-$\underline{alkyl}$), 0.89-0.81 (m, 30H, CH_3); ^{13}C NMR

(125 MHz, $CDCl_3$) δ 156.50, 136.67, 128.49, 128.12, 128.07, 116.36, 70.19, 70.02, 69.66, 69.61,69.53, 69.48, 69.06, 68.97, 67.74, 67.68, 67.62, 67.11, 66.62, 61.81, 45.66, 45.63, 40.88, 39.37, 37.63, 37.54, 37.47, 37.42, 37.40, 37.30, 37.13, 37.04, 36.95, 36.61, 32.81, 29.95, 29.88, 29.81, 27.97, 24.80, 24.49, 24.40, 24.38, 23.48, 22.86, 22.71, 22.62, 19.74, 19.68, 19.61, 19.59, 19.53, 19.48; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ -0.293, -0.339; ES (Positive) HRMS calcd. for $C_{58}H_{107}N_2O_9PNa$ ($M + Na^+$): 1029.761192; found 1029.761176; Anal. calcd. for $C_{104}H_{136}O_{20}$: C, 69.15; H, 10.71; N, 2.78; found: C, 68.77; H, 10.35; N, 2.81.

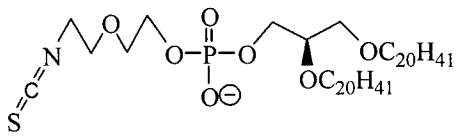
3-O-[2-(2-Aminoethoxy)ethyl phosphonato]-(2R)-1,2-di-O-[(3R/S,7R,11R)-3,7,11,15-tetramethylhexadecyl] sn-glycerol ***121***



To a solution of **120** (45 mg, 0.04 mmol) in distilled MeOH (0.5 mL) and cyclohexane (1 mL) was added 10 drops of NaOMe (1M). Palladium hydroxide (5 mg) was added after 1 h and the reaction flask was flushed with hydrogen gas. A hydrogenation balloon was fixed to the top of the round bottom flask and the reaction was stirred for 10 h. The reaction solution was passed through a 0.45 μm filter, concentrated and purified by column chromatography (6:1 dichloromethane:methanol) to a clear glass (25 mg, 69%). ^1H NMR (500 MHz, $CDCl_3$) δ 8.72 (br. s, 2H, NH_2), 4.07-4.01 (m, 2H, O-CH_2-C$\underline{H_2}$-O-P), 3.88 (t, 2H, $CH_{2(glycerol)}$, J = 5.1 Hz), 3.76 (t, 2H, NH_2-CH_2-C$\underline{H_2}$-O, J = 4.3 Hz), 3.68 (t, 2H, O-C$\underline{H_2}$-CH_2-O-P, J = 3.4 Hz), 3.64-3.50 (m, 4H, O-C$\underline{H_2}$-alkyl, $CH_{2(glycerol)}$), 3.50-3.41 (m, 3H, O-C$\underline{H_2}$-alkyl, $CH_{(glycerol)}$), 3.08 (br. s, 2H, NH_2-C$\underline{H_2}$-CH_2-O), 1.64-1.46 (m, 6H, O-CH_2-alkyl), 1.42-1.00 (m, 42H, O-CH_2-alkyl), 0.89-0.81 (m, 30H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 78.00, 70.89, 70.65, 70.03, 68.81, 68.72, 66.88, 65.54, 65.00,

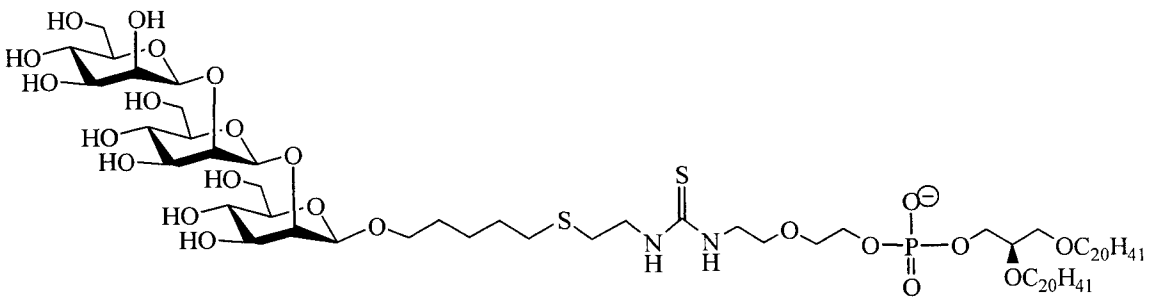
39.37, 39.15, 37.65, 37.53, 37.44, 37.30, 36.84, 36.81, 36.76, 32.82, 32.80, 29.98, 29.86, 28.00, 27.96, 24.80, 24.49, 24.45, 24.39, 22.71, 22.62, 19.74, 19.67, 19.61; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 2.19; ES (Positive) HRMS calcd. for $C_{47}H_{99}NO_7P$ (M + H): 820.7153; found 820.7150.

*3-O-[2-(2-(Isothiocyanate)ethoxy)ethyl phosphonato]-(2*R*)-1,2-di-O-[(3*R/S*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl] sn-glycerol* ***122***



Compound **121** (53 mg, 0.064 mmol) was dissolved in anhydrous dichloromethane (3 mL) under argon. To this stirring solution was added 1,1'-thiocarbonyldi-2(1*H*)-pyridone (18 mg, 0.077 mmol) and DMAP (cat.). After 12 h, the solution was concentrated and applied directly to a silica gel column (9:1 dichloromethane:methanol) and the purified product was obtain as a clear glass (41 mg, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.09 (br. s, 2H, $CH_{2(ethanolethylamine)}$), 4.02-3.88 (m, 2H, $CH_{2(glycerol)}$), 3.74 (br s., 6H, 3 x $CH_{2(ethanolethylamine)}$), 3.67-3.44 (m, 7H, 2 x O-C<u>H</u>$_2$-alkyl, $CH_{2(glycerol)}$, $CH_{(glycerol)}$), 1.64-1.46 (m, 6H, O-CH_2-<u>alkyl</u>), 1.42-1.00 (m, 42H, O-CH_2-<u>alkyl</u>), 0.89-0.81 (m, 30H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 132.30, 70.75, 70.70, 70.21, 69.17, 69.06, 68.86, 64.90, 45.27, 39.39, 37.88, 37.75, 37.63, 37.51, 37.44, 37.33, 36.94, 36.85, 32.90, 32.85, 30.11, 30.01, 29.69, 27.98, 24.81, 24.56, 22.72, 22.63, 19.74, 19.67, 19.60, 19.53; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ -2.25; ES (Negative) HRMS calcd. for $C_{48}H_{95}NO_7PS$: 860.6572; found 860.6570.

