SYNTHESIS OF C-PHOSPHONIC ACID, C-PHOSPHINIC ACID, AND C-SULFONE ANALOGS OF DECAPRENOLPHOSPHOARABINOSE: INHIBITORS OF MYCOBACTERIAL ARABINOSYLTRANSFERASES

DISSERTATION

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By
Charla A. Centrone, B.S.

The Ohio State University
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Dissertation Committee:
Professor Todd L. Lowary, Adviser
Professor Leo A. Paquette
Professor T. V. RajanBabu

Approved by

Adviser
Department of Chemistry
ABSTRACT

Tuberculosis, the disease arising from infection by *Mycobacterium tuberculosis*, is the single most lethal pathogen, killing nearly three million people every year. The resurgence of this disease, and other mycobacterial diseases, has prompted interest in developing new antimycobacterial agents. These investigations have focused on synthesizing inhibitors of arabinosyltransferases (AraT’s) involved in the biogenesis of the D-arabinofuranose-containing polysaccharides that are critical components of two cell wall polysaccharides, arabinogalactan (AG) and lipoarabinomannan (LAM). The syntheses of metabolically stable C-glycosyl substrate analogs of decaprenolphosphoarabinose (DPA), which is utilized by AraT’s to build the arabinan portion of the AG and LAM, are presented.

A series of C-phosphonic acid analogs was synthesized in 15 steps from D-glucitol. The key steps involve the formation of a protected C-phosphonate allyl ester via an intramolecular Mitsunobu reaction on a 1,4-diol, followed by coupling with disubstituted alkenes possessing long alkyl chains in an olefin cross metathesis reaction. Screening of these compounds *in vitro* against *M. tuberculosis* revealed that one of the compounds possessed significant antimycobacterial activity.

The synthesis of C-phosphinic acid and C-sulfone DPA analogs has been explored. An efficient method has been developed to synthesize a panel of C-phosphinic acids from a C-phosphonate diethyl ester. Reduction of the phosphonate ester afforded a phosphine, which, in the key step, was subsequently alkylated with long chain alkyl iodides using a
phosphazene base. Oxidation of the resulting secondary phosphine and deprotection afforded the targets. A series of C-sulfone DPA analogs has been synthesized from a protected 2,5-anhydroglucityl iodide. In the key step, a sugar thiol is alkylated with various long chain alkyl iodides to form the corresponding sulfides. Oxidation of the sulfide and deprotection yielded the target compounds. Both series of DPA analogs are currently being tested for activity against *M. tuberculosis*.

It has been postulated that a sugar nucleotide may be involved in arabinan biosynthesis. Thus far, no arabinose-derived sugar nucleotide has been unequivocally identified and isolated from *M. tuberculosis*, and the labile nature of these species may be the reason. A goal of this research was to synthesize nucleotide diphosphate-C-D-arabinose analogs, which, if found to be AraT inhibitors, would provide indirect evidence for the involvement of a sugar nucleotide in arabinan biosynthesis. In these investigations, attempts to synthesize these compounds were unsuccessful.
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VITA

October 28, 1974    Born-Canton, OH, USA
May 1998    B. S. Chemistry, Kent State University, Kent OH
September 1998-December 2002    Graduate Teaching Associate, The Ohio State University
December 2002-present    Graduate Research Associate, The Ohio State University

PUBLICATIONS


FIELDS OF STUDY

Major Field: Chemistry
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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Tuberculosis (TB) is a disease caused by a bacterium called *Mycobacterium tuberculosis*. *Mycobacterium tuberculosis* is the world’s most lethal pathogen, infecting one third of the world’s population. There are 8 million new infections and 3 million deaths from TB every year.\(^1\) This is an alarming reality for a disease in which both a vaccine and effective chemotherapeutic regimens exist. People with compromised immune systems such as the Human Immune Deficiency Virus (HIV) are at an especially high risk for contracting TB. About one third of the 36 million HIV-infected people at the end of the year 2000 were infected with *Mycobacterium tuberculosis*.\(^2\) The highest incidences of TB are seen in Africa, Asia, and Latin America, although the incidence in industrialized countries has risen in recent years, and is believed to be partially attributed to a high rate of immigration from these underdeveloped countries.\(^3\)
The resurgence of *M. tuberculosis* has prompted interest in the development of new antimycobacterial agents. It has been more than a quarter of a century since a new class of tuberculosis drugs was last introduced, and new strains of *M. tuberculosis* resistant to many currently effective antibiotics are constantly emerging. It has been estimated that at least 50 million people are infected with drug-resistant TB.4

There has been much progress over the last few years in determining the structures of a number of *M. tuberculosis* enzymes, many of which are potential drug targets. Drugs that target enzymes involved in the biosynthesis of the mycobacterium’s nearly impenetrable cell wall have made very effective treatments. Ethambutol, a drug currently used as a TB treatment, is an inhibitor of one class of cell-building enzymes, the arabinosyltransferases.5 Thus, there is precedence for developing new compounds that act as ethambutol does in disrupting the cell wall biosynthesis.

The focus of our investigations has been on developing arabinosyltransferase inhibitors. Thus far, one of our compounds has been shown to possess significant antimycobacterial activity against *M. tuberculosis*. This dissertation will describe the chemical synthesis of this, as well as other, inhibitors.

1.2 Biological background

1.2.1 General aspects of tuberculosis

Tuberculosis is a disease primarily affecting the lungs, but can attack any organ of the body. It is spread though the air when a person with the disease coughs or sneezes. People nearby may become infected, however not everyone who is infected will show clinical signs. As a result, two types of TB exist: latent TB infection and active TB disease. In most people
who breathe in TB bacteria and become infected, the body’s immune system is able to fight
the bacteria and stop them from growing. The bacteria are inactive, but remain alive in the
body and can become active later. There are an estimated 10 to 15 million people in the
United States alone with latent TB infection, many of which never develop the active form.6
People with latent TB cannot spread the disease to others, and a simple skin test can diagnose
the infection so that treatment can be administered before it has the chance to become active.

People with active TB disease may experience symptoms such as fever, weight loss, and a
cough that lasts for more than 2 weeks. Some common drugs used to treat TB and their
mechanisms of action (MOA) are shown in Figure 1.1. The drug usually used for treatment
of latent TB infection is Isoniazid. The usual drugs to treat active TB disease are Isoniazid,
Rifampin, Pyrazinamide, Ethambutol, and Streptomycin. The chemotherapy treatment is
very lengthy requiring multiple antibiotics for at least 6 months.7
Figure 1.1: Structures of some commonly used antituberculosis drugs and their mechanisms of action (MOA) against *M. tuberculosis*.

A major factor contributing to the resurgence of TB is the emergence of multi-drug resistance. The low potency of the drugs, side effects, and long duration of therapy (resulting in noncompliance) are a major cause of this resistance. In the past few years a great deal of progress has been made towards the understanding of the molecular basis of drug resistance. The development of molecular genome sequencing for mycobacteria has played an important role in identifying genes involved in drug resistance, of which the mechanisms to all the first-line tuberculosis drugs are known.
As a result of the increasing incidence of *Mycobacterium tuberculosis* in both the industrialized and developing world and the occurrence of many drug-resistant strains of *M. tuberculosis*, there has been much interest in the development of new anti-TB drugs. One area that has attracted attention is the development of compounds that inhibit the arabinosyltransferases (AraT’s) responsible for the biosynthesis of the mycobacterial cell wall.\(^9\) The cell wall, which is very thick and waxy, is a formidable barrier to the passage of antibacterial agents into the organism. Successful drug regimens employ at least one antibiotic that prevents cell wall biosynthesis (*e.g.*, ethambutol or isoniazid) in combination with others that have intracellular targets (*e.g.*, rifampicin or streptomycin). These drug-cocktail regimens have been very successful for treating tuberculosis, provided the patient takes the full course of medication.
1.2.2 Cell wall structure of *Mycobacterium tuberculosis*

The mycobacterial cell wall, also called the envelope, is an intricately designed structure composed of polysaccharides, proteins, lipids, and glycolipids. A schematic illustration of the general structural motifs is shown in Figure 1.2.  

![Schematic illustration of the mycobacterial cell wall](image)

Figure 1.2: Schematic representation of the mycobacterial cell wall.

There are five major components of the cell wall: 1) Peptidoglycan; 2) Arabinogalactan (AG); 3) Mycolic acid esters bound to the AG; 4) Glycolipids bound noncovalently to the mycolic acid esters; and 5) Lipoarabinomannan (LAM). The peptidoglycan, which forms the backbone of the cell wall skeleton, consists of chains of a polysaccharide formed from alternating units of *N*-acetylglucosamine and *N*-glycolylmuramic acid (Figure 1.3). Tetrapeptide side chains are attached to the muramic acid residues and cross-link the polysaccharide chains.
The arabinogalactan is a polymer of galactofuranosyl and arabinofuranosyl residues covalently attached to the peptidoglycan by way of a $\alpha$-L-Rha-(1→3)$\alpha$-GlcNAc-OPO$_3$ diglycosylphosphoryl bridge. The backbone of the AG is made up of approximately 30 galactofuranose (Gal$_f$) residues with alternating $\beta$-(1→5) and $\beta$-(1→6) linkages. Attached to this galactan backbone is the arabinan, which consists of approximately 70 arabinofuranose (Ara$_f$) residues. Chains of arabinose residues are linked at branch points on the galactan backbone by way of linear $\alpha$-(1→5) linkages. At the nonreducing termini of these arabinan chains is a branched hexasaccharide. Mycolic acids, a homologous series of C$_{60}$ to C$_{90}$ $\alpha$-alkyl $\beta$-hydroxy fatty acids, are bound covalently to the hexasaccharide by ester linkages. For every hexasaccharide there are four mycolic acids linked to the 5-positions of the arabinose residues. The packing of these hydrophobic tetracycolylated hexa-arabinose units gives the cell wall its waxy texture and its impermeable nature. Schematic representations of this mycolyl-AG complex are illustrated in Figure 1.4 and Figure 1.5, and the tetracycolyl-hexa-arabinoside unit is shown in Figure 1.6.
Figure 1.4: Schematic representation of the mycolyl-AG complex.
Figure 1.5: Structural motifs found in the AG.
The final part of the cell wall is the lipoarabinomannan, which is a polymer of mannoxyranosyl and arabinofuranosyl residues intercalated in between the AG branches. The LAM is noncovalently bound to the cytoplasmic membrane by the lipid portion of a phosphatidylinositol linker (Figure 1.6). The backbone of the LAM consists primarily of α-(1→6) mannosyl residues, and about half of these residues contain an α-(1→2) mannoxyranosyl branch. To the termini of the mannan is linked an arabinan polymer with α-(1→5) linkages similar to that found in the AG. The LAM differs from the AG in that there are fewer branches and there are no mycolic acids esterified to the terminal hexaccharide. In the case of LAM isolated from \textit{M. tuberculosis}, the arabinan termini are extensively capped with mannoxyranosyl residues and the whole entity is called the ManLAM.
1.2.3 Roles of the mycolyl-AG and LAM in disease progression

The mycolyl-AG complex and LAM are key components for the survival of mycobacteria in the human host.\textsuperscript{10,14} The mycolyl-AG complex is the major structural component of the cell wall. The mycolic acid ester chains are tightly packed and parallel to one another, perpendicular to the plane of the cytoplasmic membrane.\textsuperscript{11} The tight packing results in very low fluidity of the cell wall. In addition, the arabinose and galactose residues adopt the furanose (five-membered ring) conformation instead of the thermodynamically more stable pyranose (six-membered ring) conformation. It has been proposed that this allows for increased flexibility of the polysaccharide, which allows the tight packing of the
mycolic acid esters to occur. The mycolyl-AG complex, along with the peripheral glycolipids, creates an impenetrable hydrophobic barrier to prevent the passage of antibiotics into the organism, and also protects the organism from the body’s immune cells.

While the AG functions in a structural capacity, the LAM’s major function is antigenic. The LAM has the ability to inhibit the activation of macrophages (immune cells that inject pathogens with toxins) and induce T-cell chemotaxis. The LAM has also been implicated in many other immunomodulatory events that suppress the body’s immune system and enable the organism to survive in the host.

1.2.4 Arabinan biosynthesis

Much of the early research on M. tuberculosis has focused on the elucidation of the structure of the mycobacterial cell wall. Now that the structures of the mycolic acid-arabinogalactan-peptidoglycan complex are fairly well understood, emphasis has been shifting to the study of cell wall biosynthesis and the identification of enzymes that are essential to the viability of the organism. The complete M. tuberculosis genome has now been sequenced, and as a result, thirteen enzymes involved in the synthesis of the AG-peptidoglycan complex have been identified and at least partially characterized.

The general features of arabinan biosynthesis are known, however many of the finer details are still yet to be determined. The $\beta-(1 \rightarrow 2)$ and $\alpha-(1 \rightarrow 5)$ linked arabinofuranose residues are incorporated into the polymers from decaprenolphosphoarabinose (DPA, Figure 1.8). It has been postulated that the elongation of the arabinan polymer is catalyzed by an array of arabinosyltransferases (AraT’s) that utilize this DPA glycosyl donor and recognize different arabinofuranose glycosyl acceptors. In AG biosynthesis, it is believed that the entire polysaccharide is assembled as a polypreenol diphosphate.
intermediate, which is then transferred to the peptidoglycan prior to the addition of the mycolicate esters. In LAM biosynthesis, it is thought that the arabinan is synthesized as a polyprenol phosphate, which transferred to the lipomannan.²¹

Figure 1.8: Prototypical AraT catalyzed reaction for arabinan biosynthesis.

To date, no AraT’s have been unequivocally identified in M. tuberculosis. These enzymes are membrane bound and thus difficult to characterize and study. Although no AraT’s have been purified, an assay for AraT activity has been developed that utilizes mycobacterial membrane preparations as the enzyme source.²⁰,²² This assay measures the incorporation of radiolabeled arabinose into the arabinan, and has been used to screen for AraT substrates and inhibitors.
It has been established that the origin of the D-arabinose in DPA is 5-phosphoribose pyrophosphate (pRpp). The ppRp is synthesized from D-glucose in a pentose phosphate shunt pathway (Figure 1.9) and then epimerized at C-2 to form 5-phosphoarabinose pyrophosphate. It is not known whether this epimerization takes place at the level of ppRp or at the level of polypropylphosphate-5-phosphoribose (pathways A and B, respectively). However, it has been demonstrated that epimerization does not take place at the decaprenolphosphoribose level, because in vitro attempts to convert $^{14}$C-labeled decaprenyl-P-ribose into $^{14}$C-labeled decaprenyl-P-arabinose were unsuccessful.
Figure 1.9: The biosynthetic pathway to polyrenylphosphate arabinose.
Although it is widely accepted that DPA is the major, if not only, glycosyl donor for the incorporation of arabinofuranose residues into the mycobacterial cell wall, it has been suggested that sugar nucleotides could be involved in arabinan biosynthesis. The classical pathway of sugar biosynthesis involves the formation of a sugar nucleotide diphosphate (NDP, Figure 1.10).

Figure 1.10: Two potential pathways for D-arabinose biosynthesis involving a sugar nucleotide diphosphate.

D-Glucose is converted to a D-pentose 5-phosphate via the pentose phosphate pathway. The pentose could then be converted to D-arabinose by one of two pathways. The
D-pentose 5-phosphate could be epimerized to form D-arabinose 5-phosphate and subsequently converted to NDP-D-arabinose. Another possibility involves the conversion to an NDP-D-pentose before the pentose is epimerized to form NDP-D-arabinose.

If a sugar nucleotide is involved in arabinan biosynthesis, then one would expect to find either a phosphorylated form of D-arabinose or a nucleotide form of D-arabinose. There is some evidence of the formation of a nucleoside diphosphate arabinose from mycobacterial cells, and a partially purified uridine nucleotide of arabinose was reported. However, the identification of the nucleotide or sugar has not been conclusive. It is not known whether the arabinose was of the D or L configuration, or whether the arabinose had the furanose or pyranose ring structure (e.g. eukaryotic parasites possess D-arabinopyranosides). Furanose-containing sugar nucleotides are labile species, and the failure to isolate these compounds does not rule out their presence.

In a recent report, an attempt was made to isolate NDP-arabinose from *Mycobacterium smegmatis* (closely related to *M. tuberculosis*) incubated with [1-$^3$H]glucose. Arabinan biosynthesis involves the conversion of D-glucose to D-arabinose, and thus detection of NDP-[1-$^3$H]arabinose would suggest that a sugar nucleotide is involved in arabinan biosynthesis. The result of this incubation was that no [1-$^3$H]arabinose was found even in a large-scale extraction of the sugar nucleotide fraction from 300 g of cells. In addition, incubation of [$^{14}$C]glucose 6-phosphate and various nucleoside triphosphates (ATP, CTP, GTP, TTP, and UTP) with cytosolic or membrane fractions from mycobacterial cells did not result in formation of NDP-arabinose. These negative results suggest that if a NDP-arabinose is involved in arabinan biosynthesis, this species is preexisting and not formed via the classical pathway from D-glucose.
1.3 Design of inhibitors of arabinosyltransferases

An ideal candidate for a chemotherapeutic treatment is a drug that targets a specific type of pathogenic cell without harming healthy cells. Carbohydrates in the cell wall of mycobacteria present ideal targets for the development of new treatments for tuberculosis. α-D-Galactofuranose, D-arabinofuranose, and L-rhamnose are important components of the cell wall, but have no role in mammalian metabolism. As mentioned earlier, the galactose and arabinose residues present in the AG and LAM adopt a furanosidic conformation. Furanose oligosaccharides are not found in mammalian glycoconjugates and, thus, compounds that interfere with the biosynthesis of these fragments should not have significant deleterious effect on humans.\(^{27}\)

Our strategy for designing arabinosyltransferase inhibitors was to make substrate analogs based on the primary glycosyl donor decaprenolphosphoarabinose (DPA). In developing inhibitors specific for a particular human glycosyltransferase, little effort has been expended on donor analogs because a single glycosyl donor is usually a substrate for many glycosyltransferases and, thus donor analogs would also show non-specific inhibition. In bacterial systems, however, this lack of specificity can be exploited in developing glycosyltransferase inhibitors that act upon sugars that are foreign to humans. Donor analogs of DPA would be expected to be especially potent antimycobacterial agents because, unlike the glycosyl acceptors that are recognized by specific AraT’s, DPA is a substrate for many AraT’s and would be expected to block a number of biosynthetic steps.

Our initial goal was to synthesize a panel of C-phosphonic acid analogs of DPA in which the glycosidic oxygen is replaced with a methylene unit (e.g., \(\text{1.4, Figure 1.11}\)). C-Phosphonic acids are metabolically stable phosphate mimetics that have been used as phosphatase inhibitors and have also been shown to bind to carbohydrate processing
enzymes.\textsuperscript{28-30} The AraT catalyzed reactions of both the natural DPA glycosyl donor 1.1 and a C-phosphonic DPA analog are shown in Figure 1.12A&B. By replacing the anomeric oxygen with a carbon atom, 1.4 no longer has a good leaving group and thus chain elongation to form arabinan 1.3 cannot occur.

![Figure 1.11: C-Phosphonic acid substrate analogs of DPA.](image)

R = long alkyl chain

C-phosphonic acid DPA analog
Figure 1.12: AraT catalyzed reaction with DPA (A) and with C-phosphonic acid analog (B).

In designing C-phosphonic acids such as 1.4, we opted to first synthesize compounds with simple long-chain alkyl groups, instead of compounds with the long isoprenyl chain of the natural substrate. Ideally, an analog would contain only one structural variation from the parent substrate, in this case the substitution of a carbon-phosphorous linkage in place of the normal phosphate ester linkage. However, decaprenol and other polyprenols are extremely expensive ($700/50 mg), and our hope was that the arabinosyltransferases would recognize these more simplified structures as substrates, despite the significantly modified lipid chain. In addition to significantly reducing the cost, the use of simple and commercially available long-chain alkyl groups would greatly reduce the synthetic complexity of our substrate analogs.
In addition to making C-phosphonic acid DPA analogs, we chose to prepare C-phosphinic and C-sulfone acid derivatives (1.5 and 1.6, Figure 1.13). C-Phosphinic acids are structurally similar to the C-phosphonic acids but are stable to ester hydrolysis. These derivatives are commonly used in peptide synthesis, however, there have been very few reports of phosphate mimics of this type in carbohydrate chemistry. C-Sultones have also been explored as phosphate mimics in nucleic acids. Like phosphinic acids, sulfones are stable to biochemical hydrolysis. However, these compounds are easier to work with because they are nonionic.

![C-Phosphinic acid](image1)

**Figure 1.13:** C-Phosphinic and C-sulfone acid DPA analogs.

Another goal of this project was to synthesize a series of C-phosphonic acid sugar nucleotides of arabinose (e.g., 1.7, Figure 1.14). C-Phosphonic acid analogs of sugar nucleotides are not as labile as their phosphate counterparts. If one of these compounds were found to be biologically active against the enzyme, then this would provide indirect evidence for the involvement of these species in arabinan biosynthesis.
1.4 Background of glycosyl phosphate mimics

Glycomimetics are molecules that mimic a natural sugar or the transition state in an enzymatic transformation, and can replace the natural substrate in interactions with receptors and active sites of enzymes. There has been a growing interest in glycomimetics with the knowledge that carbohydrates play a key role in biological events, especially in cell-cell and cell-molecule recognition events. The modifications made to the natural sugar are usually more functional than structural, giving rise to compounds unable to undergo normal metabolic transformations, which ultimately interrupts metabolism.

Glycosyl phosphates play a central role in carbohydrate metabolism and are involved in the biosynthetic pathways of primary metabolism, for example glycolysis and gluconeogenesis. Glycosyl phosphates act as glycosyl donors in the biosynthesis of oligosaccharides, polysaccharides, glycoconjugates, and sometimes are regulators of metabolic processes.
1.4.1 Mimics of natural phosphates

There are two main classes of phosphono analogs of natural phosphonates. The first is the isosteric analog, in which one of the oxygens of the phosphate ester is replaced by one carbon atom, without any appreciable change in the geometry of the molecule. The second class is defined as the non-isosteric analog, in which the phosphoesteric oxygen is either removed or replaced by two or more carbon atoms. Examples of these two types of phosphono analogs are the isosteric analog of ribose 5-phosphate, 1.8, and the non-isosteric analog of glucose 6-phosphate, 1.9, illustrated in Figure 1.15.35,36

![Figure 1.15: Example of an isosteric and non-isosteric analog of natural phosphates.](image-url)
There are several factors that must be considered upon substitution of a phosphate with a phosphonic acid, aside from rendering the phosphorus not labile to hydrolysis. The first, and most important, factor of change is that of physical size and shape.\textsuperscript{37} The substitution of the phosphoester oxygen with a carbon atom does not significantly alter the geometry of a phosphate. This allows the phosphate analogs to fit the active site or receptor of the parent phosphate. The bond lengths and angles for both a phosphate and a phosphonate are shown in Figure 1.16.\textsuperscript{37}

![Figure 1.16: Bond length (in Å) and angles of a phosphate and a phosphonate.](image)

Because an isosteric phosphate analog only differs in that an oxygen atom is replaced with a carbon, it is expected that an isosteric analog would be a better analog than a non-isosteric analog. The omission or addition of an atom would have a much greater effect on the shape of the molecule and could greatly affect the biochemical or physiological activity unrelated to the simple substitution of a carbon-phosphorus linkage for a phosphate ester.