*5-(2-[3-O-[(2-(2-(thiourea)ethoxy)ethyl) phosphonato]-(2*R*)-1,2-di-O-[(3*R/S*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl] sn-glycerol]ethylthio)-pentyl (*β-D-*mannopyranosyl)(1→2)(*β-D-*mannopyranosyl)(1→2)-*β-D-*mannopyranoside*
123



Compounds **118** (12 mg, 0.018 mmol) and **122** (36 mg, 0.042 mmol) were co-evaporated with toluene (5 mL x 2) and dried in vacuo for 2 h. The mixture was then dissolved into a dimethylformamide and dichloromethane mixture (1:1, 1 mL) under argon. Pyridine (1 mL) and DMAP (cat.) were added and the solution was stirred for 12 hours. TLC showed that the trisaccharide had been completely consumed. The reaction mixture was concentrated *in vacuo*. The crude product purified by Iatrobeads column chromatography (100% chloroform to 85% chloroform/13% MeOH/2% H_2O). The product was passed through a LH-20 Sephadex column using 1:1 mixture of chloroform and methanol. The purified product was passed through a 0.45 μm filter and concentrated to yield a clear glass (21 mg, 72%). ^{1}H NMR (500 MHz, 50% $CDCl_3$ and 50% CD_3OD) δ 4.93 (s, 1H, H-1```), 4.84 (s, 1H, H-1``), 4.50 (s, 1H, H-1`), 4.21 (d, 1H, H-2``, $J_{1,2}$ = 3.6 Hz), 4.10 (d, 1H, H-2```, $J_{1,2}$ = 3.2 Hz), 4.07 (d, 1H, H-2`, $J_{1,2}$ = 1.7 Hz), 4.01-3.97 (m, 2H, $CH_{2(ethanolethylamine)}$), 3.92 (dt, 1H, S-$CH_2$-$CH_2$-$CH_2$-$CH_2$-C$\underline{H_2}$-O, $J$ = 5.5, 5.5, 9.1 Hz), 3.86 (t, 2H, $CH_{2(glycerol)}$), 3.84-3.76 (m, 6H, H-6a`, H-6a``, H-6a```, H-6b`, H-6b``, H-6b```), 3.73 (app. t, 1H, H-4``, $J_{3,4}$ = $J_{4,5}$ 9.7 Hz), 3.70-3.43 (m, 21H, H-3`, H-3``, H-3```, H-4`, H-4```, 2 x O-C$\underline{H_2}$-alkyl, NH-C$\underline{H_2}$-CH_2-S, S-CH_2-CH_2-CH_2-CH_2-C$\underline{H_2}$-O, $CH_{2(glycerol)}$, $CH_{(glycerol)}$, 3 x $CH_{2(ethanolethylamine)}$), 3.28-3.22 (m, 2H, H-5``, H-5```), 3.15 (ddd, 1H, H-5`) 2.72 (t, 2H, NH-CH_2-C$\underline{H_2}$-S, J = 6.92 Hz), 2.62 (t, 2H, S-C$\underline{H_2}$-CH_2-CH_2-CH_2-CH_2-O, J = 6.92 Hz), 1.67-1.44 (m, 12H, S-CH_2-C$\underline{H_2}$-CH_2-CH_2-CH_2-O, S-CH_2-CH_2-CH_2-C$\underline{H_2}$-CH_2-

O, S-CH_2-CH_2-$C\underline{H_2}$-CH_2-CH_2-O, 6 x O-CH_2-alkyl), 1.40-1.00 (m, 42H, O-CH_2-alkyl), 0.88-0.80 (m, 30H, CH_3); ^{13}C NMR (125 MHz, 50% $CDCl_3$ and 50% CD_3OD) δ 178.60, 102.41 ($^1J_{C1,H1}$ = 163.7 Hz), 102.11 ($^1J_{C1,H1}$ = 161.0 Hz), 101.90 ($^1J_{C1,H1}$ = 155.7 Hz), 79.64, 79.62, 79.55, 79.54, 79.51, 79.44, 77.85, 77.80, 77.73, 74.57, 73.94, 73.90, 72.05, 72.04, 72.03, 72.01, 71.99, 71.77, 71.37, 71.35, 71.03, 70.81, 70.36, 70.35, 70.14, 68.70, 67.90, 67.89, 67.84, 67.82, 66.39, 66.37, 66.35, 66.33, 66.17, 66.16, 66.15, 66.12, 62.32, 61.97, 61.96, 61.66, 40.83, 38.82, 38.81, 38.78, 38.77, 38.76, 38.73, 38.71, 38.69, 38.67, 38.66, 38.63, 38.57, 38.08, 38.02, 34.15, 34.12, 34.10, 34.07, 34.05, 33.29, 33.28, 31.25, 31.23, 31.11, 30.50, 30.41, 29.25, 26.64, 26.07, 25.75, 25.70, 23.73, 23.63, 20.85, 20.82, 20.80, 20.78, 20.75, 20.74, 20.72, 20.71, 20.69, 20.67, 20.64; ^{31}P NMR (202.3 MHz, 50 % $CDCl_3$ and 50 % CD_3OD) δ 1.47; ES (Negative) HRMS calcd. for $C_{73}H_{142}N_2O_{23}PS_2$: 1509.9188; found 1509.9196

6.2 Immunological Experimental:

Phosphate buffered saline (PBS) was made with milli-Q water and adjusted to the desired pH 7.2. PBS used in the preparation of the liposomes, washing of the lipid pellet after centrifugation and resuspension of the lipid pellet was filtered through a 0.22 μm filter. The round bottom flasks used to rehydrate the lipid film were placed in a 250 °C oven for at least 6 h prior to use. The ovalbumin (Grade V, Sigma-Aldrich) used in all experiments was used without further purification. The conventional lipids phosphatidylcholine, DSPC, and cholesterol (Avanti Polar Lipids) were all used without further purification.