A second factor to consider is the change in acidity of the phosphorus-containing acid function upon the introduction of an electron donating alkyl group. This could result in the existence of different dissociation states for both the phosphate analog and natural phosphate at physiological pH. When one compares the acidity of a phosphonic acid with a simple
phosphate, it is the second pK$_a$ that is of importance.$^{37}$ For either system, the first pK$_a$ represents a strong acid. Crofts and Kosolapoff have measured the second pK$_a$’s for a series of phosphonic acids and found them to be in the range of 7.7-8.2 when a primary alkyl group is attached to the phosphorus.$^{38}$ Slightly lower values were reported for 2’-deoxythymidine 5’-phosphate, 1.10, and an isoteric phosphonic acid analog, 1.11, (Figure 1.17).$^{39}$ As expected, there was a decrease in acidity for the phosphonic acid analog compared with the natural phosphate. For phosphonic acids in which one of the hydroxyl groups has been replaced with methyl group (CH$_3$P(O)(OH)OR), there remains an ester linkage and a single acidic hydrogen of low pK$_a$. Under physiological conditions this class of compounds is considered completely dissociated.$^{37}$ It can be expected that phosphinic acids, in which there is only one acidic hydroxy group and no ester linkages would also be completely dissociated.

Figure 1.17: Acidity differences for 2’-deoxythymidine 5’-phosphate and phosphinic acid analog.
A final factor is the loss of the binding function of the oxygen of the esterified phosphate as a result of its absence in the analog. This is particularly a problem in those systems where a pyrophosphate linkage is modified. However, it is hard to distinguish this factor from that of size and shape.

1.4.2 Mimics of glycosyl phosphates

A glycosyl phosphate mimetic is one in which the phosphoesteric oxygen removed or replaced is also the anomeric oxygen of the sugar. Despite the importance of glycosyl phosphates, it was not until 1981 that examples of phosphono analogs of these compounds were described. At least part of the reason lies in the chemical and stereochemical difficulties faced in the process of C-glycosylation. Some of the known C-phosphonic acids, 1.12-1.17, are shown in Figure 1.18.

![Figure 1.18: Several known C-phosphonic acids.](image)
1.4.3 Chemical synthesis of C-phosphonates

The field of C-glycoside chemistry has grown enormously over recent years and there are numerous methods for the preparation of these compounds.\(^{45}\) In contrast, growth in the field of C-phosphonate chemistry has been much more sporadic over the last twenty years. Only a few strategies towards the synthesis of these compounds have been developed, and none are general, especially with regard to stereochemistry. The most common method for the preparation of C-phosphonates involves the use of Wittig chemistry on a sugar lactol, followed by cyclization of the resulting olefin. Halocyclization and mercuriocyclization are both frequently used to achieve cyclization to afford either the furanose or pyranose product. These reactions generally give good stereoselectivities, with the 1,2-\textit{cis} diastereomer as the major product.\(^{46,47}\) The sugar halide or mercurioderivative, which is easily converted to the halide, is reacted at high temperature with a trialkyl phosphite to afford the phosphonate. An example of this method is shown in Scheme 1.1.\(^{44}\) Nicotra and co-workers treated ribose-derived lactol 1.18 with methylenetriphenylphosphorane to afford olefin 1.19 in 96% yield. Mercuriocyclization of 1.19 gave an 82% yield of mainly the \(\alpha\)-anomer. Treatment with iodine, followed by Michaelis Arbuzov reaction with triethylphosphite afforded the phosphono analog 1.22 in 70% yield.
Scheme 1.1: Ribose-derived C-phosphonate synthesized by Nicotra and co-workers via mercuriocyclization/Michaelis Arbuzov method.

Similarly, Reitz and co-workers reported the synthesis of a C-phosphonic acid analog of β-D-arabinose 1,5-bisphosphate (Scheme 1.2).\textsuperscript{29b} Wittig olefination of tri-O-benzyl-D-arabinofuranose, 1.23, followed by halocyclization with N-bromosuccinimide, afforded 1.25 as an inseparable mixture. Bromide 1.26 was formed in several steps and then reacted with diphenylethyl phosphite. Phosphonate 1.27 was formed in 40% yield as a solid, which was separable from the α-anomer.
Scheme 1.2: β-D-Arabinose 1,5-bisphosphate analog synthesized by Reitz and co-workers via bromocyclization/Michaelis Arbuzov method.

The deprotection of 1.27 proved to be particularly difficult. The standard methods for removal of benzyl groups (e.g., lithium-ammonia reduction, metal-catalyzed hydrogenation) resulted in either decomposition or formation of a cyclized, cis-fused 1,2-oxaphospholane, which is also known as a phostone. The authors suggested that the free adjacent hydroxyl group cyclized onto the diphenylphosphoryl ester group of the phosphonate, a side-reaction that was observed in a related system. The desired product, 1.28, was successfully obtained in a 3 step sequence, which involved treatment of 1.27 with tetramethylammonium hydroxide, followed by hydrogenolysis of the benzyl groups with palladium and removal of the remaining phenyl esters with hydrogen and platinum. The problem of intramolecular cyclization was avoided by the presence of the ammonium base, which “protected” the phosphonate group as a monoanion.
A more direct method for the formation of C-phosphonates involves the use of Horner-Emmons reagents in the presence of base, although the stereoselectivity of these reactions are generally poor (Scheme 1.3). Meyer and co-workers reported the synthesis of phosphonate 1.30 from lactol 1.29, which was separable from the β-anomer. However, Reitz and co-workers reported an inseparable mixture of phosphonate 1.31 after treating lactol 1.23 with the anion derived from tetraethyl methylenebisphosphonate.

Scheme 1.3: Horner-Emmons route to C-phosphonates.

A different C-glycosylation approach towards C-phosphonate involves the reaction of a stabilized ylide or Horner-Emmons reagent with an aldose, followed by Michael cyclization of the product. Reitz and co-workers used this approach as an alternative method for the preparation of compound 1.31, as described above. t-Butyldimethylsilyl protected aldose 1.32 reacted with tetraethyl diphosphonate to give α/β-unsaturated phosphonate 1.33 in 40% yield (Scheme 1.4). Treatment of 1.33 with fluoride ion afforded the cyclized product, albeit in low yield and enriched in the undesired α-anomer.
Scheme 1.4: Olefination/Michael cyclization route to C-phosphonates.

A more recent, and stereoselective, approach to the synthesis of C-phosphonate azasugars was reported by Eustache and co-workers (Scheme 1.5). Lactam 1.35 was obtained in 81% yield from N-benzyloxycarbonyl protected sugar 1.34 (prepared in 4 steps from 1.23). Diethyl methylphosphonate anion was added to 1.35, and the benzyloxycarbonyl group was removed to afford 1.36a and 1.36b in equilibrium. Catalytic hydrogenation of 1.36 afforded phosphonoazsugar 1.37 in 80% yield as a single β-anomer.
Scheme 1.5: Synthesis of a C-phosphoazasugar reported by Eustache and co-workers.

The examples presented above represent a few of the limited number of methods for the preparation of C-phosphonates and C-phosphonic acids. Unfortunately, none of these methods are general for all furanose/pyranose ring systems. The limited stereoselectivity, resulting in inseparable anomeric mixtures, remains a key concern. There are even fewer examples of C-phosphonic acids in which a hydroxyl group is replaced with an ester linkage. Selective removal of the methyl or ethyl esters after an Arbuzov reaction to obtain the free phosphonic acids can be problematic, and the use of sterically hindered phosphites, such as diphenylethyl phosphite have lower reactivity.

1.5 Phosphinic acids as phosphate mimics

C-Phosphinic acids are analogs of glycosyl phosphates in which two of the P-O bonds have been replaced with P-C bonds. To date, there have been very few reports of phosphinic acid analogs of naturally occurring carbohydrate phosphates, and C-phosphinic acid analogs of glycosyl phosphates have not been reported. The synthesis of a 3-furanosyl-
6'-furanosylphosphinate was recently reported by Piettre and co-workers (1.42, Scheme 1.6). The key step involved a radical reaction between an H-phosphinate 1.40b and olefin 1.41. Alkene 1.38 reacted with hypophosphorus 1.39 to produce phosphinous acid 1.40a, which was esterified with diazomethane to form 1.40b. This product was coupled with 1.41 using tert-butylperoxypivalate, and then esterified to afford 1.42.

Scheme 1.6: Synthesis of a 3-furanosyl-6’-furanosylphosphinate via a tandem radical process reported by Piettre and co-workers.
Similarly, Collingwood reported the synthesis of a dinucleotide containing a phosphinate group. Phosphinate 1.45 was formed by the addition of the phosphorus anion of 1.43 to aldehyde 1.44. Subsequent functional group transformations afforded target 1.46.

Scheme 1.7: Synthesis of a phosphinate-containing oligonucleotide via addition of a phosphorus anion onto an aldehyde reported by Collingwood.

1.6 Sulfones as phosphate mimics

1.6.1 Sulfones in nucleic acids

Sulfones have been explored as non-ionic backbone linkers in DNA and RNA. Natural oligonucleotides are sensitive to hydrolysis and are easily degraded in vivo. Because of their polyanionic phosphate backbone, they do not readily pass through biological
membranes, thus getting oligonucleotides to their intracellular targets is difficult. Therefore, analogs of natural oligonucleotides with increased biological stability and membrane permeability have been sought. Benner and co-workers reported the synthesis of sulfone-linked nucleic acid analogs of RNA (rSNAs). Oligomers of RNA were synthesized in which the phosphodiester linkages were replaced by dimethylene sulfone units (e.g., 1.47, Figure 1.19). The absence of the polyanionic backbone, however, had profound structural effects important for molecular recognition.

Figure 1.19: Sulfone-linked nucleic acid analogs of RNA (rSNAs) synthesized by Benner and co-workers.
1.6.2 Sulfones as glycosyl phosphate mimics

In addition to being explored as nucleotide and peptide analogs, sulfones have been employed as carbohydrate mimics. However, examples of sugars in which the anomeric position has been replaced with a methylenesulfonyl (CH$_2$SO$_2$R) group have been only sparingly described. Rollins and Lorin reported the synthesis of a series of non-anomeric sugar sulfones (e.g., 1.51a-e, Scheme 1.8) using a trans-sulfonylation process.$^{55}$ Sulfone 1.49 was prepared via a Mitsunobu reaction on alcohol 1.48, followed by oxidation with $m$-CPBA. Deprotection of the benzothiazol-2-yl (Btz) group using sodium methoxide, followed by alkylation with various electrophiles (RX) afforded sulfones 1.51a-e. For the D-galactopyranosyl template 1.51, the yields were all very good except in the case of the farnesyl derivative 1.51d.

Scheme 1.8: Rollin’s synthesis of sugar sulfones via trans-sulfonylation process.

In another report, azasugar-thio analogs of decaprenolphosphoarabinose (DPA) were synthesized and tested in vitro against M. tuberculosis strain H37Ra and several strains of M. avium.$^{56}$ The synthesis of sulfide, sulfoxide, and sulfone azasugars is shown Scheme 1.9.
Pyrrolidine 1.52, derived from 5-ketofructose, was converted in 4 steps to 1.53. Alcohol 1.53 was converted to the iodide, and treated with various alkyl sodium sulfides (NaSR) to afford 1.54. Deprotection of 1.54 was achieved using potassium hydroxide, followed by tetraethylammonium fluoride to afford sulfide 1.56. Sulfoxide 1.57 and sulfone 1.58 were prepared by oxidation of 1.55 and 1.54, respectively, followed by deprotection. The minimum inhibition concentrations (MIC) for three of these DPA analogs (1.56a, 1.57a, 1.58a, where R = (CH\(_2\))\(_n\)(CH\(_3\))) are shown in Figure 1.20. All three compounds were only weakly active against the organism. Surprisingly, sulfide 1.56a was the most active, which suggests that the polar oxygen groups may not be necessary for enzyme recognition.

Scheme 1.9: Synthesis of sulfide, sulfoxide, and sulfone DPA analogs reported by Maddry and Reynolds.
A related area of interest is the development of sulfone analogs of sugar nucleotides (e.g., 1.59, Figure 1.21). Sulfone analogs are expected to be exceptionally stable towards glycosidase activity, and thus they would make potent glycosidase inhibitors. Gervay and co-workers have reported some preliminary investigations towards the synthesis of sugar nucleotides analogs like 1.59.57 The synthesis of a C-sulfone derivative of L-fucopyranose, 1.62, is illustrated in Scheme 1.10. Radical addition of thioacetic acid to olefin 1.60 afforded the β-C-glycoside 1.61 in 78% yield. Deprotection of 1.61 and alkylation of the resulting thiol provided the thiomethyl C-glycoside, which was oxidized with dimethyldioxirane to afford 1.62.

Figure 1.20: Minimum inhibition concentration (MIC) for sulfide, sulfoxide, and sulfone DPA analogs.

Figure 1.21: Sulfone analogs of sugar nucleotides.
Scheme 1.10: Synthesis of C-sulfone derivative of L-fucopyranose reported by Gervay and co-workers.

This route for the preparation of sulfone derivatives of sugar nucleotides has been further explored with attempts to alkyate methyl C-sulfone 1.63 and methyl C-gem-disulfone 1.64 with various electrophiles RX (Scheme 1.11). However, attempts to alkylate these compounds under a variety of reaction conditions resulted in the formation of complex mixtures. This was attributed to the competing formation of the methyl and methylene anions, either of which could be alkylated.

Scheme 1.11: Gervay and co-worker’s attempt to alkylate methyl C-sulfone and methyl C-gem-disulfone.
1.7  C-Phosphonic acid sugar nucleotide analogs

Complex carbohydrates and their conjugates are involved in many biological recognition processes. Glycosyltransferases, which catalyze the biosynthesis of these important structures, usually employ sugar nucleotides as glycosyl donors. For example, D-galactofuranosyltransferases mediate the incorporation of D-galactofuranose into immunologically important glycoconjugates from bacteria, mycobacteria (including *Mycobacterium tuberculosis*), protozoa, and fungi.\(^5\) These glycoconjugates are essential for the survival and pathogenicity of many microorganisms. The biosynthetic glycosyl donor used to incorporate D-galactofuranose residues is uridine 5’-diphospho-α-D-galactofuranose (UDP-gal, 1.65) shown in Figure 1.22.

![Figure 1.22](image)

Figure 1.22: Uridine 5’-diphospho-α-D-galactofuranose (UDP-gal).

Sugar nucleotides like 1.65 are labile species, and there has been interest in the development of C-sugar nucleotides, which are stable pharmacophores. The synthesis of UDP-C-D-galactofuranose 1.71 was reported by Sinaý and co-workers (Scheme 1.12).\(^6\) Phosphonate 1.69 was synthesized using standard methods. Hydrolysis of methyl glycoside 1.66, followed by a Wittig olefination on the resulting lactol afforded 1.67. Olefin 1.67 was
cyclized using iodine to form 1.68, which was subsequently heated with trimethyl phosphite to afford 1.69. Treatment of 1.69 with iodo(trimethyl)silane in carbon tetrachloride afforded the free phosphonic acid, which was co-evaporated with pyridine and reacted with uridine 5’-monophosphate morpholidate 1.70. After 5 days, 1.71 was formed in 80% yield.

Scheme 1.12: Synthesis of UDP-C-D-galactofuranose reported by Sinaï and co-workers.

1.8 General description of thesis contents

This thesis reports the syntheses of three types of decaprenolphosphoarabinose (DPA) analogs. Chapter 2 reports a novel synthesis of C-phosphonic acids (1.4, Figure 1.11) derived from D-arabinose. In our synthesis, the formation of the furanose ring is achieved in an intramolecular Mitsunobu reaction on a 1,4-diol. The lipid chain is added onto the phosphonate via a cross-metathesis reaction. Chapter 3 presents the first reported synthesis
of C-glycosyl phosphinic acids (1.5, Figure 1.13). Our strategy involves the reducing a sugar phosphonate to a phosphine, alkylating it, and finally reoxidizing it to the phosphinic acid. Chapter 4 describes the synthesis of the third type of DPA analog, the C-sulfone (1.6, Figure 1.13). The synthesis is straightforward and involves the alkylation of a sugar thiol, followed by oxidation to afford the sulfone. Chapter 5 describes our attempts to synthesize C-sugar nucleotides of the general type 1.7 illustrated in Figure 1.14.

References


CHAPTER 2

SYNTHESIS AND ANTI-TUBERCULOSIS ACTIVITY OF
C-PHOSPHONATE ANALOGS OF
DECAPRENOLPHOSPHOARABINOSE, A KEY INTERMEDIATE IN
THE BIOSYNTHESIS OF MYCOBACTERIAL ARABINOGALACTAN
AND LIPOARABINOMANNAN

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**Introduction**

Mycobacteria, including the human pathogens *Mycobacterium tuberculosis* and *Mycobacterium leprae*, possess an extraordinarily thick and complicated cell wall structure that provides the organism with a great deal of protection from its environment.\(^1\) The viability of the organism is dependent upon its ability to synthesize an intact cell wall and, consequently, inhibitors of the enzymes that are involved in the biosynthesis of various cell wall components are potential antimycobacterial agents.\(^2\) Indeed, some of the antibiotics currently used to treat tuberculosis (e.g., ethambutol and isoniazid) act by blocking cell wall assembly.\(^3\)
Key structural components of the mycobacterial cell wall are two polysaccharides, arabinogalactan (AG) and lipoarabinomannan (LAM), which are unusual in that all of the arabinose and galactose residues exist in the furanose ring form. Glycoconjugates that contain galactofuranose and arabinofuranose are xenobiotic to mammals and, hence, the enzymes that are involved in the biosynthesis of these glycans are ideal targets for drug action. Over the past few years, we have carried out synthetic and conformational investigations directed ultimately at the development of inhibitors of the glycosyltransferases that assemble the arabinan portions of AG and LAM. Our interest in mycobacterial arabinosyltransferases (AraT's) originated from studies that validated these enzymes as suitable targets for drug action. Previous reports have demonstrated that ethambutol, an antibiotic used to treat tuberculosis, acts by inhibiting the AraT's involved in AG and LAM biosynthesis.

The structure of the arabinan in AG and LAM is essentially identical. The glycan core consists of a linear \( \alpha-(1 \rightarrow 5) \) linked chain of arabinofuranose residues, with periodic \( \alpha-(1 \rightarrow 3) \)-linked branch points from which additional \( \alpha-(1 \rightarrow 5) \) linked chains are attached. Attached to this core arabinan, at the non-reducing termini of each linear chain, is the hexasaccharide 2.1 (Chart 2.1). The biosynthesis of this arabinan is postulated to involve a family of AraT's that produce \( \beta-(1 \rightarrow 2) \), \( \alpha-(1 \rightarrow 3) \), and \( \alpha-(1 \rightarrow 5) \) arabinofuranosyl linkages. A prototypical AraT catalyzed reaction (Figure 2.1) involves the coupling of an oligosaccharide acceptor (e.g., 2.2), with decaprenolphosphoarabinose (DPA, 2.3) to afford an elongated oligosaccharide, 2.4.
Chart 2.1: Hexasaccharide attached at nonreducing termini of arabinan.

Figure 2.1: Prototypical arabinosyltransferase-catalyzed reaction.

In designing potential inhibitors of these AraT's, logical first choices are analogs of the two substrates recognized by the enzyme. The majority of our previous work in this area has focused on synthesizing analogs of the acceptor oligosaccharides.\textsuperscript{5a-d} This approach has also been used widely in developing inhibitors that are specific for particular human glycosyltransferases.\textsuperscript{9} Donor analogs have been less widely studied because a single
glycosyl donor is usually a substrate for a number of glycosyltransferases, and these compounds are therefore expected to lack specificity for a particular enzyme. In bacterial systems, this lack of specificity with regard to the donor can be exploited. Because 2.3 is a substrate for more than one AraT, analogs of this compound would be expected to block a number of biosynthetic steps and, in turn, be especially potent antimycobacterials.

We describe here the synthesis of a panel of C-phosphonate-based DPA analogs. It was our expectation that replacement of the glycosidic oxygen in 2.3 with a methylene group would provide compounds (2.5, Chart 2.2) that would be bound by these AraT's, but that could not turn over. These C-phosphonates would therefore block the assembly of mycobacterial arabinan and thus prevent growth of the organism. Our goal was not to prepare compounds that contained the long isoprenoid chain present in 2.3. Rather, we endeavored to synthesize molecules in which simple long-chain linear alkyl groups replace this lipid.

![Chart 2.2: Potential C-phosphonate-based DPA analogs.](image)

Results and Discussion

**Initial approaches.** Although a number of C-phosphonate analogs of glycosyl phosphates (e.g., 2.6, Chart 2.2) have been synthesized, far fewer reports have described the
preparation of the corresponding phosphonate ester derivatives (e.g., 2.5).\textsuperscript{11} We initially explored the possibility of synthesizing these compounds from iodide 2.7 (Figure 2.2A) via a route that involves the formation of an \textit{H}-phosphonate,\textsuperscript{12} esterification with the appropriate alcohol,\textsuperscript{13} oxidation of the \textit{H}-phosphonate\textsuperscript{14} and subsequent deprotection. Thus (Scheme 2.1), 2.7 was prepared\textsuperscript{15} and then treated with bis(trimethylsilyl)phosphonite.\textsuperscript{12} However, despite a number of attempts under different reaction conditions, only unreacted 2.7 was isolated following the reaction.

![Diagram of retrosynthesis]

Figure 2.2: Retrosynthesis of 2.5.
Scheme 2.1\textsuperscript{a}: Synthesis of 2.8.

We therefore explored an approach in which the carbon-phosphorous bond was installed via a Michaelis-Arbuzov reaction\textsuperscript{16} (Figure 2.2B). The long chain alkyl moiety was to be introduced via an esterification reaction following the selective cleavage of the ethyl groups. With this route in mind (Scheme 2.2), reaction of 2.7 with triethylphosphite at 156 °C (reflux) provided the diethyl phosphonate derivative 2.9\textsuperscript{17} in 91% yield. Next, the conversion of 2.9 to 2.10 via the selective deprotection of the ethyl groups using TMSI and TMSBr was explored. We were unsuccessful, however, as the cleavage of the ethyl groups was also accompanied by loss of one of more of the benzyl groups. It was extremely difficult to separate the products of the reaction and we therefore abandoned this approach.
Scheme 2.2\textsuperscript{a}: Synthesis of 2.10.

We next explored a route starting from lactone 2.11\textsuperscript{18} (Figure 2.2C). To assess the viability of this approach (Scheme 2.3), 2.11 was reacted with 2.12\textsuperscript{19} to provide the adduct 2.13\textsuperscript{20} in 93% yield. Deoxygenation of 2.13 with triethylsilane and trimethylsilyl trifluoromethanesulfonate as the Lewis acid\textsuperscript{21} provided a deoxygenated product in nearly quantitative yield. Unfortunately, the product produced from this reaction was shown to be 2.14, which possessed the incorrect stereochemistry at the "anomeric" center.\textsuperscript{22}
Scheme 2.3\textsuperscript{a}: Synthesis of 2.14.

Having been unsuccessful in the synthesis of 2.5 via what we considered to be the most direct routes, a number of other approaches were explored, also without success. Therefore, after considerable investigation, we slightly modified the structure of the targets and developed a successful route for their synthesis. The retrosynthesis for this approach is provided in Figure 2.3. The modified targets (2.15) differ from 2.5 in that an oxygen atom is present in the long alkyl chain. We anticipated that this modification would not significantly influence the inhibitory potential of 2.15 relative to 2.5. In this approach, the long chain alkyl group was to be introduced via an olefin cross metathesis reaction\textsuperscript{23} of 2.16 and 2.17. Subsequent reduction of the double bond and deprotection would afford the targets. Phosphonate 2.16 can be accessed from 2.19 and the protected L-xylose derivative 2.18.\textsuperscript{24}
Preparation of phosphonate 2.19. For the synthesis of 2.19 we employed methodology developed by Gagne and coworkers (Scheme 2.4).\textsuperscript{25} Dimethyl methylphosphonate (2.20) was converted to the corresponding dibenzyl ester 2.21\textsuperscript{26} in 90% yield by reaction with benzyl acetate and potassium tert-butoxide. Subsequent transesterification of 2.21 with allyl acetate and potassium tert-butoxide under controlled conditions afforded 2.22 in 47% yield. The synthesis of 2.22 from the commercially available dichloride 2.23 was also possible, but the yield of the product was lower than the route from 2.20. Lithiation of 2.22 with $n$-BuLi in THF afforded 2.19.
Scheme 2.4a: Synthesis of 2.19.

*a Conditions: (a) BnOAc, t-BuOK, THF, rt, 90%; (b) AllOAc, t-BuOK, THF, rt, 47%; (c) BnOH, pyridine, then AllOH 0 °C, 33%; (d) n-BuLi, THF, -78 °C.
Synthesis of 2.16 from 2.18 and 2.19. On the basis of previous investigations, we envisioned that C-phosphonate 2.16 could be obtained via a Mitsunobu reaction of acyclic diol 2.24 (Figure 2.4). In this earlier study, diols of this general type were shown to undergo Mitsunobu cyclization with inversion of the stereochemistry at C-5, presumably via formation of a phosphonium ion such as 2.25.

![Figure 2.4: Proposed formation of 2.16 from 2.24 via Mitsunobu cyclization.](image)

The synthesis of 2.16 is illustrated in Scheme 2.5. Reaction of the L-xylose derivative 2.18 with 2.19 afforded a 90% yield of ketose 2.26, which was subsequently reduced, in 87% yield, with sodium borohydride. This reaction was only marginally stereoselective affording the two possible diastereomeric products (2.24 and 2.27) as a 2:1 mixture, which was impossible to separate. Other reducing agents were explored to carry out this reduction (e.g., L-Selectride and (+) diisopinocamphenylborane), but with no improvement in stereoselectivity. To separate the diastereomers, the mixture was treated with benzaldehyde dimethyl acetal and p-TsOH, which provided all four possible
diastereomeric benzylidene derivatives 2.28A and 2.28B. These were separated by chromatography and the acetal protecting group was cleaved under acidic conditions to provide 2.24 and 2.27 as pure compounds. Each of the diols was cyclized upon treatment with triphenylphosphine and diisopropyl-azodicarboxylate (DIAD) affording 2.16 and its stereoisomer 2.29, in 90 and 72% yields, respectively. The stereochemistry of 2.16 was proven by complete deprotection, which afforded a product (2.6) that was identical to that obtained by total deprotection of 2.9 (Scheme 2.6).

Scheme 2.5*: Synthesis of 2.16.

* Conditions: (a) 2.19, THF, -78 °C, 90%; (b) NaBH₄, THF, rt, 87%; (c) benzaldehyde dimethyl acetal, p-TsOH, CH₃CN, 75% from 2.26 (combined 2.28A and 2.28B); (d) 80% AcOH, 50 °C, 93%; (e) 80% AcOH, 50 °C, 81%; (f) Ph₃P, DIAD, THF, rt, 90%; (g) Ph₃P, DIAD, THF, rt, 72%.
Synthesis of 2.17 and cross metathesis reactions. The synthesis of the targets required as a key step an olefin cross metathesis reaction between 2.16 and a panel of alkenes with the general structure 2.17. The synthesis of these alkenes was achieved by alkylation of cis-2-buten-1,4-diol (2.30) under standard conditions (Scheme 2.7). Each was then reacted with 2.16 in the presence 20 mol% of the Grubbs catalyst (2.31, Scheme 2.8) in refluxing dichloromethane, which provided the cross metathesis products 2.32 in 51–66% yield as a mixture of cis/trans isomers. Subsequent reduction of the alkene was achieved by reaction with diimide to afford the reduced products 2.33 in 74–95% yield. We also explored the use of (Ph₃P)₃RhCl/H₂ and Raney Nickel/H₂ to carry out this reduction, but found the diimide reaction gave better yields of the products.²⁹ The final targets 2.15 were obtained by cleavage of the benzyl groups by hydrogenation. Following chromatography on Iatrobeads,³⁰

Scheme 2.6³: Synthesis of 2.6.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂, Pd/C, AcOH</td>
<td>then TMSBr, CH₂Cl₂, rt</td>
<td>81%</td>
</tr>
<tr>
<td>H₂, Pd/C, AcOH</td>
<td>then TMSBr, CH₂Cl₂, rt</td>
<td>75%</td>
</tr>
</tbody>
</table>
the products were obtained in low to modest yield (20–68%). We are unsure as to why the yield of the deprotection step is relatively poor in some cases.

TLC of the crude reaction mixtures indicated that in some cases a significant amount of very polar material was formed in addition to the desired product. Previous work with C-phosphonate analogs of arabinofuranosyl pyrophosphates (e.g., 2.32, Figure 2.5) demonstrated the formation of phostone 2.33. The formation of a compound analogous to 2.33 is also possible here, although we could not detect the presence of such a species in the eluants obtained by washing the Iatrobead column with 3:1 dichloromethane:methanol.

\[
\begin{align*}
\text{HO} & \xrightarrow{a} \text{RO} \\
2.30 & \\
2.17a & \text{R} = (\text{CH}_2)_6\text{CH}_3 \\
2.17b & \text{R} = (\text{CH}_2)_7\text{CH}_3 \\
2.17c & \text{R} = (\text{CH}_2)_8\text{CH}_3 \\
2.17d & \text{R} = (\text{CH}_2)_9\text{CH}_3 \\
2.17e & \text{R} = (\text{CH}_2)_{10}\text{CH}_3 \\
2.17f & \text{R} = (\text{CH}_2)_{11}\text{CH}_3
\end{align*}
\]

\[\text{Conditions: (a) NaH, RI, DMF, rt}\]

Scheme 2.7a: Synthesis of 2.17.
Scheme 2.8:Synthesis of 2.15.

Figure 2.5: Formation of phostone 2.33 from 2.32 (see ref 31).
**Screening of final compounds as anti-tuberculosis agents.** Compounds 2.15a–2.15f were tested *in vitro* for the ability to prevent growth of *Mycobacterium tuberculosis* strain H37Rv (ATCC 27294) using the Alamar Blue Microplate assay. Only one of the analogs, 2.15f, possessed activity, and was shown to have an MIC of 3.13 µg/mL. It appears therefore that a fairly lengthy alkyl chain is required for compounds of this type to possess anti-tuberculosis activity.

In conclusion, we report here the synthesis of six C-phosphonate analogs of decaprenolphosphoarabinose. These compounds were synthesized via a route that had as a key step an olefin cross metathesis between allyl C-phosphonate 2.16 and an alkene 2.17. One of the six compounds was shown to prevent growth of mycobacteria in an *in vitro* assay. Screening of the compounds as inhibitors of mycobacterial arabinosyltransferases is currently in progress.

**Experimental**

**General.** Solvents were distilled from the appropriate drying agents before use. Unless stated otherwise, all reactions were carried out at room temperature under a positive pressure of argon and were monitored by TLC on silica gel 60 F254. Spots were detected under UV light or by charring with 10% H2SO4 in ethanol. Solvents were evaporated under reduced pressure and below 40 °C (bath). Organic solutions of crude products were dried over anhydrous Na2SO4. Unless otherwise indicated, column chromatography was performed on silica gel 60 (40–60 µM). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). Optical rotations were measured at 21±2 °C. 1H NMR spectra were recorded at 250, 400, or 500 MHz, and chemical shifts are referenced to either TMS (0.0, CDCl3) or CD3OH (4.78, CD3OD). 13C NMR spectra were recorded at 62.5, 100, or 125 MHz and chemical shifts are referenced to CDCl3 (77.00, CDCl3) or CH3OH (49.00,
CD$_3$OD). $^{31}$P NMR spectra were recorded at 101, 162, or 202 MHz and chemical shifts are referenced to external phosphoric acid (0.0, CDCl$_3$, CD$_3$OD). Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. Electrospray mass spectra were recorded on samples suspended in mixtures of THF and CH$_3$OH with added trifluoroacetic acid or NaCl.

**2,5-Anhydro-D-glucityl phosphonic acid (2.6).** Phosphonate 2.9$^{17}$ (2.7 g, 4.87 mmol) was dissolved in glacial HOAc (10 mL). Palladium (10% on activated carbon, 500 mg) was added and the reaction mixture was stirred under hydrogen overnight at atmospheric pressure. The solid was then filtered off and the filtrate was concentrated to a clear residue that was purified by chromatography (9:1, CH$_2$Cl$_2$:CH$_3$OH) affording 1-diethyl-2,5-anhydroglucityl phosphonate (1.13 g, 83%): $R_f$ 0.33 (9:1, CH$_2$Cl$_2$:CH$_3$OH). The diethyl phosphonate from above was dissolved in CH$_2$Cl$_2$ (5 mL). Bromotrimethylsilane (1.7 mL, 12.9 mmol) was added and the reaction mixture stirred overnight. Methanol (1 mL) was added to the orange reaction mixture and the solvent was evaporated to give a crude oil. The oil was purified by ion-exchange chromatography (AG 1-X8 resin) and was eluted with a 0.2 M solution of triethylammonium bicarbonate. After evaporation of the eluant, the clear oil was dissolved in CH$_3$OH (5 mL) and treated with Amberlite H$^+$ resin. The resin was then filtered to afford 2.6 (853 mg, 91%) as a clear oil. Compound 6 was also prepared (in 81% yield) by subjecting phosphonate 2.16 to these reaction conditions: $^1$H NMR (400 MHz, D$_2$O) $\delta_H$ 2.10–1.90 (m, 2 H), 3.66–3.47 (m, 3 H), 3.93–3.83 (m, 2 H), 4.15–4.08 (m, 1 H); $^{13}$C NMR (100 MHz, D$_2$O) $\delta_C$ 25.8 (d, $J = 135.3$ Hz), 61.0, 75.6, 76.7 (d, $J = 8.8$ Hz), 77.4, 84.3; $^{31}$P NMR (162 MHz, D$_2$O) $\delta_P$ 28.0; HRMS calcd for [C$_6$H$_{13}$O$_7$P]Na$: 251.0291. Found: 251.0425.
1-(Butyl-4’-O-heptyl)-2,5-anhydro-D-glucityl phosphonate (2.15a). Phosphonate 2.33a (95 mg, 0.125 mmol) was dissolved in a mixture of CH$_3$OH and AcOH (5 mL:10 µL). Palladium (10% on activated carbon, 22 mg) was added and the reaction mixture was stirred under hydrogen overnight at atmospheric pressure. After filtering off the solid and evaporating the solvent, the residual oil was purified by chromatography on Iatrobeads (CH$_2$Cl$_2$→3:1, CH$_2$Cl$_2$:CH$_3$OH). The pure product was then redissolved in water and then lyophilized to give 2.15a (13 mg, 26%) as an off-white solid: $R_f$ 0.25 (2:1, CH$_2$Cl$_2$:CH$_3$OH); [α]$^\text{D}_{25}$ +11.8 (c 2.2, CH$_3$OH); $^1$H NMR (500 MHz, CD$_3$OD) $\delta$H 0.83 (t, 3 H, $J$ = 7.1 Hz), 1.29–1.23 (m, 8 H), 1.50–1.44 (m, 2 H), 1.70–1.57 (m, 4 H), 2.16–2.01 (m, 2 H), 3.35 (t, 2 H, $J$ = 6.6 Hz), 3.39 (t, 2 H, $J$ = 6.3 Hz), 3.55 (dd, 1 H, $J$ = 11.5, 5.0 Hz), 3.61 (dd, 1 H, $J$ = 11.5, 3.6 Hz), 3.69–3.67 (m, 1 H), 3.83–3.82 (m, 1 H), 3.89–3.88 (m, 1 H), 3.98 (dd, 2 H, $J$ = 6.5, 6.5, 6.5 Hz), 4.25–4.17 (m, 1 H); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$C 14.4, 23.7, 27.0, 27.1 (d, $J$ = 138.8 Hz), 27.3, 28.5 (d, $J$ = 6.6 Hz), 30.3, 30.8, 33.0, 63.5, 66.4 (d, $J$ = 6.3 Hz), 71.3, 72.0, 77.7, 79.1 (d, $J$ = 7.8 Hz), 80.1, 87.5; $^{31}$P NMR (202 MHz, CD$_3$OD) $\delta$P 27.9; HRMS calcd for [C$_{17}$H$_{35}$O$_8$P]Na$^+$: 421.1962. Found: 421.1953.

1-(Butyl-4’-O-octyl)-2,5-anhydro-D-glucityl phosphonate (2.15b). Phosphonate 2.33b (132 mg, 0.171 mmol) was hydrogenated as described for 2.33a. Purification of the product was done as outlined for 2.15a to provide 2.15b (33 mg, 47%) as an off-white solid: $R_f$ 0.23 (2:1, CH$_2$Cl$_2$:CH$_3$OH); [α]$^\text{D}_{25}$ +12.2 (c 1.2, CH$_3$OH); $^1$H NMR (400 MHz, CD$_3$OD) $\delta$H 0.81 (t, 3 H, $J$ = 6.8 Hz), 1.33–1.14 (m, 10 H), 1.50–1.43 (m, 2 H), 1.70–1.54 (m, 4 H), 2.17–1.97 (m, 2 H), 3.34 (t, 2 H, $J$ = 6.6 Hz), 3.37 (t, 2 H, $J$ = 6.0 Hz), 3.54 (dd, 1 H, $J$ = 11.5, 5.1 Hz), 3.59 (dd, 1 H, $J$ = 11.6, 3.7 Hz), 3.69–3.65 (m, 1 H), 3.83–3.79 (m, 1 H), 3.89–3.85 (m, 1 H), 3.96 (dd, 2 H, $J$ = 6.5, 6.5, 6.5, 6.5 Hz), 4.23–4.15 (m, 1 H); $^{13}$C NMR
(100 MHz, CD$_3$OD) $\delta_C$ 14.4, 23.7, 27.0, 27.1 (d, $J = 139.2$ Hz), 27.3, 28.5 (d, $J = 6.4$ Hz), 30.4, 30.6, 30.8, 33.0, 63.5, 66.3 (d, $J = 6.4$ Hz), 71.3, 72.0, 77.7, 79.1 (d, $J = 7.8$ Hz), 80.1, 87.5; $^{31}$P NMR (162 MHz, CD$_3$OD) $\delta_p$ 29.4; HRMS calcd for [C$_{18}$H$_{37}$O$_8$P]Na$: 435.2118$. Found: 435.2079.

1-(Butyl-4’-O-nonyl)-2,5-anhydro-D-glucityl phosphonate (2.15c). Phosphonate $^{2.33c}$ (190 mg, 0.254 mmol) was hydrogenated as described for $^{2.33a}$. Purification of the product was done as outlined for $^{2.15a}$ to provide $^{2.15c}$ (56 mg, 54%) as an off-white solid: $R_f$ 0.19 (3:1, CH$_2$Cl$_2$:CH$_3$OH); $[\alpha]^{25}_D$ +12.2 (c 1.1, CH$_3$OH); $^{1}$H NMR (400 MHz, CD$_3$OD) $\delta_H$ 0.81 (t, 3 H, $J = 7.0$ Hz), 1.30–1.10 (m, 12 H), 1.50–1.42 (m, 2 H), 1.70–1.53 (m, 4 H), 2.16–1.97 (m, 2 H), 3.34 (t, 2 H, $J = 6.6$ Hz), 3.37 (t, 2 H, $J = 6.4$ Hz), 3.54 (dd, 1 H, $J = 11.5$, 5.2 Hz), 3.60 (dd, 1 H, $J = 11.5$, 3.6 Hz), 3.68–3.64 (m, 1 H), 3.82–3.80 (m, 1 H), 3.88–3.86 (m, 1 H), 3.96 (dddd, 2 H, $J = 6.5$, 6.5, 6.5, 6.5 Hz), 4.23–4.15 (m, 1 H); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta_C$ 14.4, 23.7, 27.0, 27.1 (d, $J = 139.0$ Hz), 27.3, 28.5 (d, $J = 6.4$ Hz), 30.4, 30.6, 30.8, 33.0, 63.5, 66.4 (d, $J = 6.2$ Hz), 71.3, 72.0, 77.7, 79.1 (d, $J = 8.0$ Hz), 80.1, 87.5; $^{31}$P NMR (162 MHz, CD$_3$OD) $\delta_p$ 29.4; HRMS calcd for [C$_{19}$H$_{39}$O$_8$P]Na$: 449.2275$. Found: 449.2271.

1-(Butyl-4’-O-decyl)-2,5-anhydro-D-glucityl phosphonate (2.15d). Phosphonate $^{2.33d}$ (99 mg, 0.124 mmol) was hydrogenated as described for $^{2.33a}$. Purification of the product was done as outlined for $^{2.15a}$ to provide $^{2.15d}$ (11 mg, 20%) as an off-white solid: $R_f$ 0.13 (3:1, CH$_2$Cl$_2$:CH$_3$OH); $[\alpha]^{25}_D$ +10.8 (c 1.0, CH$_3$OH); $^{1}$H NMR (400 MHz, CD$_3$OD) $\delta_H$ 0.83 (t, 3 H, $J = 6.9$ Hz), 1.30–1.15 (m, 14 H), 1.55–1.49 (m, 2 H), 1.72–1.55 (m, 4 H), 2.17–1.99 (m, 2 H), 3.35 (t, 2 H, $J = 6.6$ Hz), 3.39 (t, 2 H, $J = 6.3$ Hz), 3.56 (dd, 1 H, $J = 11.5$, 5.1 Hz), 3.61 (dd, 1 H, $J = 11.5$, 3.9 Hz), 3.71–3.66 (m, 1 H), 3.84–3.82 (m, 1 H),
3.90–3.88 (m, 1 H), 3.98 (dddd, 2 H, \( J = 6.5, 6.5, 6.5, 6.5 \) Hz), 4.24–4.17 (m, 1 H); \(^{13}\)C NMR (100 MHz, CD\(_3\)OD) \( \delta \) C 14.4, 23.7, 27.0, 27.1 (d, \( J = 143.0 \) Hz), 27.3, 28.5 (d, \( J = 6.7 \) Hz), 30.4, 30.6, 30.7, 30.8, 33.1, 63.5, 66.4 (d, \( J = 6.3 \) Hz), 71.3, 72.0, 77.7, 79.1 (d, \( J = 7.8 \) Hz), 80.1, 87.5; \(^{31}\)P NMR (162 MHz, CD\(_3\)OD) \( \delta \) P 29.4; HRMS calcd for \([\text{C}_{20}\text{H}_{41}\text{O}_{8}\text{P}]\)Na\(^+\): 463.2431. Found: 463.2430.

1-(Butyl-4’-O-dodecyl)-2,5-anhydro-D-glucityl phosphonate (2.15e). Phosphonate 2.33e (132 mg, 0.159 mmol) was hydrogenated as described for 2.33a. Purification of the product was done as outlined for 2.15a to provide 2.15e (51 mg, 68%) as an off-white solid: \( R_f \) 0.20 (3:1, CH\(_2\)Cl\(_2\):CH\(_3\)OH); \([\alpha]^{25}_{D} \) +10.8 (c 1.1, CH\(_3\)OH); \(^1\)H NMR (400 MHz, CD\(_3\)OD) \( \delta \) H 0.79 (t, 3 H, \( J = 7.0 \) Hz), 1.30–1.10 (m, 18 H), 1.50–1.39 (m, 2 H), 1.68–1.52 (m, 4 H), 2.15–1.95 (m, 2 H), 3.31 (t, 2 H, \( J = 6.6 \) Hz), 3.35 (t, 2 H, \( J = 6.2 \) Hz), 3.52 (dd, 1 H, \( J = 11.6, 5.2 \) Hz), 3.57 (dd, 1 H, \( J = 11.6, 3.6 \) Hz), 3.66–3.61 (m, 1 H), 3.80–3.77 (m, 1 H), 3.85–3.82 (m, 1 H), 3.94 (dddd, 2 H, \( J = 6.5, 6.5, 6.5 \) Hz), 4.20–4.12 (m, 1 H); \(^{13}\)C NMR (100 MHz, CD\(_3\)OD) \( \delta \) C 14.4, 23.7, 27.0, 27.1 (d, \( J = 139.2 \) Hz), 27.3, 28.5 (d, \( J = 6.6 \) Hz), 30.5, 30.6, 30.7, 30.8, 33.1, 63.5, 66.4 (d, \( J = 6.3 \) Hz), 71.3, 72.0, 77.7, 79.1 (d, \( J = 7.8 \) Hz), 80.1, 87.4; \(^{31}\)P NMR (162 MHz, CD\(_3\)OD) \( \delta \) P 29.4; HRMS calcd for \([\text{C}_{22}\text{H}_{45}\text{O}_{8}\text{P}]\)Na\(^+\): 491.2744. Found: 491.2728.