6.3 Liposome Preparation:

General procedure: Lipids (according to Table 6.1) were weighed and dissolved into chloroform or a chloroform/methanol mixture and pipetted into a 50 mL round bottom flask. The solvent was evaporated and the lipid film was further dried in vacuo for no less than 3 h. The lipid film was hydrated using a solution of the protein antigen OVA in PBS (2 mL) for 6-10 h. Lipid films that consisted of archaeal lipids needed longer hydration times. The lipid mixture was briefly sonicated. The lipid mixture was passed through a liposome extruder (Avanti Polar Lipids) with a minimum of 15 passes. The lipid mixture was centrifuged (100,000 x g_{max}, 25 min or 20,000 rpm, 45 min) and the lipid pellet was washed with PBS (5-8 mL) and centrifuged again for a total of three washes. The resulting lipid pellet was taken up in PBS and stored at 4 °C until used. Encapsulation of the protein antigen was quantified using a Micro BCA Protein Assay Kit (MJS Biolynx Inc.) and was compared to a standard curve constructed for protein concentrations ranging from 2.5 – 40 μg/mL.

Table 6.1: Liposome formulations

Formu -lation	Adjuvant	Archaea Lipid Amount	PC	DSPC	Chol- estrol	Final Extrusion Filter Size	Antigen Dose (ug)	# of mice
1	Freunds	NA	0	0	0	NA	1^{st} and 2^{nd} - 25	4
2	NA	NA	0	0	0	NA	1^{st} and 2^{nd} - 25	4
3	Conven- tional Lipids	0	1.33 eq.	1 eq.	1 eq.	400 nm	1^{st} and 2^{nd} -10	7
4	**5**	1 eq.	1.5 eq.	1 eq.	1.5 eq.	400 nm	1^{st} and 2^{nd} - 28	7
5	**6**	1 eq.	1.5 eq.	1 eq.	1.5 eq.	400 nm	1^{st} and 2^{nd} - 12	7
6	**6 + 8**	1 eq.	0	1.17 eq.	1.33 eq.	400 nm	1^{st}-22.5 2^{nd}-25	5
7	**8 + 9**	1 eq.	0	1.17 eq.	1.33 eq.	400 nm	1^{st}-22.5 2^{nd}-24	5
8	7	1.87 eq.	0	1 eq.	1.14 eq.	400 nm	1^{st}-18.6 2^{nd}-12	5
9	**8**	1.87 eq.	0	1 eq.	1.14 eq.	400 nm	1^{st}-18 2^{nd}-17	11
10	β- mannan archaeol phospho -lipid **123**	1 eq.	0	1.86 eq.	1.14 eq.	100 nm	1^{st}-10 2^{nd}-8	5

6.4 Animal Injection:

BALB/c mice were housed and cared for in the Biological Sciences Animal Services Laboratory. Mice were immunized on day 0 (1^{st} injection) and day 21 (2^{nd} injection) with the liposome formulation containing antigen *via* combined subcutaneous and intraperitoneal injections. On day 28 the mice were sacrificed and their blood collected. The blood was centrifuged for 4 min at 14,000 rpm and the sera collected. Sera were centrifuged again for 4 min at 14,000 rpm and placed into eppendorf tubes and frozen until further use.

6.5 General ELISA procedure for the quantification of IgM and IgG antibody titers against ovalbumin:

A 96-well plate was coated with a stock solution (10 μg/mL) of ovalbumin in PBS (0.02 M PBS, 0.15M NaCl, pH 7.4) for 24 h at 4 °C. The OVA solution was then removed from the wells and the plate was washed with PBS-tween (0.01 M PBS, 0.15 M, 0.05 % tween). The plate was then blocked for 1 h by adding a solution of milk (100 μL, 2.5 % in PBS) to each well including controls. The wells were emptied and washed with the PBS-tween solution. The sera prebleeds and sera from the immunizations were diluted from 1:100 to 1:1,000,000 using the PBS-tween solution. Diluted sera were then added to the plate and it was allow to stand for 2 h. The sera dilutions were added to the plate in duplicates such that the IgM and IgG titers are displayed on the same plate. Next the sera dilutions were emptied from the wells and the plate was washed with PBS-tween. Goat anti mouse IgM/HRP and Goat anti mouse IgG/HRP were diluted 1:2,000 (3 μL in 6 mL PBS-tween) and added (100 μL) to the appropriate wells. After 1 h, the wells were emptied and the plate was washed with PBS-tween. A solution of TMB peroxidase substrate and perixodase solution B (100 μL, 1:1 mixture, 12 mL) was pipetted to each

well. After 1 min the colour developed and the reaction was terminated by adding phosphoric acid (100 μL) to each well. The plate was then read at 450 nm. The endpoint of the titration was read as the dilution giving an optical density reading of 0.2.