1-(Butyl-4’-O-hexadecyl)-2,5-anhydro-D-glucityl phosphonate (2.15f). Phosphonate 2.33f (122 mg, 0.138 mmol) was hydrogenated as described for 2.33a. Purification of the product was done as outlined for 2.15a (except that a gradient of CH\(_2\)Cl\(_2\)→CH\(_2\)Cl\(_2\):CH\(_3\)OH, 5:1 was used in the chromatography) to provide 2.15f (38 mg, 53%) as an off-white solid: \( R_f \) 0.42 (3:1, CH\(_2\)Cl\(_2\):CH\(_3\)OH); \([\alpha]^{25}_{D} \) +14.0 (c 1.1, CH\(_3\)OH); \(^1\)H NMR (400 MHz, CD\(_3\)OD) \( \delta \) H 0.83 (t, 3 H, \( J = 7.1 \) Hz), 1.35–1.15 (m, 26 H), 1.53–1.44 (m, 2
H), 1.72–1.56 (m, 4 H), 2.19–1.90 (m, 2 H), 3.35 (t, 2 H, J = 6.6 Hz), 3.39 (t, 2 H, J = 6.3 Hz), 3.55 (dd, 1 H, J = 11.4, 5.1 Hz), 3.61 (dd, 1 H, J = 11.6, 3.7 Hz), 3.70–3.66 (m, 1 H), 3.84–3.82 (m, 1 H), 3.91–3.87 (m, 1 H), 3.98 (dddd, 2 H, J = 6.5, 6.5, 6.5, 6.5 Hz), 4.24–4.17 (m, 1 H); \(^{13}\)C NMR (100 MHz, CD\(_3\)OD) \(\delta\) C 14.4, 23.7, 27.0, 27.1 (d, \(J = 139.3\) Hz), 27.3, 28.5 (d, \(J = 6.0\) Hz), 30.5, 30.6, 30.8, 33.1, 63.5, 66.4 (d, \(J = 6.4\) Hz), 71.3, 72.0, 77.7, 79.1 (d, \(J = 7.9\) Hz), 80.1, 87.5; \(^{31}\)P NMR (162 MHz, CD\(_3\)OD) \(\delta\) P 27.9; HRMS calcd for [C\(_{26}\)H\(_{53}\)O\(_8\)P]Na\(^+\): 547.3370. Found: 547.3395.

1-(Allylbenzyl)-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucityl phosphonate (2.16).

Acyclic phosphonate 2.24 (638 mg, 0.987 mmol) was dissolved in THF (12 mL) and stirred before Ph\(_3\)P (311 mg, 1.19 mmol) and diisopropyl azodicarboxylate (0.39 mL, 1.97 mmol) were added. The reaction mixture was stirred for 2 h and then the solvent was then evaporated to give a clear yellow residue that was purified by chromatography (2:1, hexane:EtOAc) to yield 2.16 (547 mg, 90%) as a clear oil: \(R_f\) 0.37 (1:1, hexane:EtOAc); \([\alpha]^{25}\)\(_D\) +17.7 (c 1.0, CHCl\(_3\)); \(^1\)H NMR (250 MHz, CDCl\(_3\)) \(\delta\) H 2.47–2.20 (m, 2 H), 3.61–3.43 (m, 2 H), 3.92–3.88 (m, 2 H), 4.10–4.04 (m, 1 H), 4.58–4.31 (m, 9 H), 5.08–5.00 (m, 2 H), 5.17 (d, 2 H, \(J = 10.3\) Hz), 5.28 (dd, 1 H, \(J = 17.1, 1.4\) Hz), 5.95–5.80 (m, 1 H), 7.33–7.18 (m, 20 H); \(^{13}\)C NMR (62.5 MHz, CDCl\(_3\)) \(\delta\) C 26.5 (d, \(J = 140.0\) Hz), 66.6 (d, \(J = 6.1\) Hz), 66.7 (d, \(J = 6.1\) Hz), 67.5 (d, \(J = 6.1\) Hz), 67.7 (d, \(J = 6.1\) Hz), 71.0, 71.9 (d, \(J = 6.9\) Hz), 73.7, 76.5, 83.2, 83.4 (d, \(J = 6.4\) Hz), 84.1, 118.3, 118.5, 128.0, 128.1, 128.2, 128.4, 128.6, 128.8, 128.9, 129.0, 129.0, 133.4 (d, \(J = 6.2\) Hz), 133.5 (d, \(J = 6.2\) Hz), 136.8 (d, \(J = 6.6\) Hz), 136.9 (d, \(J = 6.6\) Hz), 138.2, 138.6; \(^{31}\)P NMR (101 MHz, CDCl\(_3\)) \(\delta\) P 30.9; Anal. Calcd for C\(_{37}\)H\(_{41}\)O\(_7\)P: C, 70.69; H, 6.57. Found: C, 70.51; H, 6.68.
**cis-2-Butenyl-1,4-diheptyl ether (2.17a).** Sodium hydride (874 mg, 36.4 mmol) was added to a solution of cis-2-buten-1,4-diol (2.30, 803 mg, 9.1 mmol) in DMF (20 mL). After ~10 min, 1-iodoheptane (6.0 mL, 36.4 mmol) was added and the reaction mixture was stirred for 1 h. Water (20 mL) was added to dissolve the salt precipitate and the solution was diluted with hexane (75 mL). The organic layer was separated, washed with brine (20 mL), and dried over Na$_2$SO$_4$. After evaporating the solvent, the yellowish residue was purified by chromatography (hexane→6:1, hexane:EtOAc) to afford cis-2-butenyl-1,4-diheptyl ether (2.48 g, 96%) as a clear liquid: $R_f$ 0.48 (6:1, hexane:EtOAc); $^1$H NMR (250 MHz, CDCl$_3$) $\delta$H 0.88 (t, 6 H, $J=6.8$ Hz), 1.45–1.20 (m, 16 H), 1.68–1.50 (m, 4 H), 3.41 (t, 4 H, $J=6.6$ Hz), 4.03 (d, 4 H, $J=4.6$ Hz), 5.75–5.65 (m, 2 H); $^{13}$C NMR (62.5 MHz, CDCl$_3$) $\delta$C 14.0, 22.6, 26.1, 29.1, 29.7, 31.8, 66.4, 70.5, 129.4; HRMS calcd for [C$_{18}$H$_{36}$O$_2$]Na$^+$: 307.2607. Found: 307.2613.

**cis-2-Butenyl-1,4-dioctyl ether (2.17b).** The preparation of 2.17b was achieved by the reaction of 2.30 (500 mg, 5.67 mmol) and 1-iodooctane (4.1 g, 17.01 mmol) as described for the preparation of 2.17a. The product was purified by chromatography (8:1, hexane:EtOAc) to afford cis-2-butenyl-1,4-dioctyl ether (1.63 g, 92%) as a clear oil: $R_f$ 0.50 (8:1, hexane:EtOAc); $^1$H NMR (250 MHz, CDCl$_3$) $\delta$H 0.88 (t, 6 H, $J=6.7$ Hz), 1.40–1.20 (m, 20 H), 1.65–1.51 (m, 4 H), 3.41 (t, 4 H, $J=6.6$ Hz), 4.03 (d, 4 H, $J=4.6$ Hz), 5.75–5.65 (m, 2 H); $^{13}$C NMR (62.5 MHz, CDCl$_3$) $\delta$C 14.1, 22.7, 26.2, 29.3, 29.5, 29.8, 31.8, 66.5, 70.6, 129.5; HRMS calcd for [C$_{20}$H$_{40}$O$_2$]Na$^+$: 335.2920. Found: 335.2943.

**cis-2-Butenyl-1,4-dinonyl ether (2.17c).** The preparation of 2.17c was achieved by the reaction of 2.30 (500 mg, 5.67 mmol) and 1-iodononane (4.3 g, 17.01 mmol) as described for the preparation of 2.17a. The product was purified by chromatography (9:1, hexane:EtOAc) to afford 2.17c (1.60 mg, 83%) as a clear oil: $R_f$ 0.52 (9:1, hexane:EtOAc); $^1$H
NMR (250 MHz, CDCl$_3$) $\delta_H$ 0.88 (t, 6 H, $J = 6.9$ Hz), 1.42–1.18 (m, 24 H), 1.65–1.48 (m, 4 H), 3.39 (t, 4 H, $J = 6.6$ Hz), 4.01 (d, 4 H, $J = 4.6$ Hz), 5.75–5.65 (m, 2 H); $^{13}$C NMR (62.5 MHz, CDCl$_3$) $\delta_C$ 14.0, 22.7, 26.2, 29.3, 29.5, 29.6, 29.8, 31.8, 66.5, 70.4, 129.4; HRMS calcd for [C$_{22}$H$_{44}$O$_2$]Na$: 363.3233$. Found: 363.3256.

* cis-2-Butenyl-1,4-didecyl ether (2.17d).* The preparation of 2.17d was achieved by the reaction of 2.30 (535 mg, 6.07 mmol) and 1-iododecane (6.5 g, 6.07 mmol) as described for the preparation of 2.17a. The product was purified by chromatography (10:1, hexane:EtOAc) to afford 2.17d (2.0 g, 91%) as a clear oil: $R_f$ 0.55 (10:1, hexane:EtOAc); $^1$H NMR (250 MHz, CDCl$_3$) $\delta_H$ 0.88 (t, 6 H, $J = 6.9$ Hz), 1.41–1.18 (m, 28 H), 1.65–1.49 (m, 4 H), 3.41 (t, 4 H, $J = 6.6$ Hz), 4.03 (d, 4 H, $J = 4.7$ Hz), 5.75–5.65 (m, 2 H); $^{13}$C NMR (62.5 MHz, CDCl$_3$) $\delta_C$ 14.1, 22.7, 26.2, 29.3, 29.5, 29.6, 29.7, 31.9, 66.4, 70.6, 129.5; HRMS calcd for [C$_{24}$H$_{48}$O$_2$]Na$: 391.3546$. Found: 391.3547.

* cis-2-Butenyl-1,4-didodecyl ether (2.17e).* The preparation of 2.17e was achieved by the reaction of 2.30 (500 mg, 5.67 mmol) and 1-iodododecane (6.72 g, 22.68 mmol) as described for the preparation of 2.17a. The product was purified by chromatography (12:1, hexane:EtOAc) to afford 2.17e (2.39 g, 99%) as a clear oil: $R_f$ 0.30 (12:1, hexane:EtOAc); $^1$H NMR (250 MHz, CDCl$_3$) $\delta_H$ 0.88 (t, 6 H, $J = 6.8$ Hz), 1.40–1.18 (m, 36 H), 1.65–1.50 (m, 4 H), 3.40 (t, 4 H, $J = 6.6$ Hz), 4.03 (d, 4 H, $J = 4.6$ Hz), 5.75–5.65 (m, 2 H); $^{13}$C NMR (62.5 MHz, CDCl$_3$) $\delta_C$ 14.1, 22.7, 26.2, 29.8, 29.9, 30.0, 30.1, 30.2, 30.3, 31.9, 66.4, 70.5, 129.4; HRMS calcd for [C$_{28}$H$_{56}$O$_2$]Na$: 447.4172$. Found: 447.4162.

* cis-2-Butenyl-1,4-dihexadecyl ether (2.17f).* The preparation of 2.17f was achieved by the reaction of 2.30 (500 mg, 5.67 mmol) and 1-iodohexadecane (8.0 g, 22.68 mmol) as described for the preparation of 2.17a. The product was purified by chromatography (12:1,
hexane:EtOAc) to afford 2.17e (1.6 g, 52%) as a clear oil: \( R_f \) 0.45 (12:1, hexane:EtOAc); \(^1\)H NMR (250 MHz, CDCl\(_3\)) \( \delta \) 0.88 (t, 6 H, \( J = 6.7 \) Hz), 1.43–1.15 (m, 52 H), 1.65–1.50 (m, 4 H), 3.41 (t, 4 H, \( J = 6.6 \) Hz), 4.03 (d, 4 H, \( J = 4.3 \) Hz), 5.75–5.65 (m, 2 H); \(^13\)C NMR (62.5 MHz, CDCl\(_3\)) \( \delta \)C 14.1, 22.7, 26.2, 29.8, 29.9, 30.0, 30.1, 30.2, 30.3, 31.9, 66.4, 70.6, 129.5; HRMS calcd for \([\text{C}_{36}\text{H}_{72}\text{O}_2]\)Na\(^+\): 559.5424. Found: 559.5453.

**Allylbenzyl methylphosphonate (2.22).** THF (40 mL) was added to a stirring solution of 2.21\(^{25}\) (36.7 g, 0.133 mol) and allyl acetate (28.7 mL, 0.266 mol). A 1M solution of potassium \( t \)-butoxide (20 mL) was prepared and then added in 5 mL aliquots approximately every 15 min. The reaction mixture was then neutralized with AcOH and diluted with EtOAc (100 mL). The organic layer was washed with water (100 mL) followed by brine (100 mL) and then dried over Na\(_2\)SO\(_4\). Evaporation of the solvent provided a thick yellow liquid. The product was distilled at 135 \(^\circ\)C (1 mm Hg) affording 2.22 (14.1 g, 47%) as a clear liquid: \( R_f \) 0.33 (3:1 hexane:EtOAc); \(^1\)H NMR (250 MHz, CDCl\(_3\)) \( \delta \) 1.49 (d, 3 H, \( J = 17.5 \) Hz), 4.58–4.39 (m, 2 H), 5.16–4.99 (m, 2 H), 5.23 (dd, 1 H, \( J = 9.8, 1.3 \) Hz), 5.33 (dd, 1 H, \( J = 17.5, 1.5 \) Hz), 5.99–5.82 (m, 1 H), 7.50–7.35 (m, 5 H); \(^13\)C NMR (62.5 MHz, CDCl\(_3\)) \( \delta \)C 11.5 (d, \( J = 143.1 \) Hz), 65.9 (d, \( J = 24.3 \) Hz), 67.0 (d, \( J = 24.3 \) Hz), 117.8, 127.8, 128.3, 128.5, 132.9 (d, \( J = 25.0 \) Hz), 136.3 (d, \( J = 25.0 \) Hz); \(^{31}\)P NMR (101 MHz, CDCl\(_3\)) \( \delta \)P 32.8; HRMS calcd for \( \text{C}_{11}\text{H}_{15}\text{O}_3\text{PNa}^+\): 249.0651. Found: 249.0659.

**1-(Allylbenzyl)-3,4,6-tri-O-benzyl-D-glucityl phosphonate (2.24).** Benzylidene 2.28 (559 mg, 0.761 mmol) was dissolved in an 80:20 mixture of AcOH/H\(_2\)O (20 mL) and heated at 50 \(^\circ\)C overnight. After cooling to rt, the reaction mixture was diluted with CH\(_2\)Cl\(_2\) (30 mL) and the product was extracted from the aqueous layer. The organic layer was dried over Na\(_2\)SO\(_4\) and the solvent was evaporated. The residue was purified by chromatography...
(1:3, hexane:EtOAc) to give 2.24 (456 mg, 93%) as a clear oil: $R_f$ 0.18 (1:3, hexane:EtOAc); $[\alpha]^{25}_D +19.6$ (c 0.9, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 1.96–1.84 (m, 1 H), 2.24–2.12 (m, 1 H), 3.18–3.05 (m, broad, 1 H), 3.38 (dd, 1 H, $J = 9.3$, 6.6 Hz), 3.51 (dd, 1 H, $J = 9.3$, 6.4 Hz), 3.64 (dd, 1 H, $J = 6.8$, 1.7 Hz), 3.74 (d, 1 H, $J = 6.8$ Hz), 3.88–3.78 (m, broad, 1 H), 4.11–4.05 (m, 1 H), 4.37–4.26 (m, 1 H), 4.55–4.38 (m, 6 H), 4.70–4.61 (m, 2 H), 5.10–5.00 (m, 2 H), 5.22–5.17 (m, 1 H), 5.33–5.25 (m, 1 H), 5.93–5.80 (m, 1 H), 7.40–7.18 (m, 20 H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 30.9 (d, $J = 138.7$ Hz), 31.0 (d, $J = 138.6$ Hz), 64.9 (d, $J = 2.6$ Hz), 65.0 (d, $J = 2.8$ Hz), 66.2 (d, $J = 6.2$ Hz), 66.4 (d, $J = 6.1$ Hz), 67.3 (d, $J = 6.2$ Hz), 67.5 (d, $J = 6.3$ Hz), 68.0, 71.2, 73.2, 74.2 (d, $J = 6.1$ Hz), 77.3, 79.6 (d, $J = 12.7$ Hz), 118.1, 118.3, 127.7, 127.9, 128.0, 128.1, 128.4, 128.4, 128.4, 128.5, 128.6, 128.7, 132.8 (d, $J = 6.0$ Hz), 132.9 (d, $J = 6.3$ Hz), 136.2 (d, $J = 5.5$ Hz), 136.3 (d, $J = 5.7$ Hz), 137.8, 137.9, 138.0; $^{31}$P NMR (162 MHz, CDCl$_3$) $\delta$P 32.2; Anal. Calcd for C$_{37}$H$_{43}$O$_8$P: C, 68.72; H, 6.70. Found: C, 68.55; H, 6.72.

1-(Allylbenzylphosphinyl)-1-deoxy-3,4,6-tri-O-(benzyl)-D-fructofuranose (2.26).

Phosphonate 2.22 (1.5 g, 6.7 mmol) was dissolved in 5 mL of THF and stirred at $-78 \, ^{\circ}C$. $n$-BuLi (11.7 mL of a 1.6 M solution in hexane) was added and, after 5 min, a solution of lactone 2.18$^{24}$ (4.9 g, 11.7 mmol) in THF (10 mL) was added in one aliquot. After 1 h, the solution was removed from the dry ice bath and stirred for another 30 min. The reaction mixture was then neutralized with AcOH and diluted with EtOAc (100 mL). The organic layer was washed with water (75 mL) followed by brine (75 mL) and then dried over Na$_2$SO$_4$. The solvent was evaporated and the residue was purified by chromatography (1:1, hexane:EtOAc) to give 2.26 (6.8 g, 90%) as a clear oil: $R_f$ 0.35 (1:1, hexane:EtOAc); Selected NMR data: $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 32.1 (d, $J = 137.7$ Hz), 35.9 (d, $J = 135.9$ Hz),
101.89 (d, $J = 5.4$ Hz), 101.93 (d, $J = 6.0$ Hz), 105.79 (d, $J = 2.5$ Hz), 105.82 (d, $J = 2.8$ Hz);

$^{31}$P NMR (162 MHz, CDCl$_3$) $\delta_p$ 28.1, 28.2, 29.5, 29.6; Anal. Calcd for C$_{37}$H$_{41}$O$_8$P: C, 68.72; H, 6.70. Found: C, 68.55; H, 6.72.

1-(Allylbenzyl)-3,4,6-tri-O-benzyl-D-mannityl phosphonate (**2.27**). Benzyldiene 2.29 (336 mg, 0.457 mmol) was dissolved in an 80:20 mixture of AcOH/H$_2$O (10 mL) and heated at 40 °C for 3 h. After cooling to rt, the reaction mixture was diluted with CH$_2$Cl$_2$ (30 mL) and the product was extracted from the aqueous layer. The organic layer was dried over Na$_2$SO$_4$ and the solvent was evaporated. The residue was purified by chromatography (1:3, hexane:EtOAc) to give 2.27 (240 mg, 81%) as a clear oil: $R_f$ 0.18 (1:3, hexane:EtOAc); $[\alpha]^{25}_D +17.3$ (c 1.0, CHCl$_3$); $^1$H NMR (250 MHz, CDCl$_3$) $\delta_h$ 2.30–1.93 (m, 2 H), 3.57–3.40 (m, 3 H), 3.81–3.68 (m, 2 H), 4.14–4.03 (m, broad, 1 H), 4.59–4.30 (m, 7 H), 4.78–4.64 (m, 3 H), 5.13–5.05 (m, 2 H), 5.38–5.19 (m, 2 H), 5.99–5.83 (m, 1 H), 7.30–7.14 (m, 20 H); $^{13}$C NMR (62.5 MHz, CDCl$_3$) $\delta_C$ 29.6 (d, $J = 138.6$ Hz), 66.1, 66.2, 66.3, 67.2, 67.3, 67.3, 67.4, 67.4, 67.5, 69.5, 71.1, 73.2, 73.7, 74.2, 77.2, 78.2 (d, $J = 2.5$ Hz), 80.8 (d, $J = 2.3$ Hz), 81.1 (d, $J = 2.9$ Hz), 117.7, 117.7, 127.4, 127.4, 127.5, 127.7, 127.7, 128.0, 128.1, 128.3, 132.6, 132.7, 136.0, 136.1, 137.7, 137.8, 137.9; $^{31}$P NMR (162 MHz, CDCl$_3$) $\delta_p$ 33.6; HRMS calcd for [C$_{37}$H$_{43}$O$_8$P]Na$: 669.2588. Found: 669.2638.

1-(Allylbenzyl)-3,4,6-tri-O-benzyl-2,5-O-benzylidene-D-glucityl phosphonate (2.28A). and 1-(Allylbenzyl)-3,4,6-tri-O-benzyl-2,5-O-benzylidene-D-mannityl-phosphonate (2.28B). Sodium borohydride (963 mg, 25.5 mmol) was added to a solution of lactol 2.26 (13.7 g, 21.2 mmol) in THF (200 mL) and the reaction mixture was stirred for 2 h. Upon completion, the reaction mixture was neutralized with AcOH, diluted with EtOAc (100 mL), and washed with water (100 mL) followed by brine (100 mL). The organic layer was dried over Na$_2$SO$_4$ and the solvent was evaporated affording a crude reduction product (14.6
g, 87%), which was a 2:1 mixture of \textit{2.24} and \textit{2.27}. This crude material was used in the next step without any further purification. Benzaldehyde dimethyl acetal (9.5 mL, 63.6 mmol) and \textit{p}-toluenesulfonic acid (193 mg, 1.01 mmol) were added to a solution of the crude diol in CH\textsubscript{3}CN (60 mL) and the mixture stirred overnight. The solvent was evaporated and the residue, which was a mixture of 4 diastereomers, was was purified by chromatography (hexane:EtOAc, 1:1) to afford the major diasteromers \textit{2.28A} (5.9 g, 38\% over both steps) and \textit{2.28B} (5.8 g, 37\% over both steps) as clear oils. It was possible to obtain more of these products by resubjecting the minor diastereomers and mixed fractions from the column to the reaction. Data for \textit{2.28A}: \textit{R} \textsubscript{f} 0.30 (1:1, hexane:EtOAc); [\alpha] \textsubscript{25}^\circD –8.0 (c 0.9, CHCl\textsubscript{3}); \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \delta \textit{H} 2.01–1.89 (m, 1 H), 2.60–2.46 (m, 1 H), 3.37 (dd, 1 H, \textit{J} = 9.4, 5.8 Hz), 3.55–3.47 (m, 2 H), 3.70–3.67 (m, 1 H), 4.21–4.16 (m, 1 H), 4.31–4.25 (m, 2 H), 4.55–4.37 (m, 6 H), 4.63 (dt, 1 H, \textit{J} = 9.1, 4.8 Hz), 5.04–4.93 (m, 2 H), 5.26–5.09 (m, 2 H), 5.87– 5.75 (m, 2 H), 7.13–7.10 (m, 2 H), 7.33–7.20 (m, 21 H), 7.53–7.40 (m, 2 H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \delta \textit{C} 29.9 (d, \textit{J} = 140.7 Hz), 66.0 (d, \textit{J} = 6.2 Hz), 66.1 (d, \textit{J} = 6.2 Hz), 67.0, 67.1, 68.8, 70.3, 72.4, 72.7 (d, \textit{J} = 3.3 Hz), 74.9, 77.2, 79.1 (d, \textit{J} = 11.9 Hz), 99.1(d, \textit{J} = 3.1 Hz), 118.0, 118.1, 126.6, 126.6, 127.4, 127.4, 127.9, 128.1, 128.3, 128.4, 128.5, 132.8 (d, \textit{J} = 6.3 Hz), 132.9 (d, \textit{J} = 6.9 Hz), 136.2 (d, \textit{J} = 6.1 Hz), 136.2 (d, \textit{J} = 5.9 Hz), 137.8, 137.9, 138.0, 138.7; \textsuperscript{31}P NMR (161.9 MHz, CDCl\textsubscript{3}) \delta \textit{P} 31.2; HRMS calcd for C\textsubscript{44}H\textsubscript{47}O\textsubscript{8}PNa\textsuperscript{+}: 757.2901. Found: 757.2920. Data for \textit{2.28B}: \textit{R} \textsubscript{f} 0.37 (1:1, hexane:EtOAc); [\alpha] \textsubscript{25}^\circD +8.4 (c 1.0, CHCl\textsubscript{3}); \textsuperscript{1}H NMR (250 MHz, CDCl\textsubscript{3}) \delta \textit{H} 2.35–2.10 (m, 2 H), 3.90–3.57 (m, 4 H), 5.20–4.15 (m, 14 H), 5.95–5.59 (m, 2 H), 7.60–7.00 (m, 25 H); \textsuperscript{13}C NMR (62.5 MHz, CDCl\textsubscript{3}) \delta \textit{C} 31.3 (d, \textit{J} = 140.9 Hz), 65.8 (d, \textit{J} = 6.1 Hz), 66.1 (d, \textit{J} = 6.1 Hz), 66.9 (d, \textit{J} = 6.2 Hz), 67.1 (d, \textit{J} = 6.2 Hz), 69.5, 72.1, 72.5, 73.0, 76.3, 78.0 (d, \textit{J} = 4.6 Hz), 78.9, 83.5, 83.7, 105.3 (d, \textit{J} = 5.8 Hz), 117.6, 117.8, 126.4, 127.5, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.1, 128.2,
1-(Allylbenzyl)-3,4,6-tri-O-benzyl-2,5-anhydro-D-mannityl phosphonate (2.29).

Acyclic phosphonate 2.27 (240 mg, 0.371 mmol) was dissolved in THF (5 mL) and stirred. Triphenylphosphine (107 mg, 0.408 mmol) was added, followed by diisopropyl azodicarboxylate (0.15 mL, 0.742 mmol), and the reaction mixture was stirred for 2 h. The solvent was then evaporated to give a clear yellow residue which was purified by chromatography (1:1, hexane:EtOAc) to afford 2.29 as a clear oil (168 mg, 72%): R$_f$ 0.37 (1:1, hexane:EtOAc); [α]$^25_D$ +1.4 (c 1.1, CHCl$_3$); $^1$H NMR (250 MHz, CDCl$_3$) δ H 2.49–2.13 (m, 2 H), 3.66–3.42 (m, 2 H), 3.97–3.95 (m, 1 H), 4.11–4.08 (m, 1 H), 4.28–4.22(m, 1 H), 4.56–4.35 (m, 9 H), 5.09–4.96 (m, 2 H), 5.30–5.12 (m, 2 H), 5.91–5.75 (m, 1 H), 7.45–7.18 (m, 20 H); $^{13}$C NMR (62.5 MHz, CDCl$_3$) δ C 30.0 (d, J = 136.7 Hz), 65.7 (d, J = 6.1 Hz), 66.0 (d, J = 6.1 Hz), 66.7 (d, J = 6.2 Hz), 67.0 (d, J = 6.2 Hz), 70.2, 71.2, 71.4, 73.1, 75.8, 77.8, 82.1, 82.7, 84.8, 86.3, 86.4, 117.6, 117.7, 127.3, 127.4, 127.5, 127.5, 127.6, 128.1, 128.1, 128.1, 128.3, 132.6, 132.7, 132.8, 136.0, 136.1, 136.2, 137.4, 137.5, 137.6, 137.6, 137.9; $^{31}$P NMR (101.2 MHz, CDCl$_3$) δ P 29.7; HRMS calcd for [C$_{37}$H$_{41}$O$_7$P]Na$: 651.2482. Found: 651.2515.

1-[Benzyl((E)-2'-butenyl-4'-O-heptyl)]-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucityl phosphonate (2.32a). Alkene 2.17a (366 mg, 1.29 mmol) was added to a solution of 2.16 (269 mg, 0.43 mmol) in dry CH$_2$Cl$_2$ (10 mL). The Grubbs’ catalyst 2.31$^{28}$ (70 mg, 20 mol%) was added and the reaction mixture was heated at reflux for 3 h. After cooling to room temperature, Pb(OAc)$_4$ (114 mg, 0.26 mmol) was added and the mixture was stirred overnight. The solvent was evaporated and the black residue was purified by chromatography (2:1, hexane:EtOAc) to give 2.32a (179 mg, 55%) as a clear oil. The
product was a mixture of cis and trans isomers: \( R_f \) (cis) 0.53, \( R_f \) (trans) 0.44 (1:1, hexane:EtOAc); \([\alpha]^{25}_{D} +16.9 \) (c 1.1, CHCl\(_3\)); (data for trans isomer) \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \)H 0.87 (t, 3 H, \( J = 6.8 \) Hz), 1.34–1.20 (m, 8 H), 1.60–1.50 (m, 2 H), 2.42–2.22 (m, 2 H), 3.38 (t, 2 H, \( J = 6.6 \) Hz), 3.50–3.44 (m, 1 H), 3.60–3.54 (m, 1 H), 3.92–3.86 (m, 4 H), 4.10–4.04 (m, 1 H), 4.56–4.33 (m, 9 H), 5.08–4.99 (m, 2 H), 5.85–5.72 (m, 2 H), 7.38–7.18 (m, 20 H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \)C 14.0, 22.6, 26.0 (d, \( J = 139.9 \) Hz), 26.1, 29.1, 29.7, 31.8, 65.3 (d, \( J = 6.0 \) Hz), 65.5 (d, \( J = 5.9 \) Hz), 67.0 (d, \( J = 6.1 \) Hz), 67.2 (d, \( J = 6.2 \) Hz), 70.2 (d, \( J = 1.4 \) Hz), 70.5, 70.7, 71.4 (d, \( J = 7.1 \) Hz), 73.2, 76.0, 77.2, 82.8, 83.0 (d, \( J = 6.2 \) Hz), 83.6, 126.8 (d, \( J = 6.3 \) Hz), 126.9 (d, \( J = 6.1 \) Hz), 127.6, 127.6, 127.6, 127.7, 127.7, 127.8, 127.9, 128.3, 128.3, 128.3, 128.4, 128.5, 128.5, 131.1 (d, \( J = 11.6 \) Hz), 136.3 (d, \( J = 6.8 \) Hz), 136.5 (d, \( J = 6.8 \) Hz), 137.7, 137.8, 138.1; \(^{31}\)P NMR (162 MHz, CDCl\(_3\)) \( \delta \)P 30.9; Anal. Calcd for C\(_{45}\)H\(_{57}\)O\(_8\)P: C, 71.41; H, 7.59. Found: C, 71.70; H, 7.66.

1-[Benzyl((E)-2'-butenyl-4'-O-octyl)]-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucityl phosphonate (2.32b). The preparation of 2.32b was achieved by the reaction of 2.16 (210 mg, 0.334 mmol) and 2.17b (313 mg, 1.00 mmol) as described for the preparation of 2.32a. The product was purified by chromatography (2:1, hexane:EtOAc) to afford 2.32b (163 mg, 63%) as a clear oil. The product was a mixture of cis and trans isomers: \( R_f \) (cis) 0.22 \( R_f \) (trans) 0.17, (2:1, hexane:EtOAc); \([\alpha]^{25}_{D} +11.2 \) (c 0.25, CHCl\(_3\)); (data for trans isomer) \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \)H 0.87 (t, 3 H, \( J = 6.9 \) Hz), 1.36–1.20 (m, 10 H), 1.60–1.48 (m, 2 H), 2.47–2.18 (m, 2 H), 3.37 (t, 2 H, \( J = 6.7 \) Hz), 3.50–3.44 (m, 1 H), 3.62–3.53 (m, 1 H), 3.92–3.86 (m, 4 H), 4.10–4.04 (m, 1 H), 4.58–4.30 (m, 9 H), 5.10–4.99 (m, 2 H), 5.82–5.75 (m, 2 H), 7.38–7.16 (m, 20 H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \)C 14.0, 22.6, 25.9 (d, \( J = 139.8 \) Hz), 26.1, 29.1, 29.4, 29.7, 31.7, 65.2 (d, \( J = 6.1 \) Hz), 65.4 (d, \( J = 6.2 \) Hz), 67.0 (d, \( J = 6.1 \) Hz).
Hz), 67.2 (d, J = 6.1 Hz), 70.1 (d, J = 1.4 Hz), 70.4, 70.7, 71.4 (d, J = 5.0 Hz), 73.2, 76.0, 77.2, 82.7, 82.9 (d, J = 6.5 Hz), 83.5, 126.7 (d, J = 6.4 Hz), 126.8 (d, J = 6.5 Hz), 127.5, 127.6, 127.6, 127.7, 127.8, 127.9, 128.2, 128.3, 128.4, 131.1 (d, J = 7.3 Hz), 136.3 (d, J = 5.7 Hz), 136.4 (d, J = 6.3 Hz), 137.6, 137.7, 138.1; 31P NMR (162 MHz, CDCl3) δP 30.9; Anal. Calcd for C46H59O8P: C, 71.67; H, 7.65. Found: C, 71.47; H, 7.78.

1-[Benzyl((E)-2’-butenyl-4’-O-nonyl)]-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucityl phosphonate (2.32c). The preparation of 2.32c was achieved by the reaction of 2.16 (235 mg, 0.373 mmol) and 2.17c (508 mg, 1.49 mmol) as described for the preparation of 2.32a. The product was purified by chromatography (2:1, hexane:EtOAc) to afford 2.32c (168 mg, 57%) as a clear oil. The product was a mixture of cis and trans isomers: Rf (cis) 0.27, Rf (trans) 0.21 (2:1, hexane:EtOAc); [α]25D +16.0 (c 1.1, CHCl3); (data for trans isomer) 1H NMR (400 MHz, CDCl3) δH 0.88 (t, 3 H, J = 6.9 Hz), 1.36–1.20 (m, 12 H), 1.60–1.50 (m, 2 H), 2.42–2.22 (m, 2 H), 3.38 (t, 2 H, J = 6.6 Hz), 3.49–3.44 (m, 1 H), 3.60–3.54 (m, 1 H), 3.92–3.86 (m, 4 H), 4.09–4.05 (m, 1 H), 4.57–4.33 (m, 9 H), 5.08–4.89 (m, 2 H), 5.85–5.72 (m, 2 H), 7.38–7.18 (m, 20 H); 13C NMR (100 MHz, CDCl3) δC 14.0, 22.6, 25.9 (d, J = 139.9 Hz), 26.1, 29.2, 29.4, 29.5, 29.7, 31.8, 65.2 (d, J = 5.9 Hz), 65.4 (d, J = 6.1 Hz), 67.0 (d, J = 6.1 Hz), 67.2 (d, J = 6.2 Hz), 70.2 (d, J = 1.7 Hz), 70.5, 70.7, 71.4 (d, J = 6.0 Hz), 73.2, 76.0, 77.2, 82.7, 82.9 (d, J = 5.7 Hz), 83.6, 126.8 (d, J = 6.5 Hz), 126.9 (d, J = 6.7 Hz), 127.6, 127.6, 127.7, 127.7, 127.9, 127.9, 128.2, 128.3, 128.4, 128.5, 128.5, 131.1 (d, J = 12.0 Hz), 136.3 (d, J = 6.1 Hz), 136.4 (d, J = 6.8 Hz), 137.7, 137.8, 138.1; 31P NMR (161.9 MHz, CDCl3) δP 30.9; Anal. Calcd for C47H61O8P: C, 71.92; H, 7.83. Found: C, 71.64; H, 7.83.

1-[Benzyl((E)-2’-butenyl-4’-O-decyl)]-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucityl phosphonate (2.32d). The preparation of 2.32d was achieved by the reaction of 2.16 (268 mg,
mg, 0.426 mmol) and 2.17d (628 mg, 1.70 mmol) as described for the preparation of 2.32a. The product was purified by chromatography (2:1, hexane:EtOAc) to afford 2.32d (174 mg, 51%) as a clear oil. The product was a mixture of cis and trans isomers: $R_f$ (cis) 0.28, $R_f$ (trans) 0.23 (2:1, hexane:EtOAc); $[\alpha]_D^{25} +14.7$ (c 1.0, CHCl$_3$); (data for trans isomer) $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 0.88 (t, 3 H, $J = 6.9$ Hz), 1.36–1.20 (m, 14 H), 1.59–1.50 (m, 2 H), 2.43–2.22 (m, 2 H), 3.38 (t, 2 H, $J = 6.6$ Hz), 3.50–3.44 (m, 1 H), 3.60–3.54 (m, 1 H), 3.92–3.86 (m, 4 H), 4.09–4.04 (m, 1 H), 4.57–4.32 (m, 9 H), 5.08–4.98 (m, 2 H), 5.85–5.72 (m, 2 H), 7.40–7.15 (m, 20 H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 14.1, 22.7, 26.0 (d, $J = 140.0$ Hz), 26.2, 29.3, 29.5, 29.6, 29.6, 29.8, 31.9, 65.3 (d, $J = 6.0$ Hz), 65.5 (d, $J = 6.1$ Hz), 67.1 (d, $J = 6.1$ Hz), 67.3 (d, $J = 6.2$ Hz), 70.2 (d, $J = 1.6$ Hz), 70.5, 70.8, 71.4 (d, $J = 7.3$ Hz), 73.3, 76.1, 77.2, 82.8, 83.0 (d, $J = 6.2$ Hz), 83.6, 126.9 (d, $J = 6.5$ Hz), 127.0 (d, $J = 6.2$ Hz), 127.6, 127.6, 127.7, 127.7, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 128.4, 128.5, 128.6, 131.1 (d, $J = 11.5$ Hz), 136.4 (d, $J = 6.2$ Hz), 136.5 (d, $J = 6.6$ Hz), 137.7, 137.8, 138.2; $^{31}$P NMR (162 MHz, CDCl$_3$) $\delta$P 30.9; Anal. Calcd for C$_{48}$H$_{63}$O$_8$P: C, 72.16; H, 7.95. Found: C, 72.55; H, 8.24.

1-[Benzyl((E)-2’-butenyl-4’-O-dodecyl)]-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucityl phosphonate (2.32e). The preparation of 2.32e was achieved by the reaction of 2.16 (237 mg, 0.377 mmol) and 2.17e (640 mg, 1.51 mmol) as described for the preparation of 2.32a. The product was purified by chromatography (2:1, hexane:EtOAc) to afford 2.32e (205 mg, 66%) as a clear oil. The product was a mixture of cis and trans isomers: $R_f$ (cis) 0.26, $R_f$ (trans) 0.20 (2:1, hexane:EtOAc); $[\alpha]_D^{25} +14.4$ (c 1.1, CHCl$_3$); (data for trans isomer) $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 0.88 (t, 3 H, $J = 7.2$ Hz), 1.40–1.24 (m, 18 H), 1.63–1.50 (m, 2 H), 2.43–2.22 (m, 2 H), 3.37 (t, 2 H, $J = 6.6$ Hz), 3.49–3.40 (m, 1 H),
3.60–3.54 (m, 1 H), 3.93–3.86 (m, 4 H), 4.09–4.04 (m, 1 H), 4.56–4.32 (m, 9 H), 5.08–4.97 (m, 2 H), 5.85–5.71 (m, 2 H), 7.40–7.15 (m, 20 H); $^{13}$C NMR (100 MHz, CDCl$_3$) δC 14.1, 22.7, 26.0 (d, $J = 139.9$ Hz), 26.2, 29.4, 29.5, 29.6, 29.6, 29.8, 31.9, 65.3 (d, $J = 6.0$ Hz), 65.5 (d, $J = 6.1$ Hz), 67.1 (d, $J = 6.2$ Hz), 67.3 (d, $J = 6.1$ Hz), 70.3 (d, $J = 1.5$ Hz), 70.5, 70.8, 71.4 (d, $J = 7.2$ Hz), 73.3, 76.1, 77.2, 82.8, 83.0 (d, $J = 6.0$ Hz), 83.6, 126.9 (d, $J = 6.3$ Hz), 127.0 (d, $J = 6.4$ Hz), 127.6, 127.6, 127.7, 127.7, 127.7, 127.8, 127.9, 128.0, 128.3, 128.3, 128.4, 128.5, 128.6, 131.1 (d, $J = 11.6$ Hz), 136.4 (d, $J = 6.3$ Hz), 136.5 (d, $J = 6.7$ Hz), 137.7, 137.8, 138.2; $^{31}$P NMR (162 MHz, CDCl$_3$) δP 30.9; Anal. Calcd for C$_{50}$H$_{67}$O$_8$P: C, 72.61; H, 8.17. Found: C, 72.72; H, 8.48.

1-[Benzyl((E)-2’-butenyl-4’-O-hexadecyl)]-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucityl phosphonate (2.32f). The preparation of 2.32f was achieved by the reaction of 2.16 (218 mg, 0.347 mmol) and 2.17f (746 mg, 1.39 mmol) as described for the preparation of 2.32a. The product was purified by chromatography (2:1, hexane:EtOAc) to afford 2.32f (188 mg, 62%) as a clear oil. The product was a mixture of cis and trans isomers: $R_f$ (cis) 0.29, $R_f$ (trans) 0.20 (2:1, hexane:EtOAc); [$\alpha$]$^\circ_{D25}$ +14.0 (c 0.9, CHCl$_3$); (data for trans isomer) $^1$H NMR (400 MHz, CDCl$_3$) δH 0.88 (t, 3 H, $J = 6.7$ Hz), 1.37–1.18 (m, 26 H), 1.60–1.51 (m, 2 H), 2.42–2.22 (m, 2 H), 3.38 (t, 2 H, $J = 6.7$ Hz), 3.49–3.43 (m, 1 H), 3.60–3.52 (m, 1 H), 3.93–3.85 (m, 4 H), 4.09–4.03 (m, 1 H), 4.57–4.32 (m, 9 H), 5.08–4.98 (m, 2 H), 5.85–5.72 (m, 2 H), 7.38–7.20 (m, 20 H); $^{13}$C NMR (100 MHz, CDCl$_3$) δC 14.1, 22.7, 25.9 (d, $J = 139.7$ Hz), 26.2, 29.3, 29.5, 29.6, 29.6, 29.7, 29.7, 31.9, 65.3 (d, $J = 6.1$ Hz), 65.5 (d, $J = 6.3$ Hz), 67.1 (d, $J = 6.2$ Hz), 67.3 (d, $J = 6.1$ Hz), 70.2 (d, $J = 1.5$ Hz), 70.5, 70.7, 71.4 (d, $J = 5.6$ Hz), 73.2, 76.0, 77.2, 82.7, 82.9 (d, $J = 6.7$ Hz), 83.6, 126.8 (d, $J = 6.2$ Hz), 126.9 (d, $J = 6.8$ Hz), 127.6, 127.6, 127.6, 127.7, 127.7, 127.8, 127.9, 127.9, 128.3, 128.5, 128.6, 131.1 (d, $J = 11.6$ Hz), 136.4 (d, $J = 6.3$ Hz), 136.5 (d, $J = 6.7$ Hz), 137.7, 137.8, 138.2; $^{31}$P NMR (162 MHz, CDCl$_3$) δP 30.9; Anal. Calcd for C$_{50}$H$_{67}$O$_8$P: C, 72.61; H, 8.17. Found: C, 72.72; H, 8.48.
128.3, 128.3, 128.4, 128.5, 128.5, 131.1 (d, \(J = 11.7\) Hz), 136.3 (d, \(J = 6.3\) Hz), 136.5 (d, \(J = 6.8\) Hz), 137.7, 137.8, 138.1; \(^{31}\)P NMR (162 MHz, CDCl\(_3\)) \(\delta_p\) 30.9; Anal. Calcd for C\(_{54}\)H\(_{75}\)O\(_8\)P: C, 73.44; H, 8.56. Found: C, 73.18; H, 8.77.

1-[Benzyl(butyl-4’-O-heptyl)]-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucityl phosphonate (2.33a). Dipotassium azodicarboxylate (DAPA, 20 mg, 0.123 mmol) was added to a solution of 2.32a (149 mg, 0.197 mmol) in methanol (10 mL). Glacial HOAc (10 \(\mu\)L) was added and the reaction mixture was heated to 40 \(^{\circ}\)C open to the atmosphere. As the yellow color of the solution started to fade, more DAPA (20 mg) and HOAc (10 \(\mu\)L) were added. This addition procedure was repeated several times over the course of 10 h, and the reaction progress was monitored by NMR. Upon completion of the reaction, the reaction mixture was cooled and then a saturated aqueous solution of NaHCO\(_3\) (15 mL) was added. The product was then extracted into CH\(_2\)Cl\(_2\) (3 x 25 mL) and the organic layer dried over Na\(_2\)SO\(_4\). After evaporation of the solvent, the clear residue was purified by chromatography (2:1, hexane:EtOAc) to give 2.33a (130 mg, 87%) as a clear oil: \(R_f\) 0.18 (2:1, hexane:EtOAc); \([\alpha]_{D}^{25}\) +11.5 (c 0.9, CHCl\(_3\)); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta_h\) 0.87 (t, 3 H, \(J = 6.8\) Hz), 1.35–1.20 (m, 8 H), 1.62–1.49 (m, 4 H), 1.71–1.63 (m, 2 H), 2.42–2.19 (m, 2 H), 3.39–3.31 (m, 4 H), 3.49–3.44 (m, 1 H), 3.60–3.54 (m, 1 H), 4.08–3.85 (m, 5 H), 4.58–4.30 (m, 7 H), 5.09–5.00 (m, 2 H), 7.40–7.10 (m, 20 H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta_c\) 14.0, 22.6, 25.8 (d, \(J = 140.1\) Hz), 25.8, 26.1, 27.3 (d, \(J = 3.3\) Hz), 27.4 (d, \(J = 3.2\) Hz), 29.1, 29.7, 31.8, 65.5 (d, \(J = 6.8\) Hz), 65.6 (d, \(J = 6.3\) Hz), 67.1 (d, \(J = 6.2\) Hz), 67.2 (d, \(J = 6.2\) Hz), 70.0, 70.5, 71.0, 71.4 (d, \(J = 6.5\) Hz), 73.2, 76.1, 77.2, 82.7, 83.0 (d, \(J = 3.1\) Hz), 83.1 (d, \(J = 3.8\) Hz), 83.6, 127.6, 127.6, 127.7, 127.7, 127.8, 127.8, 127.9, 127.9, 128.2, 128.2, 128.3, 128.4, 128.5, 128.6, 136.5 (d, \(J = 6.0\) Hz), 136.6 (d, \(J = 6.9\) Hz), 137.7, 137.8, 138.1; \(^{31}\)P
NMR(161.9 MHz, CDCl₃) δP 30.4; Anal. Calcd for C₄₅H₅₉O₈P: C, 71.22; H, 7.84. Found: C, 72.39; H, 7.83.