Chapter 7

Bibliography

1. Janeway, C. A.; Travers, P. *Immunobiology: The Immune System in Health and Disease*. Third ed.; Current Biology Ltd./Garland Publishing Inc.: New York, NY, **1997**.
2. Schijns, V. Mechanisms of vaccine adjuvant activity: initiation and regulation of immune responses by vaccine adjuvants. *Vaccine* **2003**, *21*, 829-831.
3. Marciani, D. Vaccine adjuvants: role and mechanisms of action in vaccine immunogenicity. *Drug Discovery Today* **2003**, *8*, (20), 934-942.
4. Singh, M.; O'Hagan, D. Advances in vaccine adjuvants. *Nature Biotechnology* **1999,** *17*, (11), 1075-1081.
5. Jennings, V. Review of selected adjuvants used in antibody production. *Institute for Laboratory Animal Research Journal* **1995**, *37*, (3).
6. Lindblad, E. Aluminium adjuvants-in retrospect and prospect. *Vaccine* **2004**, *22*, 3658-3668.
7. Becaria, A.; Campbell, A.; Bondy, S. Aluminum as a toxicant. *Toxicology and Industrial Health* **2002**, *18*, 309-320.
8. Gregoriadis, G. Liposomes as immunoadjuvants and vaccine carriers: Antigen entrapment. *Immunomethods* **1994**, *4*, 210-216.
9. Allison, A.; Gregoriadis, G. Liposomes as immunological adjuvants. *Nature* **1974**, *252*, 252.
10. Fries, L.; Gordon, D.; Richards, R.; Egan, J.; Hollingdale, M.; Gross, M.; Silverman, C.; Alving, C. Liposomal malaria vaccine in humans: A safe and potent adjuvant strategy. *Proceedings of the National Academy of Science of the United States of America* **1992**, *89*, 358-362.
11. Fox, G.; Magrum, L.; Balch, W.; Wolfe, R.; Woese, C. Classification of methanogenic bacteria by 16S ribosomal RNA characterization. *Proceedings of the National Academy of Science of the United States of America* **1977**, *74*, (10), 4537-4541.
12. Woese, C.; Fox, G. Phylogenetic structure of the prokaryotic domain: The primary kingdoms. *Proceedings of the National Academy of Science of the United States of America* **1977**, *74*, (11), 5088-5090.
13. Kushwaha, S.; Kates, M.; Sprott, G.; Smith, I. Novel complex polar lipids from the methanogenic archaebacterium *Methanospirillum hungatei*. *Science* **1981**, *211*, 1163-1164.
14. Kates, M.; Palameta, B.; Yengoyan, L. Aliphatic diether analogs of glyceride-derived lipids. II. Synthesis of naturally occuring L-2,3,-Di-*O*-3',7',11',15'-

tetramethylhexadecyl glycerol and its D isomer. *Biochemistry* **1965**, *4*, (8), 1595-1599.

15. Heathcock, C.; Finkelstein, B.; Jarvi, E.; Radel, P.; Hadley, C. 1,4- and 1,5-Stereoselection by sequential aldol addition to a α,β-unsaturated aldehydes followed by Claisen rearrangement. Application to total synthesis of the vitamin Eside chain and the archaebacterial C40 diol. *Journal of Organic Chemistry* **1988**, *53*, (9), 1922-1942.

16. Montenegro, E.; Gabler, B.; Paradies, G.; Seemann, M.; Helmchen, G. Determination of the configuration of an Archaea membrane lipid containing cyclopentane rings by total synthesis. *Angewandte Chemie International Edition* **2003**, *42*, 2419-2421.

17. Zhang, D.; Daniels, L.; Poulter, C. Biosynthesis of Archaebacterial membranes. Formation of isoprene ethers by a prenyl transfer reaction. *Journal of the American Chemical Society* **1990**, *112*, (3), 1264-1265.

18. Kakinuma, K.; Yamagishi, M.; Fujimoto, Y.; Ikekawa, N.; Oshima, T. Biosynthetic mechanism of *sn*-2,3-di-*O*-phytanyl glycerol, core membrane lipid Archaebacterium *Halobacterium halobium*. *Journal of the American Chemical Society* **1990**, *112*, (7), 2740-2745.

19. Zhang, D.; Poulter, C. Biosythesis of archaebacterial ether lipids. Formation of ether linkages by prenyltransferases. *Journal of the American Chemical Society* **1993**, *115*, (4), 1270-1277.

20. Koga, Y.; Nishihara, M.; Morii, H.; Akagawa-Matsushita, M. Ether polar lipids of methanogenic bacteria: Structure, comparative aspects, and biosytheses. *Microbiological Reviews* **1993**, *57*, (1), 164-182.

21. Morii, H.; Nishihara, M.; Koga, Y. CTP:2,3-di-*O*-geranylgeranyl-sn-glycero-1-phosphate cytidyltransferase in the methanogenic archaeon *Methanothermobacter thermoauttrophicus*. *The Journal of Biological Chemistry* **2000**, *275*, (47), 36568-36574.

22. Morii, H.; Koga, Y. CDP-2,3-di-*O*-geranylgeranyl-sn-glycerol:L-Serine *O*-archaetidyltransferase (archaetidylserine synthase) in the metanogenic Archaeon *Methanothermobacter thermautotrophicus*. *Journal of Bacteriology* **2003**, *185*, (4), 1181-1189.

23. Eguchi, T.; Morita, M.; Kakinuma, K. Multigram synthesis of mevalonolactone-d_9 and its application to stereochemical analysis by ^{1}H NMR of the saturation reaction in the biosynthesis of the 2,3-di-*O*-phytanyl-*sn*-glycerol core of the archaeal membrane lipid. *Journal of the American Chemical Society* **1998**, *120*, (22), 5427-5433.

24. Eguchi, T.; Takyo, H.; Morita, M.; Kakinuma, K.; Koga, Y. Unusual double-bond migration as a plausible key reaction in the biosynthesis of the isoprenoidal membrane lipids of methanogenic archaea. *Chemical Communications* **2000**, 1545-1546.

25. Choquet, C.; Patel, G.; Beveridge, T.; Sprott, G. Stability of pressure-extruded liposomes made from archaeobacteria ether lipids. *Applied Microbiology and Biotechnology* **1994**, *42*, 375-384.

26. Gambacorta, A.; Gliozzi, A.; De Rosa, M. Archaeal lipids and their biotechnological applications. *World Journal of Microbiology & Biotechnology* **1995**, *11*, 115-131.

27. Choquet, C.; Patel, G.; Beveridge, T.; Sprott, G., Formation of unilamellar liposomes from total polar lipid extracts of methanogens. *Applied and Environmental Microbiology* **1992**, 2894-2900.

28. Tolson, D.; Latta, R.; Patel, G.; Sprott, G. Uptake of archaeobacterial liposomes and conventional liposomes by phagocytic cells. *Journal of Liposome Research* **1996**, *6*, (4), 755-776.

29. Sprott, G.; Brisson, J.; Dicaire, C.; Pelletier, A.; Deschatelets, L.; Krishnan, L.; Patel, G. A structural comparison of the total polar lipids from the human archaea *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* and its relevance to the adjuvant activities of their liposomes. *Biochimica et Biophysica Acta* **1999**, *1440*, 275-288.

30. Krishnan, L.; Dicaire, C.; Patel, G.; Sprott, G. Archaeosome vaccine adjuvants induce strong humoral, cell-mediated, and memory responses: Comparison to conventional liposomes and alum. *Infection and Immunity* **2000**, *68*, (1), 54-63.

31. Krishnan, L.; Sad, S.; Patel, G.; Sprott, G. Archaeosomes induce long-term CD8+ cytotoxic T-cell response to entrapped soluble protein by the exogenous cytosolic pathway, in the absence of CD4+ T-cell help. *The Journal of Immunology* **2000**, *165*, 5177-5185.

32. Krishnan, L.; Sad, S.; Patel, G.; Sprott, G. The potent adjuvant activity of archaeosomes correlates to the recruitment and activation of macrophages and dendretic cell in vivo. *The Journal of Immunology* **2001**, *166*, 1885-1893.

33. a) Krishnan, L.; Sad, S.; Patel, G.; Sprott, G. Archaeosomes induce enhanced cytotoxic T lymphocyte responses to entrapped soluble protein in the absence of interleukin 12 and protect against tumor challenge. *Cancer Research* **2003**, *63*, 2526-2534. b) Patel, G.; Omri, A; Deschatelets, L; Sprott, G. Safety of archaeosome adjuvants evaluated in a mouse model. *Journal of Liposome Research* **2002**, *12*, (4), 353-372.

34. Kataoka, K.; Muta, T.; Yamazaki, S.; Takeshige, K. Activation of macrophages by linear (1-->3)-β-D-Glucans. *Journal of Biological Chemistry* **2002**, *277*, (39), 36825-36831.

35. Fadok, V. The Sirens' Call. *Nature Cell Biology* **2003**, *5*, (8), 697-699.

36. Sprott, G.; Sad, S.; Fleming, L.; Dicaire, C.; Patel, G.; Krishnan, L. Archaeosomes varying in lipid composition differ in receptor-mediated endocytosis and differentially adjuvant immune responses to entrapped antigen. *Archaea* **2003**, *1*, (3), 151-164.

37. Gurnani, K.; Kennedy, J.; Sad, S.; Sprott, G.; Krishnan, L. Phosphatidylserine receptor-mediated recognition of archaeosome adjuvant promotes endocytosis and MHC class I cross-presentation of the entrapped antigen by phagosome-to-cytosol transport and classical processing. *The Journal of Immunology* **2004**, *173*, 566-578.

38. Nishihara, M.; Morii, H.; Matsuno, K.; Ohga, M.; Stetter, K.; Koga, Y., Structural analysis by reductive cleavage with $LiAlH_4$ of an allyl ether choline-phospholipid, archaetidylcholine, from the hyperthermophilic methanoarchaeon *Methanopyrus kandleri*. *Archaea* **2003**, *1*, (2), 123-131.

39. Aoki, T.; Poulter, C. Archaebacterial isoprenoids. Synthesis of 2,3-Di-*O*-diphytanyl-sn-glycerol and its 1,2-isomer. *Journal of Organic Chemistry* **1985**, *50*, (26), 5634-5636.

40. Eguchi, T.; Arakawa, K.; Terachi, T.; Kakinuma, K. Total synthesis of archaeal 36-membered macrocyclic diether lipid. *Journal of Organic Chemistry* **1997**, *62*, (7), 1924-1933.

41. Eguchi, T.; Ibaragi, K.; Kakinuma, K. Total synthesis of archaeal 72-membered macrocyclic tetraether lipids. *Journal of Organic Chemistry* **1998**, *63*, (8), 2689-2698.

42. Eguchi, T.; Arakawa, K.; Kakinuma, K.; Rapp, G.; Ghosh, S.; Nakatani, Y.; Ourisson, G. Giant vesicles from 72-membered macrocyclic archaeal phospholipid analogues: Initiation of vesicle formation by molecular recognition between membrane components. *Chemistry - A European Journal* **2000**, *6*, (18), 3351-3358.

43. Ohta, T.; Takaya, H.; Kitamura, M.; Nagai, K.; Noyori, R. Asymmetric hydrogenation of unsaturated carboxylic acids catalyzed by BINAP-ruthenium(II) complexes. *Journal of Organic Chemistry* **1987**, *52*, (14), 3174-3176.

44. Ohta, T.; Takaya, H.; Noyori, R. BINAP-Ruthenium(II) dicarboxylate complexes - New, highly efficient catalysts for asymmetric hydrogenations. *Inorganic Chemistry* **1988**, *27*, (3), 566-569.

45. Takaya, H.; Ohta, T.; Mashima, K.; Noyori, R. New chiral ruthenium complexes for asymmetric catalytic hydrogenations. *Pure and Applied Chemistry* **1990**, *62*, (6), 1135-1138.

46. Takaya, H.; Ohta, T.; Sayo, N.; Kumobayashi, H.; Akutagawa, S.; Inoue, S.; Kasahara, I.; Noyori, R. Enantioselective hydrogenation of allylic and homoallylic alcohols. *Journal of the American Chemical Society* **1987**, *109*, (5), 1596-1597.

47. Sita, L. Convenient highly stereoselective syntheses of (3*R*, 7*R*, 11*R*)- and (3*S*, 7*R*, 11*R*)-3,7,11,15-tetramethylhexadecanoic acid (phytanic acid) and the corresponding 3,7,11,15-tetramethylhexadecan-1-ols. *Journal of Organic Chemistry* **1993**, *58*, (19), 5285-5287.

48. Burns, C.; Field, L.; Hashimoto, K.; Petteys, B.; Ridley, D.; Rose, M. Synthesis of stereomerically pure monoether lipids. *Australian Journal of Chemistry* **1999**, *52*, 387-394.

49. Huo, S.; Negishi, E. A convenient and asymmetric protocol for the synthesis of natural products containing chiral alkyl chains *via* Zr-catalyzed asymmetric carboalumination of alkenes. Synthesis of phytol and vitamins E and K. *Organic Letters* **2001**, *3*, (21), 3253-3256.

50. Fischer, H.; Baer, E. Uber Aceton-glycerinaldehyd II; Darstellung des Aceton-d-glycerinaldehyds. *Helvetica Chimica Acta* **1934**, *17*, 622-632.