1-[Benzyl(butyl-4'-O-octyl)-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucityl phosphonate (2.33b). Alkene 2.32b (149 mg, 0.193 mmol) was converted to 2.33b as described for the preparation of 2.33a. Purification by chromatography (2:1, hexane:EtOAc) afforded the product (132 mg, 89%) as a clear oil: Rf 0.20 (2:1, hexane:EtOAc); [α]²⁵/D +14.7 (c 1.2, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δH 0.87 (t, 3 H, J = 6.8 Hz), 1.34–1.21 (m, 10 H), 1.62–1.48 (m, 4 H), 1.72–1.63 (m, 2 H), 2.42–2.20 (m, 2 H), 3.38–3.33 (m, 4 H), 3.49–3.44 (m, 1 H), 3.60–3.54 (m, 1 H), 4.09–3.86 (m, 5 H), 4.57–4.32 (m, 7 H), 5.08–5.01 (m, 2 H), 7.37–7.18 (m, 20 H); ¹³C NMR (100 MHz, CDCl₃) δC 14.1, 22.7, 25.7 (d, J = 140.3 Hz), 25.8, 26.2, 27.3 (d, J = 3.2 Hz), 27.4 (d, J = 3.0 Hz), 29.3, 29.5, 29.7, 31.8, 65.5 (d, J = 6.4 Hz), 65.6 (d, J = 6.6 Hz), 67.0 (d, J = 6.0 Hz), 67.1 (d, J = 6.1 Hz), 70.0, 70.5, 71.0, 71.4 (d, J = 6.4 Hz), 73.3, 76.1, 77.2, 82.8, 82.9 (d, J = 3.1 Hz), 83.0 (d, J = 3.1 Hz), 83.6, 127.6, 127.6, 127.7, 127.7, 127.8, 127.9, 127.9, 128.3, 128.3, 128.4, 128.4, 128.5, 128.5, 136.5 (d, J = 6.9 Hz), 136.6 (d, J = 6.7 Hz), 137.7, 137.8, 138.1; ³¹P NMR (162 MHz, CDCl₃) δP 30.4; Anal. Calcd for C₄₆H₆₁O₈P: C, 71.48; H, 7.95. Found: C, 71.37; H, 8.01.

1-[Benzyl(butyl-4'-O-nonyl)-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucityl phosphonate (2.33c). Alkene 2.32c (135 mg, 0.172 mmol) was converted to 2.33c as described for the preparation of 2.33a. Purification by chromatography (2:1, hexane:EtOAc) afforded the product (100 mg, 74%) as a clear oil: Rf 0.17 (2:1 hexane:EtOAc); [α]²⁵/D +11.0 (c 1.1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δH 0.88 (t, 3 H, J = 6.6 Hz), 1.35–1.20 (m, 12 H), 1.62–1.49 (m, 4 H), 1.71–1.63 (m, 2 H), 2.41–2.20 (m, 2 H), 3.38–3.31 (m, 4 H), 3.49–3.43 (m, 1 H), 3.60–3.53 (m, 1 H), 4.15–3.85 (m, 5 H), 4.58–4.30 (m, 7 H), 5.10–5.00
(m, 2 H), 7.34–7.20 (m, 20 H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 14.1, 22.6, 25.7 (d, $J$ = 139.8 Hz), 25.8, 26.1, 27.2 (d, $J$ = 3.3 Hz), 27.3 (d, $J$ = 3.1 Hz), 29.2, 29.4, 29.5, 29.7, 31.8, 65.5 (d, $J$ = 6.3 Hz), 65.6 (d, $J$ = 6.4 Hz), 67.0 (d, $J$ = 6.0 Hz), 67.1 (d, $J$ = 6.1 Hz), 70.0, 70.5, 71.0, 71.4 (d, $J$ = 6.5 Hz), 73.2, 76.1, 77.2, 82.7, 82.9 (d, $J$ = 3.1 Hz), 83.0 (d, $J$ = 3.8 Hz), 83.6, 127.6, 127.6, 127.6, 127.7, 127.7, 127.9, 127.9, 128.2, 128.3, 128.3, 128.4, 128.5, 128.5, 136.4 (d, $J$ = 6.0 Hz), 136.5(d, $J$ = 6.1 Hz), 137.7, 137.8, 138.1; $^{31}$P NMR (162 MHz, CDCl$_3$) $\delta_P$ 30.4; Anal. Calcd for C$_{47}$H$_{63}$O$_8$P: C, 71.73; H, 8.07. Found: C, 71.35; H, 7.98.

1-[Benzyl(butyl-4'-O-decyl)]-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucityl phosphonate (2.33d). Alkene 2.32d (143 mg, 0.179 mmol) was converted to 2.33d as described for the preparation of 2.33a. Purification by chromatography (2:1, hexane:EtOAc) afforded the product (122 mg, 85%) as a clear oil: $R_f$ 0.23 (2:1, hexane:EtOAc); [$\alpha$]$^2$$_D$ +14.7 (c 1.2, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta_H$ 0.88 (t, 3 H, $J$ = 6.8 Hz), 1.37–1.40 (m, 14 H), 1.61–1.47 (m, 4 H), 1.73–1.62 (m, 2 H), 2.42–2.19 (m, 2 H), 3.38–3.31 (m, 4 H), 3.49–3.44 (m, 1 H), 3.60–3.54 (m, 1 H), 4.08–3.86 (m, 5 H), 4.58–4.30 (m, 7 H), 5.08–5.01 (m, 2 H), 7.34–7.24 (m, 20 H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 14.1, 22.7, 25.8 (d, $J$ = 140.0 Hz), 25.8, 26.2, 27.3 (d, $J$ = 3.2 Hz), 27.4 (d, $J$ = 3.6 Hz), 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 65.5 (d, $J$ = 6.6 Hz), 65.6 (d, $J$ = 6.6 Hz), 67.0 (d, $J$ = 6.1 Hz), 67.1 (d, $J$ = 6.0 Hz), 70.0, 70.5, 71.0, 71.4 (d, $J$ = 7.8 Hz), 73.3, 76.1, 77.2, 82.7, 82.9 (d, $J$ = 3.1 Hz), 83.0 (d, $J$ = 3.1 Hz), 83.6, 127.6, 127.6, 127.7, 127.7, 127.7, 127.8, 127.9, 127.9, 128.3, 128.3, 128.4, 128.4, 128.5, 128.6, 136.5 (d, $J$ =6.1 Hz), 136.6 (d, $J$ = 6.3 Hz), 137.7, 137.8, 138.1; $^{31}$P NMR (162 MHz, CDCl$_3$) $\delta_P$ 30.4; Anal. Calcd for C$_{48}$H$_{65}$O$_8$P: C, 71.97; H, 8.18. Found: C, 72.29; H, 8.21.
1-[Benzyl(butyl-4'-O-dodecyl)]-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucityl phosphonate (2.33e). Alkene 2.32e (175 mg, 0.212 mmol) was converted to 2.33e as described for the preparation of 2.33a. Purification by chromatography (2:1, hexane:EtOAc) affords the product (150 mg, 86%) as a clear oil: \( R_f 0.24 \) (2:1, hexane:EtOAc); \([\alpha]^{25}_D +14.5 \) (c 1.0, CHCl_3); \(^1\)H NMR (400 MHz, CDCl_3) \( \delta_H 0.88 \) (t, 3 H, \( J = 6.6 \) Hz), 1.35–1.18 (m, 18 H), 1.62–1.48 (m, 4 H), 1.71–1.63 (m, 2 H), 2.41–2.19 (m, 2 H), 3.38–3.30 (m, 4 H), 3.49–3.42 (m, 1 H), 3.60–3.53 (m, 1 H), 4.09–3.85 (m, 5 H), 4.57–4.30 (m, 5 H), 5.08–5.00 (m, 2 H), 7.33–7.16 (m, 20 H); \(^{13}\)C NMR (100 MHz, CDCl_3) \( \delta_C 14.1, 22.7, 25.8 \) (d, \( J = 140.4 \) Hz), 25.8, 26.2, 27.3 (d, \( J = 3.1 \) Hz), 27.4 (d, \( J = 3.2 \) Hz), 29.4, 29.5, 29.6, 29.7, 29.8, 31.9, 65.5 (d, \( J = 6.5 \) Hz), 65.6 (d, \( J = 6.8 \) Hz), 67.0 (d, \( J = 5.9 \) Hz), 67.2 (d, \( J = 6.1 \) Hz), 70.0, 70.5, 71.0, 71.4 (d, \( J = 8.3 \) Hz), 73.2, 76.1, 77.2, 82.7, 83.0 (d, \( J = 3.2 \) Hz), 83.1 (d, \( J = 3.0 \) Hz), 83.6, 127.6, 127.6, 127.7, 127.7, 127.8, 127.8, 127.9, 127.9, 128.2, 128.2, 128.3, 128.3, 128.4, 128.5, 136.5 (d, \( J = 6.6 \) Hz), 136.6 (d, \( J = 6.3 \) Hz), 137.7, 137.8, 138.1; \(^{31}\)P NMR (162 MHz, CDCl_3) \( \delta_P 30.4 \); Anal. Calcd for C_{50}H_{69}O_8P: C, 72.44; H, 8.39. Found: C, 72.45; H, 8.51.

1-[Benzyl(butyl-4'-O-hexadecyl)]-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucityl phosphonate (2.33f). Alkene 2.32f (158 mg, 0.179 mmol) was converted to 2.33f as described for the preparation of 2.33a. Purification by chromatography (2:1, hexane:EtOAc) afforded the product (150 mg, 95%) as a clear oil: \( R_f 0.24 \) (2:1, hexane:EtOAc); \([\alpha]^{25}_D +15.6 \) (c 0.6, CHCl_3); \(^1\)H NMR (400 MHz, CDCl_3) \( \delta_H 0.88 \) (t, 3 H, \( J = 6.7 \) Hz), 1.35–1.18 (m, 26 H), 1.62–1.48 (m, 4 H), 1.71–1.63 (m, 2 H), 2.41–2.19 (m, 2 H), 3.38–3.31 (m, 4 H), 3.49–3.43 (m, 1 H), 3.60–3.53 (m, 1 H), 4.09–3.85 (m, 5 H), 4.57–4.30 (m, 7 H), 5.08–5.00 (m, 2 H), 7.37–7.18 (m, 20 H); \(^{13}\)C NMR (100 MHz, CDCl_3) \( \delta_C 14.1, 22.7, 25.7 \) (d, \( J = 139.8 \) Hz).
Hz), 25.8, 26.2, 27.2 (d, $J = 3.4$ Hz), 27.3 (d, $J = 3.3$ Hz), 29.4, 29.5, 29.6, 29.7, 29.8, 31.9, 31.9, 65.5 (d, $J = 6.3$ Hz), 65.6 (d, $J = 6.7$ Hz), 67.0 (d, $J = 6.0$ Hz), 67.1 (d, $J = 6.1$ Hz), 70.0, 70.5, 71.0, 71.4 (d, $J = 6.6$ Hz), 73.2, 76.1, 77.2, 82.7, 82.9 (d, $J = 3.0$ Hz), 83.0 (d, $J = 3.0$ Hz), 83.6, 127.6, 127.6, 127.7, 127.7, 127.8, 127.8, 127.9, 127.9, 128.2, 128.2, 128.3, 128.3, 128.4, 128.5, 136.4 (d, $J = 6.0$ Hz), 136.5 (d, $J = 6.3$ Hz), 137.7, 137.8, 138.1; $^{31}$P NMR (162 MHz, CDCl$_3$) $\delta$P 30.5; Anal. Calcd for C$_{54}$H$_{77}$O$_8$P: C, 73.27; H, 8.77. Found: C, 73.07; H, 9.08.

Measurement of anti-tuberculosis activity of 2.15a–2.15f. Measurement of the anti-tuberculosis activity of the target compounds was carried out as previously reported using the fluorescence-based Alamar Blue Microplate assay.$^{33}$ All compounds were initially tested against Mycobacterium tuberculosis strain H$_{37}$Rv (ATCC 27294) at a concentration 6.25 µg/mL. At this concentration, only 2.15f inhibited the growth of the bacteria. The MIC for 2.15f was determined by testing this compound at lower concentrations of the compound; the MIC is defined as the lowest concentration producing a 90% reduction in fluorescence relative to controls.

References


8. Incorporation of arabinose into AG has been shown through the use of radiolabeled 3 and mycobacterial membrane extracts. In addition, incubation of small oligosaccharides substrates with 3, in the presence of a mycobacterial membrane preparation, led to the formation of an oligosaccharide with additional β-(1→2) and α-(1→5) linkages (Lee, R. E.; Brennan, P. J.; Besra, G. S. *Glycobiology* 1997, 7, 1121). The lack of α-(1→3) linkages was attributed to either instability or absence of α-(1→3) AraT activity in the membrane preparation, or the possibility that this enzyme recognizes oligosaccharide substrates larger than those investigated. It is also conceivable that another activated donor (e.g., a sugar nucleotide) is used by this AraT. The presence of UDP-Araf in mycobacteria has been reported (Singh, S.; Hogan S. E. *Microbios* 1994, 77, 217), but incorporation of this donor into arabinan has not been demonstrated.


22. The stereochemistry was proven by comparison of the $^1$H, $^{13}$C and $^{31}$P spectral data previously reported for 14: McClard, R. W.; Tsimikas, S.; Schriver, K. E. *Arch. Biochem. Biophys.* **1986**, *245*, 282.


29. We attempted simultaneous reduction of the alkene and hydrogenation of the benzyl ethers upon reaction with H$_2$, Pd/C, but were unsuccessful. Under these conditions, decomposition of the substrate was observed, which we attribute to the formation of a palladium-$\pi$-allyl complex from the allyl phosphonate.

30. Iatrobeads refers to a beaded silica gel 6RS-8060, which is manufactured by Iatron Laboratories (Tokyo).


CHAPTER 3

SYNTHESIS OF C-PHOSPHINIC ACID ANALOGS OF DECAPRENOLPHOSPHOARABINOSE

3.1 Introduction

This chapter describes the synthesis of a series of C-phosphinic acid DPA analogs (3.1, Figure 3.1) as potential antimycobacterial agents. Phosphinic acids are metabolically stable phosphate mimics in which two of the oxygen-phosphorous bonds have been replaced by carbon-phosphorous bonds. In our synthesis of the C-phosphonic acid series (Chapter 2), we obtained low yields in the deprotection step, which we postulated was due to the formation of phostones (e.g., 3.4, Figure 3.2) from cyclization of the C-3 OH group onto the phosphorous, resulting in the loss of the “lipid” chain. C-Phosphinic acids are hydrolytically stable and thus, phostone formation cannot occur.
As discussed in Chapter 1, the synthesis of glycosyl C-phosphinic acids has not been previously reported. This chapter describes the synthesis of a series of alkyl-2,5-anhydroglucityl (“β-arabino”) phosphinic acids of type 3.1, where the R group is a long saturated alkyl chain. In addition, the synthesis of octyl-2,5-anhydromannityl (“α-arabino”) phosphinic acid 3.5 (Figure 3.3) is described.
3.2 Synthesis of C-phosphinic acid DPA analogs

3.2.1 Retrosynthetic analysis of 3.1

The retrosynthetic analysis of C-phosphinic acid 3.1 is shown in Figure 3.4. Phosphonate 3.9 could be reduced to form phosphine 3.14, which could then be alkylated using an appropriate base and long chain electrophile to afford 3.17. Oxidation of 3.17 and subsequent hydrogenation of 3.18 would afford 3.1.

Figure 3.3: Octyl-2,5-anhydromannityl phosphinic acid 3.5.

Figure 3.4: Retrosynthetic analysis of 3.1.
3.2.2 Synthesis of C-phosphinic acid 3.1

The precursor to our C-phosphinic acids is phosphonate 3.9, which was synthesized as shown in Scheme 3.1. Olefin 3.7 was formed in 77% yield via a Wittig reaction on lactol 3.6, which is commercially available. Iodocyclization of 3.7 afforded 3.8 in 95% yield. Phosphonate 3.9 was formed in 85% yield via a Michaelis Arbuzov reaction on iodide 3.8 with triethylphosphite.

Scheme 3.1: Synthesis of phosphonate 3.9.

The stereoselectivity achieved in the iodocyclization to form 3.8 can be partially rationalized from examination of the transition states for this cyclization (3.10 and 3.12, Figure 3.5).\(^\text{1}\) The allylic ether oxygen controls the stereochemical outcome of the cyclization, and there is a preference for the olefin to cyclize through a transition state in which the OR residue is in the plane of the prochiral olefin. The electrophile approaches from the least hindered side, followed by trapping by the internal OH group. Cyclization of 3.10 with H-2 and H-3 cis leads to the preferred β-arabino epimer 3.11. Cyclization through conformation
3.12, in which H-2 and H-3 are *trans* and H-3 eclipses the olefin, leads to the altro (“α-arabino”) isomer 3.13. It is not fully understood why 3.10, with the OR group eclipsed with H-1, is the preferred transition state for this kinetically controlled iodocyclization. Similar stereoselectivity was observed for iodolactonizations of allylic alcohols, which resulted in the formation of *cis* iodo lactones, and it is thought that subtle conformational or electronic factors are responsible for this stereocontrol. Under the reaction conditions used, we did not observe the formation of a product corresponding with 3.13.

Figure 3.5: Transition states for iodocyclization.
The synthesis of a series of C-phosphinic acids (3.1a-e) is illustrated in Scheme 3.2. The yields of compounds 3.17, 3.18, and 3.1 are shown in Table 3.1. After rigorous drying under vacuum, phosphonate 3.9 was reduced with lithium aluminum hydride in anhydrous ether to afford 3.14. The yield of this reaction varied and benzyl alcohol was frequently obtained as a byproduct, however we are unsure of the mechanism for its formation. Phosphine 3.14 was then alkylated using phosphazene base 3.15 (Figure 3.6) and various long chain alkyl halides 3.16a-d (or ROTs, 3.16e) to produce the secondary phosphines 3.17a-e in modest to good yields (50-82%) after chromatography. The yields were lower in some cases due to oxidation of the product that occurred during chromatography. We attempted to obtain mass spectrometric data for these phosphines, but the only signal detected was for the corresponding phosphinous acid derivative (e.g., 3.19, Figure 3.7). In the $^1$H-decoupled $^{31}$P-NMR spectra for 3.16a, resonances were seen for both the secondary phosphine ($\delta_P -76.8$ ppm, -77.3 ppm, $^1J_{P,H}$ 198 Hz and 200 Hz, CDCl$_3$) and phosphinous acid 3.19 ($\delta_P$ 34.3 ppm, 33.9 ppm, $^1J_{P,H}$ 462 Hz and 476 Hz, CDCl$_3$).

![Figure 3.6: Phosphazene base 3.15.](image)
Phosphazene bases belong to a class of sterically hindered non-ionic bases that were developed by Schwesinger in the early 1980s. Compared with other well-known nitrogen containing bases such as 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU), phosphazene bases are more basic (pKa’s of the corresponding conjugate acids range from 26-42) and less nucleophilic. Before using 3.15, we attempted using \( n \)-butyl lithium and \( tert \)-butyl lithium in THF at various temperatures, but even at room temperature, no alkylation product formed when electrophile 3.16 was added. The starting material was recovered, along with a mixture of the alkyl halide and elimination byproduct.
Scheme 3.2: Synthesis of C-phosphinic acids 3.1a-e.

<table>
<thead>
<tr>
<th></th>
<th>3.17</th>
<th>3.18</th>
<th>3.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>a, R = (CH₂)₇CH₃</td>
<td>55%</td>
<td>62%</td>
<td>81%</td>
</tr>
<tr>
<td>b, R = (CH₂)₉CH₃</td>
<td>66%</td>
<td>86%</td>
<td>82%</td>
</tr>
<tr>
<td>c, R = (CH₂)₁₁CH₃</td>
<td>50%</td>
<td>80%</td>
<td>88%</td>
</tr>
<tr>
<td>d, R = (CH₂)₁₅CH₃</td>
<td>82%</td>
<td>78%</td>
<td>70%</td>
</tr>
<tr>
<td>e, R = (CH₂)₁₉CH₃</td>
<td>60%</td>
<td>43%</td>
<td>80%</td>
</tr>
</tbody>
</table>

Table 3.1: Yields of 3.17, 3.18, and 3.1(a-e) from Scheme 3.2.
Oxidation of 3.17a-e was achieved with iodine in a 98:2 mixture of pyridine/H$_2$O to afford 3.18a-e in generally good yields. The reaction was slow, requiring at least 3 days to go to completion. The progress of the reaction was monitored by $^1$H-coupled $^{31}$P-NMR spectroscopy, and we were looking for the presence of a singlet at approximately 50 ppm (similar to other phosphinic acids$^6$) corresponding to phosphinic acid 3.18, and the absence of signals at 33 and 34 ppm for the phosphinous acid 3.19. In the $^1$H-decoupled $^{31}$P-NMR spectrum for 3.18, a singlet at 49 ppm was present. Similarly, in the $^{13}$C-NMR spectra, the presence of two C-P bonds were observed between 30 and 32 ppm, which appeared as doublets with $^1J_{C,P}$ values of approximately 90 Hz. As mentioned earlier, we believe that the secondary phosphines 3.17 were oxidizing during chromatography, which resulted in lower yields of the products. In an attempt to prevent the loss of material, we attempted a one-pot oxidation of the crude alkylation product 3.17 to 3.18. However, even after several attempts to purify the products by chromatography, 3.18 could not be completely separated from the byproducts of the alkylation.

Because the oxidation reaction was very slow, we also explored other oxidizing agents (e.g., H$_2$O$_2$, $^m$-CPBA), but the only product formed was the phosphinous acid derivative 3.19, which was confirmed by mass spectrometric data (exact mass = 601.3053; observed mass = 601.3055) and $^1$H-coupled $^{31}$P-NMR spectra. The $^1$H-NMR spectra for phosphinic acid 3.18a and phosphinous acid 3.19 were nearly identical. For 3.18a, a broad singlet was observed at approximately 8 ppm, and for 3.19, a more intense broad singlet appeared at approximately 9 ppm. The multiplets corresponding to each of the P-H diastereomers were not seen in the $^1$H-NMR spectra, and we think this might be because 3.19 was existing as the hydroxyphosphite tautomer 3.20.
Figure 3.8: Hydroxyphosphite 3.20 tautomer of 3.19.

A possible mechanism for the oxidation of a secondary phosphine (e.g., 3.21) to an alkyl C-phosphinic acid (3.27) is illustrated in Figure 3.9, and is based on the mechanism proposed for a phosphate system. Phosphine 3.21 reacts with iodine to form 3.22. Hydrolysis of 3.22 affords phosphinous acid 3.23, which is in equilibrium with 3.24. Hydroxyphosphite 3.24 reacts with another equivalent of iodine to form 3.25, and then loses a proton to form 3.26. Hydrolysis of 3.26 affords phosphinic acid 3.27.