51. Jung, M.; Shaw, T. Total synthesis of (*R*)-glycerol acetonide and the antiepileptic and hypotensive drug (-)-gamma-amino-β-hydroxybutyric acid (GABOB): Use of vitamin C as a chiral starting material. *Journal of the American Chemical Society* **1980**, *102*, (20), 6304-6311.

52. Lok, C.; Ward, J.; van Dorp, D. The synthesis of chiral glycerides starting from D- and L-serine. *Chemisrty and Physics of Lipids* **1976**, *16*, (2), 115-122.

53. Baer, E.; Fischer, H. Studies on acetone-glyceraldehyde. VII. Preparation of L-glyceraldehyde and L-(-)-acetone glycerol. *Journal of the American Chemical Society* **1939**, *61*, (4), 761-765.

54. Badorrey, R.; Cativiela, C.; Diaz-de-Villegas, M.; Galvez, J. Highly convergent stereoselective synthesis of chiral key intermediates in the synthesis of Palinavir from imines derived from L-glyceraldehyde. *Tetrahedron* **2002**, *58*, 341-354.

55. Ley, S.; Michel, P. Butane-2,3-diacetals of glyceraldehyde: A stable alternative to glyceraldehyde acetonide. *Angewandte Chemie International Edition* **2002**, *41*, (20), 3898-3901.

56. Ley, S.; Michel, P. Preparation of butane-1,2-diacetal-protected L-glyceraldehyde from D-mannitol. *Synthesis* **2004**, (1), 147-150.

57. Stawinski, J.; Kraszewski, A. How to get the most out of two phosphorus chemistries. Studies on H-phosphonates. *Accounts in Chemical Research* **2002**, *35*, (11), 952-960.

58. Narang, S. DNA synthesis. *Tetrahedron* **1983**, *39*, (1), 3-22.

59. Caruthers, M. Gene synthesis machines: DNA chemistry and its uses. *Science* **1985**, *230*, 281-285.

60. David, S.; Malleron, A.; Dini, C. Preparation of oligosaccharides with β-D-mannopyranosyl and 2-azido-2-deoxy-β-D-mannopyranosyl residues by inversion at C-2 after coupling. *Carbohydrate Research* **1989**, *188*, 193-200.

61. Gunther, W.; Kunz, H. Synthesis of β-D-mannosides from β-D-glucosides *via* an intramolecular S_N2 reaction at C-2. *Carbohydrate Research* **1992**, *228*, 217-241.

62. Twaddle, G.; Yashunsky, D.; Nikolaev, A. The Chemical Synthesis of β-(1→4)-Linked D-Mannobiose and D-Mannotriose. *Organic & Biomolecular Chemistry* **2003**, *1*, 623-628.

63. Alais, J.; David, S. Preparation of disaccharides having a β-D-mannopyranosyl group from *N*-phthaloyllactosamine derivatives by double or triple S_N2 substitution. *Carbohydrate Research* **1990**, *201*, 69-77.

64. Nitz, M.; Bundle, D. Synthesis of di- to hexasaccharide 1,2-linked β-mannopyranan oligomers, a terminal S-linked tetrasaccharide congener and the corresponding BSA glycoconjugates. *Journal of Organic Chemistry* **2001**, *66*, (25), 8411-8423.

65. Shaban, M.; Jeanloz, R. The synthesis of 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-glucose. *Carbohydrate Research* **1976**, *52*, 115-127.

66. Nitz, M.; Purse, B.; Bundle, D. Synthesis of a β-1,2-mannopyranosyl tetrasaccharide found in the phosphomannan antigen of *Candida albicans*. *Organic Letters* **2000**, *2*, (19), 2939-2942.

67. Warren, C.; Auge, C.; Laver, M.; Suzuki, S.; Power, D.; Jeanloz, R. The synthesis of *O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl) -(1→4)-2-acetamido-2-deoxy-D-glucopyranose. Part I. *Carbohydrate Research* **1980**, *82*, 71-83.

68. Galan, M.; Venot, A.; Glushka, J.; Imberty, A.; Boons, G. α-(2→6)-Sialyltransferase-catalyzed sialylations of conformationally constrained oligosaccharides. *Journal of the American Chemical Society* **2002**, *124*, (21), 5964-5973.

69. Galan, M.; Venot, A.; Phillips, R.; Boons, G. The design and synthesis of a selective inhibitor of fucosyltransferase VI. *Organic & Biomolecular Chemistry* **2004**, *2*, (9), 1376-1380.

70. Auge, C.; Warren, C.; Jeanloz, R. The synthesis of *O*-β-D-mannosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranose. Part II. *Carbohydrate Research* **1980**, *82*, (1), 85-95.

71. Nitz, M.; Bundle, D. Efficient synthesis of 3,6-dideoxy-β-D-*arabino*-hexopyranosyl-terminated LacdiNac glycan chains of the *Trichinella spiralis* parasite. *Journal of Organic Chemistry* **2000**, *65*, (10), 3064-3073.

72. Mathew, F.; Mach, M.; Hazen, K.; Fraser-Reid, B. Orthoester-based strategy for efficient synthesis of the virulent antigenic-1,2-linked oligomannans of *Candida albicans*. *Synlett* **2003**, (9), 1319-1322.

73. Lichtenthaler, F.; Schneider-Adams, T. 3,4,6-Tri-*O*-benzyl-α-D-*arabino*-hexapyranos-2-ulosyl bromide: A versatile glycosyl donor for the efficient generation of β-D-mannopyranosidic linkages. *Journal of Organic Chemistry* **1994**, *59*, (22), 6728-6734.

74. Lichtenthaler, F.; Schneider, A.; Immel, S. Practical synthesis of β-D-Xyl-(1→2)-β-D-Man-(1→4)-α-D-Glc-OMe, A Trisaccharide Component of the *Hyriopsis schlegelii* glycosphingolipid. *Journal of Organic Chemistry* **1994**, *59*, (22), 6735-6738.

75. Garegg, P.; Ossowski, P. Silver zeolite as promoter in glycoside synthesis. The synthesis of β-D-mannopyranosides. *Acta Chemica Scandinavica B* **1983**, *37*, (3), 249-250.

76. Srivastava, V.; Schuerch, C. A synthesis of β-D-mannopyranosides by glycosidation at C-1. *Carbohydrate Research* **1980**, *79*, C13-C16.