Figure 3.9: Possible oxidation mechanism for phosphinic acid formation.
After oxidizing 3.17a-e, the residual pyridine was removed with Amberlite H⁺ resin to afford 3.18a-e as the free phosphinic acid. Hydrogenolysis of 3.18a-e in methanol afforded 3.1a-e in good yields. For all compounds, the ¹H-decoupled ³¹P-NMR spectra showed a singlet at approximately 50 ppm. In the ¹³C-NMR spectra, the presence of two doublets (¹J_C,P ~90 Hz) arising from the carbon atoms adjacent to the phosphorous were present between 30 and 32 ppm.

Initially, we performed the hydrogenation of 3.18 in methanol containing 1% acetic acid (Figure 3.10). This reaction was repeated many times to produce the desired product 3.1, along with other products resulting from decomposition. Multiple peaks were observed in the ³¹P-NMR spectra, and the ¹H-NMR spectra of the crude product showed many impurities. This result was disturbing because we expected that the C-phosphinic acids would be more stable to basic and acidic conditions relative to the C-phosphonic acids (Chapter 2), and thus more suitable as drug candidates. We are not sure why these compounds were sensitive to the mildly acidic conditions, but when the hydrogenation was done without any acetic acid, only 3.1 was observed in the crude ³¹P-NMR spectra (~50 ppm, C₅D₅N) and the crude ¹H-NMR spectra showed that the product had few impurities. Further purification of these compounds consisted of simple filtration through an Iatrobead column.

![Figure 3.10: Initial hydrogenation attempts.](image-url)
The $^1$H-NMR spectra of compounds 3.18 and 3.1 were taken in both CD$_3$OD and C$_5$D$_5$N. We noticed that there was significant peak broadening in CD$_3$OD compared with C$_5$D$_5$N. This can be attributed to the differences between a phosphinic acid with a free OH group and one that exists as a pyridinium salt. In methanol, a phosphinic acid has a free OH group that has the capability of hydrogen bonding or possibly forming aggregates. In pyridine, this compound exists as the pyridinium salt and cannot form hydrogen bonds or aggregates. The peak broadening was even more exaggerated when the spectrum was taken in D$_2$O, but this could also be a solubility issue because these compounds were only marginally soluble in water. For compound 3.1a, where the R group was an octyl group, the signals in the $^1$H-NMR spectra recorded in D$_2$O were barely discernable.

The solubility properties were much different for the C-phosphinic acids than for the C-phosphonic acid series. Except for 3.1a, the C-phosphinic acids were completely insoluble in water. Addition of water to these compounds resulted in formation of a “soapy” opaque mixture. In contrast, all the C-phosphonic acids were water-soluble. The C-phosphonic acids, including one with a 20-carbon chain (22-atoms total) (3.28, Figure 3.11), were completely soluble in methanol at room temperature. The C-phosphinic acids with 16-carbon and 20-carbon alkyl chains, 3.1d&e (Figure 3.12) were only soluble in hot methanol. For these compounds it was possible to purify them by recrystallization from methanol. The main difference between the C-phosphonic acids (such as 3.28) and the C-phosphinic acids 3.1, is two oxygens in the long chain. These oxygens appear to make a big difference in the solubility of these compounds.
C-Phosphinic acids 3.1a-e are currently being screened against *M. tuberculosis*. Because the C-phosphonic acid 3.28 was the only biologically active compound in this series, we suspect that the length of the chain is important, but not extremely important because decaprenol has a chain that is 40 carbons in length (50 atoms total). We anticipate that the C-phosphininc acids with the longest chains (3.1d&e) have the greatest potential for activity.
3.3 Synthesis of octyl-2,5-anhydromannityl phosphinic acid

As discussed earlier, the alkyl-2,5-anhydroglucityl phosphinic acids (3.1a-e) were sensitive to the mildly acidic conditions in the hydrogenation reaction, leading to products resulting from decomposition. It is not clear why these compounds were unstable to these conditions, and we were unable to isolate or identify any of the products formed as a result of decomposition. Our initial hypothesis was that the C-3 OH group might be attacking the phosphorus leading to a mixture of isomers (3.29 & 3.30, Figure 3.13). This might occur with the “β-arabino” phosphinic acid in which the C-2/C-3 orientation is cis. This type of interaction would not be possible if the relationship was trans (“α-arabino” epimer or 2,5-mannityl phosphinic acid). We attempted to prove our hypothesis by synthesizing octyl-2,5-anhydromannityl phosphinic acid.

![Figure 3.13: Two potential isomers of alkyl-2,5-anhydroglucityl phosphinic acids.](image)

Figure 3.13: Two potential isomers of alkyl-2,5-anhydroglucityl phosphinic acids.
The synthesis of 3.36, the precursor to 3.5, is shown in Scheme 3.3. Lactone 3.31\(^9\) reacted with the anion of dimethyl methylphosphonate to afford 3.32 in 94% yield. Lactol 3.32 was deoxygenated using triethylsilane and trimethylsilyl triflate to afford the “α-arabino” phosphonate 3.33 in 84% yield.\(^10\) Phosphonate 3.33\(^11\) was reduced with lithium aluminum hydride to form phosphine 3.34 in 57% yield, which was then alkylated with 1-iodooctane using 3.15 to afford 3.35 in 42% yield. Oxidation of 3.35 afforded 3.36 in 67% yield.

Scheme 3.3: Synthesis of 3.36.
Hydrogenolysis of 3.36 was done using 10% palladium on carbon in methanol, with and without the presence of acetic acid (Scheme 3.4). In both cases, 3.5 was formed as the only product, and the $^{31}$P-NMR spectrum showed only a single phosphorous peak ($\delta_P$ 49 ppm, C$_3$D$_2$N, $\delta_P$ 55 ppm, CD$_3$OD). The $^1$H-NMR spectra for the crude products showed that these compounds were nearly pure, requiring only filtration through a silica gel plug. While this result does not directly prove our hypothesis that the C-3 OH is cyclizing onto the phosphorous, it does show that the 2,3-cis relationship of the “α-arabino” phosphinic acids of type 3.29 is likely causing an adverse effect on the reaction in the presence of acid.

Scheme 3.4: Synthesis of 3.5.
In conclusion, we have developed an efficient method for the synthesis of glycosyl C-phosphinic acids, which were previously unknown. The reduction, alkylation, oxidation sequence is novel and amenable to the preparation of many C-phosphinic acids. Our main goal was to synthesize arabinose-derived C-phosphinic acids to be screened against *M. tuberculosis*, but this methodology could also be applied to the synthesis of non-carbohydrate phosphinic acids.

3.4 Experimental

**General.** Solvents were distilled from the appropriate drying agents before use. Unless stated otherwise, all reactions were carried out at room temperature under a positive pressure of argon and were monitored by TLC on silica gel 60 F$_{254}$. Spots were detected under UV light or by charring with 10% H$_2$SO$_4$ in ethanol. Solvents were evaporated under reduced pressure and below 40 °C (bath). Organic solutions of crude products were dried over anhydrous Na$_2$SO$_4$. Unless otherwise indicated, column chromatography was performed on silica gel 60 (40–60 µM). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). Optical rotations were measured at 21±2 °C. $^1$H NMR spectra were recorded at 250, 400, or 500 MHz, and chemical shifts are referenced to either TMS (0.0, CDCl$_3$) or CD$_3$OH (4.78, CD$_3$OD). $^{13}$C NMR spectra were recorded at 62.5, 100, or 125 MHz and chemical shifts are referenced to CDCl$_3$ (77.00, CDCl$_3$) or CH$_3$OH (49.00, CD$_3$OD). $^{31}$P NMR spectra were recorded at 101, 162, or 202 MHz and chemical shifts are referenced to external phosphoric acid (0.0, CDCl$_3$, CD$_3$OD). Electrospray mass spectra were recorded on samples suspended in mixtures of THF and CH$_3$OH with added trifluoroacetic acid or NaCl.
1-(Octyl)-2,5-anhydroglucityl phosphinic acid (3.1a) Phosphinic acid 3.17a (90 mg, 0.15 mmol) was dissolved in methanol (5 mL) and 10% palladium on carbon (30 mg) was added. The reaction mixture was stirred under hydrogen at atmospheric pressure overnight before being filtered and concentrated. The resulting oil was purified by chromatography on Iatro beads (1% pyridine in CH$_2$Cl$_2$→CH$_2$Cl$_2$:CH$_3$OH, 3:1) providing a compound that was then redissolved in water and lyophilized to afford 3.1a (40 mg, 81%) as an off-white solid: $R_f$ 0.45 (1:1, CH$_2$Cl$_2$:CH$_3$OH); $[\alpha]^{25}_D$ +10.4 (c 1.0, CH$_3$OH); $^1$H NMR (500 MHz, CD$_3$OD) $\delta$H 0.82 (t, 3 H, $J = 7.1$ Hz), 1.40-1.11 (m, 10 H), 2.09-1.78 (m, 4 H), 2.72 (broad s, 2 H), 4.29-4.13 (m, 2 H), 4.47 (broad s, 1 H), 4.76 (broad s, 1 H), 4.84 (broad s, 1 H), 5.10 (broad s, 1 H), 8.29 (broad s, 4 H); $^{13}$C NMR (125 MHz, C$_5$D$_5$N) $\delta$C 14.3, 22.9, 29.5, 29.6, 31.2 (d, $J = 88.0$ Hz), 31.4 (d, $J = 13.9$ Hz), 31.4 (d, $J = 93.8$ Hz), 32.1, 63.4, 77.9, 79.8, 79.9, 87.5; $^{31}$P NMR (202.4 MHz, C$_5$D$_5$N) $\delta$P 49.6; HRMS calcd for C$_{14}$H$_{29}$O$_6$PNa$:\quad$347.1594. Found: 347.1607.

1-(Decyl)-2,5-anhydroglucityl phosphinic acid (3.1b) Phosphinic acid 3.17b (140 mg, 0.23 mmol) was hydrogenated in CH$_3$OH (5 mL) using 10% Pd/C (50 mg) as described for the preparation of 3.1a. Purification of the product was done as described for 3.1a to provide 3.1b (65 mg, 82%) as an off-white solid: $R_f$ 0.38 (2:1, CH$_2$Cl$_2$:CH$_3$OH); $[\alpha]^{25}_D$ +6.2 (c 1.0, CH$_3$OH); $^1$H NMR (500 MHz, C$_5$D$_5$N) $\delta$H 0.86 (t, 3 H, $J = 7.1$ Hz), 1.42-1.18 (m, 14 H), 2.09-1.79 (m, 4 H), 2.72 (broad s, 2 H), 4.16 (dd, 1 H, $J = 11.1$, 4.0 Hz), 4.24 (dd, 1 H, $J = 11.4$, 3.2 Hz), 4.47 (broad s, 1 H), 4.75 (broad s, 1 H), 4.83 (broad s, 1 H), 4.83 (broad s, 1 H), 5.16-5.08 (m, 1 H), 8.93 (broad s, 2 H); $^{13}$C NMR (125 MHz, C$_5$D$_5$N) $\delta$C 14.4, 23.0, 29.6, 29.7, 29.7, 29.9, 30.1, 30.8, 31.2 (d, $J = 92.4$ Hz), 31.4 (d, $J = 93.6$ Hz), 31.5 (d, $J = 13.7$ Hz), 32.2, 63.4, 78.0,
79.8, 80.0, 87.5; $^{31}$P NMR (202.4 MHz, C$_5$D$_5$N) $\delta$P 49.7; HRMS calcd for C$_{16}$H$_{33}$O$_6$PNa$^+$: 375.1907. Found: 375.1889.

1-(Dodecyl)-2,5-anhydroglucityl phosphinic acid (3.1c) Phosphinic acid 3.17c (98 mg, 0.15 mmol) was hydrogenated in CH$_3$OH (5 mL) using 10% Pd/C (40 mg) as described for the preparation of 3.1a. Purification of the product was done as described for 3.1a to provide 3.1c (50 mg, 88%) as an off-white solid: $R_f$ 0.50 (2:1, CH$_2$Cl$_2$:CH$_3$OH); [\(\alpha\)]$_D$$^25$ +7.2 (c 1.1, CH$_3$OH); $^1$H NMR (500 MHz, C$_5$D$_5$N) $\delta$H 0.87 (t, 3 H, $J$ = 7.1 Hz), 1.41-1.15 (m, 18 H), 2.09-1.76 (m, 4 H), 2.71 (broad s, 2 H), 4.28-4.10 (m, 2 H), 4.47 (broad s, 1 H), 4.75 (broad s, 1 H), 4.82 (broad s, 1 H), 5.10 (broad s, 1 H), 8.00 (broad s, 4 H); $^{13}$C NMR (125 MHz, C$_5$D$_5$N) $\delta$C 14.4, 23.1, 29.8, 29.8, 30.0, 30.1, 30.1, 30.9, 31.5 (d, $J$ = 89.0 Hz), 31.6 (d, $J$ = 13.9 Hz), 32.3, 63.5, 78.0, 79.9, 80.0, 87.6; $^{31}$P NMR (202.4 MHz, C$_5$D$_5$N) $\delta$P 49.9; HRMS calcd for C$_{18}$H$_{37}$O$_6$PNa$^+$: 403.2220. Found: 403.2218.

1-(Hexadecyl)-2,5-anhydroglucityl phosphinic acid (3.1d) Phosphinic acid 3.17d (189 mg, 0.27 mmol) was hydrogenated in CH$_3$OH (5 mL) using 10% Pd/C (70 mg) as described for the preparation of 3.1a. The product was purified by recrystallization from CH$_3$OH to provide 3.1d (81 mg, 70%) as an off-white solid: $R_f$ 0.60 (2:1, CH$_2$Cl$_2$:CH$_3$OH); $^1$H NMR (500 MHz, C$_5$D$_5$N) $\delta$H 0.85 (t, 3 H, $J$ = 7.1 Hz), 1.42-1.19 (m, 26 H), 2.05-1.78 (m, 4 H), 2.79-2.66 (m, 2 H), 4.16 (dd, 1 H, $J$ = 11.2, 4.5 Hz), 4.26 (dd, 1 H, $J$ = 11.4, 4.0 Hz), 4.49-4.46 (m, 1 H), 4.76 (broad s, 1 H), 4.84 (broad s, 1 H), 5.13-5.07 (m, 1 H), 7.56 (broad s, 5 H); $^{13}$C NMR (125 MHz, C$_5$D$_5$N) $\delta$C 14.4, 23.1, 29.8, 29.8, 30.0, 30.1, 30.1, 31.5 (d, $J$ = 88.2 Hz), 31.5 (d, $J$ = 14.8 Hz), 31.6 (d, $J$ = 93.8 Hz), 32.3, 63.5, 78.1, 79.9, 80.0, 80.0, 87.6; $^{31}$P NMR (202.4 MHz, C$_5$D$_5$N) $\delta$P 49.4; HRMS calcd for C$_{22}$H$_{45}$O$_6$PNa$^+$: 459.2846. Found: 459.2822.
1-(Eicosanyl)-2,5-anhydroglucityl phosphinic acid (3.1e) Phosphinic acid 3.17e
(29 mg, 0.038 mmol) was hydrogenated in CH$_3$OH (5 mL) using 10% Pd/C (15 mg) as described for the preparation of 3.1a. The product was purified by recrystallization from CH$_3$OH to provide 3.1e (15 mg, 80%) as an off-white solid: R$_f$ 0.62 (2:1, CH$_2$Cl$_2$:CH$_3$OH); $^1$H NMR (500 MHz, C$_5$D$_5$N) $\delta$H 0.86 (t, 3 H, $J$ = 7.1 Hz), 1.45-1.18 (m, 34 H), 2.06-1.79 (m, 4 H), 2.80-2.69 (m, 2 H), 4.17 (dd, 1 H, $J$ = 11.5, 4.7 Hz), 4.27 (dd, 1 H, $J$ = 11.5, 4.5 Hz), 4.51-4.46 (m, 1 H), 4.79-4.76 (m, 1 H), 4.87-4.83 (m, 1 H), 5.15-5.08 (m, 1 H), 6.15 (broad s, 12 H); $^{13}$C NMR (125 MHz, C$_5$D$_5$N) $\delta$C 14.3, 23.0, 29.6, 29.7, 29.9, 29.9, 30.0, 30.0, 31.3 (d, $J$ = 87.7 Hz), 31.4 (d, $J$ = 14.9 Hz), 31.5 (d, $J$ = 93.2 Hz), 32.1, 63.3, 77.9, 79.8, 79.9 (d, $J$ = 4.9 Hz), 87.4; $^{31}$P NMR (202.4 MHz, C$_5$D$_5$N) $\delta$P 49.3; HRMS calcd for C$_{26}$H$_{53}$O$_6$PNa$^+$: 515.3472. Found: 515.3504.

1-(Octyl)-2,5-anhydromannityl phosphinic acid (3.5) Phosphinic acid 3.34 (66 mg, 0.11 mmol) was dissolved in methanol (5 mL) and 10% palladium on carbon (25 mg) was added. The reaction mixture was stirred under hydrogen overnight at atmospheric pressure. After filtering the solid and evaporating the solvent, the resulting oil was purified by chromatography on Iatrobeads (1% pyridine in CH$_2$Cl$_2$→ CH$_2$Cl$_2$:CH$_3$OH, 3:1) and lyophilized to afford 3.5 (37 mg, 89%) as an off-white solid: R$_f$ 0.39 (1:1, CH$_2$Cl$_2$: CH$_3$OH); [α]$_D^{25}$ +28.1 (c 1.2, CH$_3$OH); $^1$H NMR (500 MHz, C$_5$D$_5$N) $\delta$H 0.81 (t, 3 H, $J$ = 7.0 Hz), 1.38-1.09 (m, 10 H), 2.00-1.75 (m, 4 H), 2.71-2.52 (m, 2 H), 4.17 (dd, 1 H, $J$ = 11.7, 5.3 Hz), 4.25 (dd, 1 H, $J$ = 11.8, 3.4 Hz), 4.59-4.56 (m, 1 H), 4.70-4.67 (m, 1 H), 4.92-4.83 (m, 2 H), 7.79 (broad s, 5 H); $^{13}$C NMR (125 MHz, C$_5$D$_5$N) $\delta$C 14.3, 22.8, 22.9, 29.4, 29.5, 31.3 (d, $J$ = 15.0 Hz), 31.6 (d, $J$ = 93.3 Hz), 32.0, 36.2 (d, $J$ = 86.6 Hz), 63.3, 78.4, 79.2, 83.9 (d, $J$ = 5.5 Hz), 85.2; $^{31}$P NMR (202.4 MHz, C$_5$D$_5$N) $\delta$P 49.2; HRMS calcd for C$_{14}$H$_{29}$O$_6$PNa$^+$: 347.1594. Found: 347.1598.
3,4,6-Tri-O-benzyl-1,2-dideoxy-D-arabino-hex-1-enitol (3.7) n-Butyl lithium (24 mL, 38.5 mmol) was added dropwise to a solution of methyl triphenylphosphonium bromide (13.8 g, 38.5 mmol) in THF (100 mL) stirring at rt. Lactol 3.6 (4.6 g, 11.0 mmol) was added in THF (10 mL). After 1.5 h the reaction mixture was diluted with EtOAc (100 mL) and the organic layer was washed with H₂O (100 mL), brine (100 mL), and dried over Na₂SO₄. The solvent was then evaporated to a light yellow oil which was purified by chromatography (hexane:EtOAc, 4:1) to afford 3.7 (3.6 g, 77%) as a colorless oil. The NMR data is the same as previously reported.¹²

2,5-Anhydro-1-deoxy-1-iodo-3,4,6-tri-O-p-methoxybenzyl-D-glucitol (3.8) Alkene 3.7 (5.7 g, 13.6 mmol) was dissolved in THF (20 mL) and stirred at rt. To this solution was added saturated sodium bicarbonate (170 mL, 136 mmol), followed by iodine (25.9 g, 100 mmol) in Et₂O (300 mL). After 12 h, sodium bisulfite was added until the organic layer was a pale yellow color. The organic layer was washed with H₂O (100 mL), brine (100 mL), and dried over MgSO₄. The solvent was then evaporated to a light yellow oil which was purified by chromatography (hexane:EtOAc, 6:1) to afford 3.8 (7.1 g, 95%) as a colorless oil. The NMR data is the same as previously reported.¹

Diethyl 3,4,6-tri-O-benzyl-2,5-anhydroglucityl phosphonate (3.9) Iodide 3.8 (3.85 g, 7.08 mmol) was mixed with triethyl phosphite (20 mL) and the reaction mixture was heated at reflux (156 °C) for 12 h. The excess triethyl phosphite was distilled under vacuum and the resulting oil was purified by chromatography (hexane:EtOAc, 4:1→EtOAc:hexane, 2:1) to afford 3.9 (3.34 g, 85%) as a colorless oil: R_f 0.26 (2:1, EtOAc:hexane); [α]_D +17.4 (c 1.0, CHCl₃);¹ H NMR (400 MHz, CD₃OD) δH 1.23 (t, 3 H, J = 7.1 Hz), 1.23 (t, 3 H, J = 7.0 Hz), 2.34-2.14 (m, 2 H), 3.42 (dd, 1 H, J = 9.9, 6.4 Hz), 3.51 (dd, 1 H, J = 9.9, 5.9 Hz), 3.93-3.86 (m, 2 H), 4.07-3.95 (m, 5 H), 4.27-4.19 (m, 1 H), 4.51-4.35 (m, 6 H), 7.33-7.17 (m, 15
$^{13}$C NMR (100 MHz, CD$_3$OD) δC 16.6, 16.7, 26.3 (d, $J = 141.1$ Hz), 63.2 (d, $J = 6.3$ Hz), 63.5 (d, $J = 6.3$ Hz), 71.6, 72.6, 72.7, 74.3, 77.4, 84.3, 84.4, 84.4, 128.8, 128.9, 129.0, 129.1, 129.1, 129.5, 129.5, 129.6, 139.4, 139.4, 139.6; $^{31}$P NMR (101.2 MHz, CDCl$_3$) δP 29.8; HRMS calcd for C$_{31}$H$_{39}$O$_7$PNa$: 577.2326. Found: 577.2332.

3,4,6-Tri-O-benzyl-2,5-anhydroglucityl phosphine (3.14) A solution of phosphonate 3.9 (235 mg, 0.42 mmol) in anhydrous ether (5 mL) was added dropwise to a mixture of lithium aluminum hydride (40 mg, 1.10 mmol) in anhydrous ether (5 mL) stirring at 0 °C. After 20 min. EtOAc (1 mL) was added to the reaction mixture, and after a few minutes H$_2$O (0.2 mL) was added. After all gas evolution had subsided, the mixture was filtered through Celite and the solvent was evaporated to give a clear residual oil. Purification by chromatography (hexane:EtOAc, 6:1) afforded 3.14 (132 mg, 69%) as a colorless oil: $R_f$ 0.41 (6:1, hexane:EtOAc); [α]$^D_{25}$ +33.9 (c 1.0, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) δH 1.92 (dd, 2 H, $J = 7.1$, 4.6 Hz), 3.15-2.60 (broad s, 2 H), 3.56 (dd, 1 H, $J = 9.8$, 6.8 Hz), 3.67 (dd, 1 H, $J = 9.8$, 5.8 Hz), 4.01-3.95 (m, 2 H), 4.19-4.08 (m, 2 H), 4.42 (d, 1 H, $J = 11.7$ Hz), 4.67-4.54 (m, 5 H), 7.28-7.48 (m, 15 H); $^{13}$C NMR (100 MHz, CDCl$_3$) δC 13.0 (d, $J = 10.5$ Hz), 70.5, 71.3, 71.4, 73.3, 82.2, 82.8, 83.2 (d, $J = 1.3$ Hz), 83.4, 127.5, 127.6, 127.7, 127.7, 127.8, 127.8, 127.9, 128.3, 128.4, 128.4, 128.6, 137.6, 137.7, 138.2; $^{31}$P NMR (161.9 MHz, CDCl$_3$) δP -150.6; HRMS calcd for C$_{27}$H$_{31}$O$_4$PNa$: 473.1852. Found: 473.1820.