77. Srivastava, V.; Schuerch, C. Synthesis of β-D-mannopyranosides and β-L-rhamnopyranosides by glycosidation at C-1. *Journal of Organic Chemistry* **1981**, *46*, (6), 1121-1126.

78. Abdel-Rahman, A.-H.; Jonke, S.; El Ashry, E.; Schmidt, R. Stereoselective synthesis of β-D-mannopyranosides with reactive mannopyranosyl donors possessing a nieghbouring electron-withdrawing group. *Angewandte Chemie International Edition* **2002**, *41*, (16), 2972-2974.

79. Kahne, D.; Walker, S.; Cheng, Y.; Van Engen, D. Glycosylation of unreactive substrates. *Journal of the American Chemical Society* **1989**, *111*, (17), 6881-6882.

80. a) Crich, D.; Sun, S. Formation of β-mannopyranosides of primary alcohols using the sulfoxide method. *Journal of Organic Chemistry* **1996**, *61*, (14), 4506-4507. b) Crich, D.; Sun, S. Direct synthesis of β-mannopyranosides by the sulfoxide method. Journal of Organic Chemistry. **1997**, *62*, (5), 1198-1199. c) Crich, D.; Sun, S. Are glycosyl triflates intermediates in the sulfoxide glycosylation method? A chemical and ^{1}H, ^{13}C and ^{19}F NMR spectroscopic investigation. *Journal of the American Chemical Society* **1997**, *119*, (46), 11217-11223.

81. Crich, D.; Smith, M. 1-Benzenesulfinyl piperidine/trifluoromethanesulfonic anhydride: A potent combination of shelf-stable reagents for the low-temperature conversion of thioglycosides to glycosyl triflates and for the formation of diverse glycosidic linkages. *Journal of the American Chemical Society* **2001**, *123*, (37), 9015-9020.

82. Crich, D.; Chandrasekera, N. Mechanism of 4,6-*O*-benzylidene-directed β-mannosylation as determined by α-deuterium kinetic isotope effects. *Angewandte Chemie International Edition* **2004**, *43*, 5386-5389.

83. Crich, D.; Li, H.; Yao, Q.; Wink, D.; Sommer, R.; Rheingold, A. Direct synthesis of β-mannans. A hexameric [→3)-β-D-Man-(1→4)-β-D-Man-(1]$_3$ subunit of the antigenic polysaccharide from *Leptospira biflexa* and the octameric (1→2)-Linked β-D-mannan of the *Candida alibicans* phospholipomannan. X-ray crystal structure of a protected tetramer. *Journal of the American Chemical Society* **2001**, *123*, (24), 5826-5828.

84. Crich, D.; Banerjee, A.; Yao, Q. Direct chemical synthesis of the β-D-mannans: The β-(1-->2) and β-(1-->4) series. *Journal of the American Chemical Society* **2004**, *126*, (45), 14930-14934.

85. Crich, D.; Li, W.; Li, H. Direct chemical synthesis of the β-mannans. Linear and block syntheses of the alternating β-(1→3)-β-(1→4)-mannan common to

Rhodotorula glutinis, *Rhodotorula mucilaginosa* and *Leptospira biflexa*. *Journal of the American Chemical Society* **2004**, *126*, (46), 15081-15086.

86. Weingart, R.; Schmidt, R. Can preferential β-mannopyranoside formation with 4,6-*O*-benzylidene protected mannosyl sulfoxides be reached with trichloroacetimidates? *Tetrahedron Letters* **2000**, *41*, 8753-8758.

87. Codee, J.; Hossain, L.; Seeberger, P. Efficient installation of β-mannosides using a dehydrative coupling strategy. *Organic Letters* **2005**, *ASAP*.

88. Garcia, B.; Gin, D. Dehydrative glycosylation with activated diphenyl sulfonium reagents. Scope, mode of C(1)-hemiacetal activation, and detection of reactive glycosyl intermediates. *Journal of the American Chemical Society* **2000**, *122*, (18), 4269-4279.

89. De Meo, C.; Kamat, M.; Demchenko, A. Remote participation-assisted synthesis of β-mannosides. *European Journal of Organic Chemistry* **2005**, 706-711.

90. Barresi, F.; Hindsgaul, O. Synthesis of β-mannopyranosides by intramolecular aglycon delivery. *Journal of the American Chemical Society* **1991**, *113*, 9376-9377.

91. Stork, G.; Kim, G. Stereocontrolled synthesis of disaccharides *via* the temporary silicon connection. *Journal of the American Chemical Society* **1992**, *114*, (3), 1087-1088.

92. Ito, Y.; Ogawa, T. A novel approach to the stereoselective synthesis of β-mannosides. *Angewandte Chemie International Edition* **1994**, *33*, (17), 1765-1767.

93. Dan, A.; Ito, Y.; Ogawa, T. A convergent and stereocontrolled synthetic route to the core pentasaccharide structure of asparagine-linked glycoproteins. *Journal of Organic Chemistry* **1995**, *60*, (15), 4680-4681.

94. Abdel-Rahman, A.-H.; El Ashry, E.; Schmidt, R. Efficient intramolecular β-mannoside formation using *m*-xylylene and isophthaloyl derivatives as rigid spacers. *Carbohydrate Research* **2002**, *337*, 195-206.

95. Jung, M.; Liu, C. Efficient synthesis of a head-to-head isoprenoid geochemical biomarker from phytol. *Journal of Organic Chemistry* **1986**, *51*, (26), 5446-5447.

96. Kuszmann, J.; Tomori, E.; Meerwald, I. The synthesis of 1,2:5,6-di-*O*-isopropylidene-D-mannitol: A comparative study. *Carbohydrate Research* **1984**, *128*, 87-99.

97. Schmid, C.; Bryant, J.; Dowlatzedah, M.; Phillips, J.; Prather, D.; Schantz, R.; Sear, N.; Vianco, C. Synthesis 2,3-*O*-isopropylidene in high chemical and optical purity: Observations on the development of a practical bulk process. *Journal of Organic Chemistry* **1991**, *56*, (12), 4056-4058.

98. Puff, H.; Hevendehl, H.; Hofer, K.; Reuter, H.; Schun, W. Hydroxid-Halogenid-Verbindungen Des Di-*t*-Butyl-Substituierten Zinns. *Journal of Organometallic Chemistry* **1985**, *287*, 163-178.

99. Chu, C.; Murray, J. Hydrolysis products of dibutyltin dichlorides. *Journal of the Chemical Society (A)* **1971**, 360-367.

100. Orita, A.; Hamada, Y.; Nakano, T.; Toyoshima, S.; Otera, J. Highly efficient deacetylation by use of the neutral organotin catalyst $[t\mathrm{Bu_2SnOH(Cl)}]_2$. *Chemistry - A European Journal* **2001**, *7*, (5), 3321-3327.

101. Dreef-Tromp, C.; Lefeber, A.; van der Marel, G.; van Boom, J. Synthesis and phosphorylating of hydroxyamino acid phosphoramidates. *Synthesis* **1992**, 1269-1272.

102. Morii, H.; Nishihara, M.; Ohga, M.; Koga, Y. A diphytanyl ether analog of phosphatidylserine from a methanogenic bacterium, *Methanobrevibacter arboriphilus*. *Journal of Lipid Research* **1986**, *27*, 724-730.

103. Sallusto, F.; Cella, M.; Danieli, C.; Lanzavecchia, A. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: Downregulation by cytokines and bacterial products. *Journal of Experimental Medicine* **1995**, *182*, (2), 389-400.

104. Jones, M. Carbohydrate-mediated liposomal targeting and drug delivery. *Advanced Drug Delivery Reviews* **1994**, *13*, 215-250.

105. Copland, M.; Baird, M.; Rades, T.; McKenzie, J.; Becker, B.; Reck, F.; Tyler, P.; Davies, N. Liposomal delivery antigen to human dendritic cells. *Vaccine* **2003**, *21*, 883-890.

106. Nitz, M.; Ling, C.; Otter, A.; Cutler, J.; Bundle, D. The unique solution structure and immunochemistry of the *Candida albicans* β-1,2-mannopyranan cell wall antigens. *Journal of Biological Chemistry* **2002**, *277*, (5), 3440-3446.

107. Han, Y.; van Rooijen, N.; Cutler, J. Binding of *Candida albicans* yeast cells to mouse popliteal lymph node tissue is mediated by macrophages. *Infection and Immunity* **1993**, *61*, 3244-3249.

108. Kanbe, T.; Han, Y.; Redgrave, B.; Riesselman, M.; Cutler, J. Evidence that mannans of *Candida albicans* are responsible for adherence of yeast forms to spleen and lymph node tissue. *Infection and Immunity* **1993**, *61*, 2578-2584.

109. Fradin, C.; Poulain, D.; Jouault, T. β-1,2-linked oligomannosides from *Candida albicans* bind to a 32-kilodalton macrophage membrane protein homologous to the mammalian lectin galectin-3. *Infection and Immunity* **2000**, *68*, (8), 4391-4398.

110. Perico, M.; Mezzanzanica, D.; Luison, E.; Alberti, P.; Panza, L.; Russo, G.; Canevari, S. Development of a new vaccine formulation that enhances the immunogenicity of tumor-associated antigen CaMBr-1. *Cancer Immunology and Immunotherapy* **2000**, *49*, 296-304.

111. Wu, X.; Ling, C.; Bundle, D. A new homobifunctional *p*-nitro phenyl ester coupling reagent for the preparation of neoglycoproteins. *Organic Letters* **2004**, *6*, (24), 4407-4410.

112. Unverzagt, C.; André, S.; Seifert, J.; Kojima, S.; Fink, C.; Srikrishna, G.; Freeze, H.; Kayser, K.; Gabius, H. Structure-activity profiles of complex biantennary glycans with core fucosylation and with/without additional α-2,3/α-2,6 sialylation: Synthesis of neoglycoproteins and their properties in lectin assays, cell binding, and organ uptake. *Journal of Medicinal Chemistry* **2002**, *45*, (2), 478-491.

113. Mawas, F.; Niggemann, J.; Jones, C.; Corbel, M.; Kamerling, J.; Vliegenthart, J. Immunogenicity in a mouse model of a conjugate vaccine made with a synthetic single repeating unit of type 14 pneumococcal polysaccharide coupled to CRM197. *Infection and Immunity* **2002**, *70*, (9), 5107-5114.

114. Nitz, M, Bundle, D. Ph. D. Thesis. University of Alberta, Edmonton, Canada, **2001**.

115. Charles, I.; Xue, L.; Arya, D. Synthesis of aminoglycoside-DNA conjugates. *Bioorganic and Medicinal Chemistry Letters* **2002**, *12*, (9), 1259-1262.

116. Arya, D.; Willis, B. Reaching into the major groove of B-DNA: Synthesis and nucleic acid binding of a Neomycin-Hoechst 33258 conjugate. *Journal of the American Chemical Society* **2003**, *125*, (41), 12398-12399.

117. Arya, D.; Xue, L.; Tennant, P. Combining the best in triplex recognition: Synthesis and nucleic acid binding of a BQQ-Neomycin conjugate. *Journal of the American Chemical Society* **2003**, *125*, (27), 8070-8071.

118. Kim, S.; Yi, K. 1,1'-Thiocarbonyldi-2,2'-pyridone. A new useful reagent for functional group conversions under essentially neutral conditions. *Journal of Organic Chemistry* **1986**, *51*, (13), 2613-2615.

119. Lelkes, P.; Goldenberg, D.; Gliozzi, A.; De Rosa, M.; Gambacorta, A.; Miller, I. Vesicles from mixtures of bipolar archaebacterial lipids with egg phosphotidylcholine. *Biochimica et Biophysica Acta* **1983**, *732*, 714-718.

120. Phillips, N.; Gagne, L.; Ivanoff, N.; Riveau, G. Influence of phospholipid composition on antibody responses to liposome encapsulated protein and peptide antigens. *Vaccine* **1996**, *14*, (9), 898-904.

121. Wu, X and Bundle D. Synthesis of glycoconjugate vaccines for *Candida albicans* using novel linker methodology. *Journal of Organic Chemistry,* **2005**, *70*, 7381-7388.