1-$p$-Toluenesulfonyloxy-eicosane (3.16e) 1-Eicosanol (500 mg, 1.67 mmol) was dissolved in THF (15mL) and stirred at rt (solid not soluble below rt). n-Butyl lithium (1.1 mL, 1.84 mmol) was added, followed by $p$-toluenesulfonyl chloride (639 mg, 3.35 mmol) in THF (15 mL). After 3 h, the mixture was diluted with EtOAc (20 mL), washed with H$_2$O (25 mL) and brine (25 mL), and dried over Na$_2$SO$_4$. The solvent was evaporated and the solid was purified by chromatography (hexane:EtOAc, 10:1) to afford 3.16e (501 mg, 66%) as a
white solid: $R_f$ 0.49 (10:1, hexane:EtOAc); $^1$H NMR (250 MHz, CDCl$_3$) $\delta$H 0.86 (t, 3 H, $J$ = 6.9 Hz), 1.33-1.14 (m, 34 H), 1.68-1.55 (m, 2 H), 2.43 (s, 3 H), 4.00 (t, 2 H, $J$ = 6.6 Hz), 7.31 (d, 2 H, $J$ = 8.0 Hz), 7.77 (d, 2 H, $J$ = 8.3 Hz); $^{13}$C NMR (62.5 MHz, CDCl$_3$) $\delta$C 14.1, 21.6, 22.7, 25.3, 28.8, 28.9, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 70.7, 127.9, 129.8, 133.3, 144.6; HRMS calcd for C$_{27}$H$_{48}$O$_3$SNa$: 475.3216$. Found: 475.3240.

1-(Octyl)-3,4,6-tri-O-benzyl-2,5-anhydroglucityl phosphinic acid (3.18a)

Phosphine 3.14 (245 mg, 0.54 mmol) was dissolved in Et$_2$O (10 mL) and stirred at rt. Phosphazene base 3.15 (600 $\mu$L of a 1M solution in hexane, 0.60 mmol) was added, followed by 1-iodooctane (0.1 ml, 0.60 mmol). After 30 min., the mixture was neutralized with AcOH and the salt precipitate was removed by filtration through a cotton plug, which was rinsed with anhydrous Et$_2$O. The solvent was evaporated and the residue was purified by flash chromatography (hexane:EtOAc, 6:1, $R_f = 0.36$) to afford 3.17a (169 mg, 55%) as a colorless oil.

Phosphine 3.17a (169 mg, 0.30 mmol) was dissolved in a 98:2 mixture of pyridine/H$_2$O (5 mL). Iodine (189 mg, 0.751 mmol) was added and the reaction mixture stirred for 3 d at rt. The mixture was diluted with CH$_2$Cl$_2$, washed with 5% NaHSO$_3$, and dried over Na$_2$SO$_4$. Most of the pyridine was removed by chromatography (CH$_2$Cl$_2$:CH$_3$OH, 12:1). To remove traces of pyridine, the product was dissolved in CH$_3$OH (5 mL) and stirred with Amberlite 120 (H$^+$) resin (150 mg) overnight. Filtration of the resin and evaporation of the solvent provided the product, which was again purified by chromatography (CH$_2$Cl$_2$:CH$_3$OH, 12:1) to afford 3.17a (111 mg, 62%) as a clear oil: $R_f$ 0.42 (6:1, CH$_2$Cl$_2$:CH$_3$OH); [$\alpha$]$^25_D +11.7$ (c 1.1, CHCl$_3$); $^1$H NMR (400 MHz, C$_5$D$_5$N) $\delta$H 0.84 (t, 3 H, $J$ = 7.1 Hz), 1.40-1.15 (m, 10 H), 2.07-1.80 (m, 4 H), 2.69-2.60 (m, 2 H), 3.85-3.75 (m, 2 H), 4.29-4.26 (m, 1 H), 4.42-4.32 (m, 2 H), 4.78-4.53 (m, 6 H), 4.98-4.89 (m, 1 H), 7.50-7.20 (m, 108
15 H), 12.27 (broad s, 1 H); $^{13}$C NMR (100 MHz, C$_5$D$_5$N) $\delta$C 14.3, 22.9, 29.4, 29.6, 30.5 (d, $J = 88.7$ Hz), 31.4 (d, $J = 14.5$ Hz), 31.5 (d, $J = 94.0$ Hz), 32.1, 71.6, 71.9, 73.4, 77.6, 83.4, 84.2 (d, $J = 6.0$ Hz), 84.7, 127.9, 128.0, 128.1, 128.1, 128.3, 128.7, 128.8, 138.8, 139.0, 139.1; $^{31}$P NMR (161.9 MHz, C$_5$D$_5$N) $\delta$P 48.6; HRMS calcd for C$_{35}$H$_{47}$O$_6$PNa$: 617.3002. Found: 617.3007.

1-(Decyl)-3,4,6-tri-O-benzyl-2,5-anhydroglucityl phosphinic acid (3.18b)

Phosphazene base 3.15 (500 µL of a 1M solution in hexane, 0.50 mmol) was added to phosphine 3.14 (203 mg, 0.45 mmol) in Et$_2$O (10 mL), followed by the addition of 1-iododecane (0.1 mL, 0.50 mmol) as described for the preparation of 3.17a. Purification by chromatography (9:1, hexane:EtOAc, $R_f = 0.26$) afforded 3.17b (175 mg, 66%) as a colorless oil.

Phosphine 3.17b (175 mg, 0.30 mmol) was oxidized with I$_2$ (187 mg, 0.74 mmol) in pyridine/H$_2$O (98:2, 6 mL) as described for the preparation of 3.18a. After removing the pyridine with ion-exchange resin, the product was purified by chromatography (CH$_2$Cl$_2$:CH$_3$OH, 12:1) to afford 3.18b (158 mg, 86%) as a clear oil: $R_f$ 0.57 (3:1, CH$_2$Cl$_2$:CH$_3$OH); $[\alpha]^{25}_D +12.4$ (c 1.0, CHCl$_3$); $^1$H NMR (500 MHz, C$_5$D$_5$N) $\delta$H 0.85 (t, 3 H, $J = 7.1$ Hz), 1.41-1.15 (m, 14 H), 2.08-1.81 (m, 4 H), 2.68-2.60 (m, 2 H), 3.83-3.73 (m, 2 H), 4.27-4.24 (m, 1 H), 4.39-4.31 (m, 2 H), 4.74-4.52 (m, 6 H), 4.95-4.88 (m, 1 H), 7.50-7.20 (m, 15 H), 11.22 (broad s, 1 H); $^{13}$C NMR (125 MHz, C$_5$D$_5$N) $\delta$C 14.3, 22.9, 29.6, 29.8, 29.9, 30.5 (d, $J = 88.5$ Hz), 31.4 (d, $J = 14.6$ Hz), 31.6 (d, $J = 92.7$ Hz), 32.1, 71.6 (d, $J = 1.6$ Hz), 71.9, 73.4, 77.6, 83.4, 84.2 (d, $J = 6.0$ Hz), 84.7, 127.9, 128.0, 128.1, 128.1, 128.3, 128.7, 128.8, 138.8, 139.0, 139.1; $^{31}$P NMR (161.9 MHz, C$_5$D$_5$N) $\delta$P 49.0; HRMS calcd for C$_{37}$H$_{51}$O$_6$PNa$: 645.3315. Found: 645.3344.
1-(Dodecyl)-3,4,6-tri-O-benzyl-2,5-anhydroglucityl phosphinic acid (3.18c)

Phosphazene base 3.15 (490 µL of a 1M solution in hexane, 0.49 mmol) was added to phosphine 3.14 (208 mg, 0.46 mmol), followed by the addition of 1-iododecane (125 µL, 0.51 mmol) as described for the preparation of 3.17a. Purification by chromatography (9:1, hexane:EtOAc, R_f = 0.30) afforded 3.17c (143 mg, 50%) as a colorless oil.

Phosphine 3.17c (140 mg, 0.23 mmol) was oxidized with I_2 (143 mg, 0.57 mmol) in pyridine/H_2O (98:2, 6 mL) as described for the preparation of 3.18a. After removing the pyridine with ion-exchange resin, the product was purified by chromatography (CH_2Cl_2:CH_3OH, 12:1) to afford 3.18c (118 mg, 80%) as a clear oil: R_f 0.27 (6:1, CH_2Cl_2:CH_3OH); [α]_D^25 +11.4 (c 1.1, CHCl_3); ¹H NMR (500 MHz, C_5D_5N) δ_H 0.87 (t, 3 H, J = 7.1 Hz), 1.45-1.18 (m, 18 H), 2.10-1.82 (m, 4 H), 2.69-2.60 (m, 2 H), 3.85-3.74 (m, 2 H), 4.28-4.25 (m, 1 H), 4.41-4.32 (m, 2 H), 4.78-4.55 (m, 6 H), 4.97-4.89 (m, 1 H), 7.50-7.25 (m, 15 H), 8.05 (broad s, 1 H); ¹³C NMR (125 MHz, C_5D_5N) δ_C 14.3, 23.0, 29.6, 29.8, 29.9, 30.0, 30.2, 30.6 (d, J = 89.2 Hz), 31.4 (d, J = 14.4 Hz), 31.6 (d, J = 92.2 Hz), 32.2, 71.6, 71.9, 73.4, 77.6, 83.4, 84.2 (d, J = 5.4 Hz), 84.7, 127.9, 128.0, 128.1, 128.3, 128.7, 128.8, 128.8, 138.8, 139.0, 139.1; ³¹P NMR (161.9 MHz, C_5D_5N) δ_P 48.8; HRMS calcd for C_{39}H_{55}O_6PNa^+: 673.3628. Found: 673.3622.

1-(Hexadecyl)-3,4,6-tri-O-benzyl-2,5-anhydroglucityl phosphinic acid (3.18d)

Phosphazene base 3.15 (440 µL of a 1M solution in hexane, 0.44 mmol) was added to phosphine 3.14 (189 mg, 0.42 mmol), followed by the addition of 1-iododecane (155 mg, 0.44 mmol) as described for the preparation of 3.17a. Purification by chromatography (8:1, hexane:EtOAc, R_f = 0.33) afforded 3.17d (231 mg, 82%) as a clear oil.

Phosphine 3.17d (231 mg, 0.34 mmol) was oxidized with I_2 (216 mg, 0.86 mmol) in pyridine/H_2O (98:2, 6 mL) as described for the preparation of 3.18a. After removing the...
pyridine with ion-exchange resin, the product was purified by chromatography (CH$_2$Cl$_2$:CH$_3$OH, 12:1) to afford 3.18d (189 mg, 78%) as a clear oil: R$_f$ 0.41 (6:1, CH$_2$Cl$_2$:CH$_3$OH); [α]$^{25}_D$ +10.3 (c 1.0, CHCl$_3$); $^1$H NMR (500 MHz, C$_5$D$_5$N) $\delta_H$ 0.86 (t, 3 H, $J$ = 7.1 Hz), 1.42-1.18 (m, 26 H), 2.07-1.81 (m, 4 H), 2.68-2.60 (m, 2 H), 3.83-3.73 (m, 2 H), 4.26-4.25 (m, 1 H), 4.39-4.32 (m, 2 H), 4.74-4.53 (m, 6 H), 4.96-4.89 (m, 1 H), 7.47-7.26 (m, 15 H), 9.88 (broad s, 1 H); $^{13}$C NMR (100 MHz, C$_5$D$_5$N) $\delta_C$ 14.3, 23.0, 29.7, 29.7, 29.9, 30.0, 30.0, 30.0, 30.5 (d, $J$ = 88.5 Hz), 31.0, 31.4 (d, $J$ = 14.5 Hz), 31.6 (d, $J$ = 92.7 Hz), 32.2, 71.6, 71.9, 73.4, 77.6, 83.4, 84.2 (d, $J$ = 5.7 Hz), 84.7, 127.9, 128.0, 128.1, 128.3, 128.7, 128.8, 138.8, 139.0, 139.1; $^{31}$P NMR (161.9 MHz, C$_5$D$_5$N) $\delta_P$ 48.8; HRMS calcd for C$_{43}$H$_{63}$O$_6$PNa$: 729.4254. Found: 729.4297.

1-(Eicosanyl)-3,4,6-tri-O-benzyl-2,5-anhydroglucityl phosphinic acid (3.18e)

Phosphazene base 3.15 (180 µL of a 1M solution in hexane, 0.18 mmol) was added to phosphine 3.14 (79 mg, 0.18 mmol), followed by the addition of 1-p-toluenesulfonyloxydecadecane (130 mg, 0.29 mmol) as described for the preparation of 3.17a. Purification by chromatography (12:1, hexane:EtOAc, R$_f$ = 0.26) afforded 3.17e (77 mg, 60%) as a clear oil.

Phosphine 3.17e (77 mg, 0.11 mmol) was oxidized with I$_2$ (66 mg, 0.26 mmol) in pyridine/H$_2$O (98:2, 4 mL) as described for the preparation of 3.18a. After removing the pyridine with ion-exchange resin, the product was purified by chromatography (CH$_2$Cl$_2$:CH$_3$OH, 12:1) to afford 3.18e (35 mg, 43%) as a clear oil: R$_f$ 0.29 (6:1, CH$_2$Cl$_2$:CH$_3$OH); [α]$^{25}_D$ +9.7 (c 1.1, CHCl$_3$); $^1$H NMR (500 MHz, C$_5$D$_5$N) $\delta_H$ 0.87 (t, 3 H, $J$ = 7.1 Hz), 1.45-1.17 (m, 34 H), 2.10-1.81 (m, 4 H), 2.70-2.59 (m, 2 H), 3.85-3.74 (m, 2 H), 4.27 (broad s, 1 H), 4.39-4.32 (m, 2 H), 4.76-4.54 (m, 6 H), 4.92 (broad s, 1 H), 7.50-7.26 (m, 15 H), 8.10 (broad s, 1 H); $^{13}$C NMR (100 MHz, C$_5$D$_5$N) $\delta_C$ 14.3, 23.0, 29.6, 29.8, 30.0, 30.0, 111
30.1, 30.5 (d, $J = 87.0$ Hz), 30.9, 31.5 (d, $J = 12.5$ Hz), 31.6 (d, $J = 89.6$ Hz), 32.2, 71.6, 71.6, 71.9, 73.4, 77.7, 83.3, 84.2, 84.7, 127.9, 128.0, 128.1, 128.3, 128.7, 128.8, 138.8, 139.0, 139.4; $^{31}$P NMR (202.4 MHz, C$_5$D$_5$N) $\delta$P 47.4; HRMS calcd for C$_{47}$H$_{71}$O$_6$PNa$: 785.4880. Found: 785.4893.

1-(Dimethylphosphinyl)-1-deoxy-3,4,6-tri-O-(benzyl)-D-fructofuranose (3.32) n-Butyl lithium (3.9 mL of a 1.6 M solution in hexane, 6.3 mmol) was added to a solution of dimethyl methylphosphonate (0.68 mL, 6.3 mmol) in THF (10 mL) stirring at -78 °C. After 15 min, a solution of lactone 3.31 (1.3 g, 3.1 mmol) in THF (20 mL) was added dropwise and the reaction mixture stirred for 30 min, and then was warmed to room temperature over 1 h. The mixture was neutralized with acetic acid and diluted with EtOAc (100 mL). The organic layer was washed with H$_2$O (50 mL), brine (50 mL), and dried over Na$_2$SO$_4$. The solvent was evaporated and the product was purified by chromatography (EtOAc:hexane, 3:1) to afford 3.32 (1.6 g, 94%) as a white solid. The NMR data is the same as previously reported.$^{13}$

Dimethyl 3,4,6-Tri-O-benzyl-2,5-anhydromannityl phosphonate (3.33) Trimethylsilyl triflate (0.49 mL, 2.5 mmol) was added dropwise to a mixture of lactol 3.32 (1.36 g, 2.51 mmol), triethylsilane (2.8 mL, 17.5 mmol), and CH$_2$Cl$_2$ (5 mL) stirring at rt. After 1 h, the mixture was neutralized with triethylamine and the solution was concentrated. The crude oil was purified by chromatography (EtOAc:hexane, 2.5:1) to afford 3.33 (1.2 g, 84%) as a colorless oil. The NMR data is the same as previously reported.$^{11}$

3,4,6-Tri-O-benzyl-2,5-anhydromannityl phosphine (3.34) Phosphonate 3.33 (360 mg, 0.68 mmol) was dissolved in anhydrous ether (10 mL) and stirred at rt. Lithium aluminum hydride (39 mg, 1.0 mmol) was added and the reaction mixture stirred for 30 min. before EtOAc (1 mL) was added, followed by H$_2$O (0.2 mL). The mixture was then filtered
through Celite and the solvent was evaporated to give an oily residue. Purification by chromatography (hexane:EtOAc, 6:1) afforded 3.34 (176 mg, 57%) as a colorless oil which was used immediately in the next step.

**1-(Octyl)-3,4,6-tri-O-benzyl-2,5-anhydromannityl phosphinic acid (3.36)**

Phosphine 3.34 (176 mg, 0.39 mmol) was dissolved in Et₂O (5 mL) and stirred at rt. Phosphazene base 3.15 (430 µL of a 1M solution in hexane, 0.43 mmol) was added, followed by 1-iodooctane (78 µl, 0.43 mmol). After 30 min., the mixture was neutralized with AcOH and the salt precipitate was removed by filtration through a cotton plug, which was rinsed with anhydrous Et₂O. The solvent was evaporated and the residue purified by flash chromatography (hexane:EtOAc, 6:1, R_f = 0.55) to afford 3.35 (93 mg, 42%) as a colorless oil.

Phosphine 3.35 was dissolved in a 98:2 mixture of pyridine/H₂O (5 mL). Iodine (84 mg, 0.33 mmol) was added and the reaction mixture stirred for 3 days at rt. The mixture was diluted with CH₂Cl₂, washed with 5% NaHSO₃, and dried over Na₂SO₄. The solvent was then evaporated and the residual oil purified by chromatography (CH₂Cl₂:CH₃OH, 11:1) to afford 3.36 (66 mg, 67%) as a clear oil: R_f 0.36 (6:1, CH₂Cl₂:CH₃OH); [α]$_D^{25}$ +10.2 (c 1.0, CHCl₃); $^1$H NMR (400 MHz, CDCl₃) δH 0.91 (t, 3 H, J = 7.1 Hz), 1.42-1.20 (m, 10 H), 1.85-1.54 (m, 4 H), 2.29-2.08 (m, 2 H), 3.56 (dd, 1 H, J = 9.8, 6.5 Hz), 3.62 (d, 1 H, J = 9.9, 5.9 Hz), 4.08-4.02 (m, 2 H), 4.29-4.23 (m, 1 H), 4.62-4.47 (m, 7 H), 7.36-7.27 (m, 15 H), 9.75 (broad s, 1 H); $^{13}$C NMR (125 MHz, CDCl₃) δC 14.1, 21.4 (d, J = 4.0 Hz), 22.6, 29.1, 29.9 (d, J = 91.9 Hz), 30.8 (d, J = 15.9 Hz), 31.8, 33.1 (d, J = 91.5 Hz), 70.3, 71.7 (d, J = 1.9 Hz), 73.3, 77.6, 82.2, 85.0, 87.6 (d, J = 10.0 Hz), 127.6, 127.7, 127.7, 127.7, 127.8, 128.3, 128.4, 128.4, 137.7, 137.8, 138.1; $^{31}$P NMR (202.4 MHz, CDCl₃) δP 59.3; HRMS calcd for C$_{35}$H$_{47}$O$_6$PNa$: 617.3002. Found: 617.3004.

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References


CHAPTER 4

SYNTHESIS OF C-SULFONE ANALOGS OF

DECAPRENOLPHOSPHOARABINOSE

4.1 Introduction

This chapter describes the synthesis of a series of C-sulfone DPA analogs (4.1, Figure 4.1) that are potential antimycobacterial agents. Sulfones are isoelectronic and hydrolytically stable phosphate mimics. However, unlike phosphonic and phosphinic acids, sulfones are non-ionic. As a result, they are more membrane permeable and are therefore more able to penetrate into cells.\(^1\) The cell wall of \textit{M. tuberculosis}, described in Chapter 1, is very hydrophobic and thus, our hope was that isosteric C-sulfone analogs would be good candidates for arabinosyltransferase inhibition.

![Figure 4.1: C-Sulfone DPA analogs.](image)

\(^1\) Reference needed.
4.2 Synthesis of C-sulfones with long alkyl chains

4.2.1 Retrosynthetic analysis of 4.2

Because we were successful in that one of our C-phosphonic acids with a long alkyl chain possessed antimycobacterial activity, we again opted to synthesize C-sulfones with long alkyl chains. The retrosynthesis of 4.2, where R is a long alkyl chain, is shown in Figure 4.2. We envisioned that 4.2 could be derived from iodide 4.3. Displacement of the iodide with potassium thioacetate would afford 4.4. Thioacetate 4.4 could be reduced to the thiol and subsequently alkylated to form sulfide 4.7. Oxidation of 4.7, followed by debenzylation, would afford the target compound 4.2.

R = long alkyl chain

Figure 4.2: Retrosynthesis of 4.2.

4.2.2 Synthesis of C-sulfones with alkyl chains containing 9-16 atoms

The synthesis of sulfides 4.7a-d is illustrated in Scheme 4.1. Iodide 4.3 was reacted with potassium thioacetate to afford 4.4 in 94% yield. Reduction of 4.4 was achieved with
lithium aluminum hydride to produce thiol 4.5. We also attempted this transformation with milder reaction conditions (e.g., NaBH₄/CH₃OH, NaOCH₃), however the yields were lower due to the formation of a disulfide (4.9, Figure 4.3) byproduct. Alkylation of 4.5 with sodium hydride and various commercially available iodides (4.6a-d) afforded sulfides 4.7a-d in 65-82% yield. Attempts to react long-chain alkyl thiols (RSH) with iodide 4.3 resulted in lower yields of the product.

Scheme 4.1: Synthesis of sulfides 4.7a-d.

Figure 4.3: Disulfide byproduct 4.9.
The synthesis of compounds \textbf{4.2a-d} is shown in Scheme 4.2. Sulfides \textbf{4.7a-d} were oxidized with \textit{m}-chloroperoxybenzoic acid and used in the subsequent deprotection step without purification. Hydrogenolysis of the benzylated sulfones with 10% palladium on activated carbon afforded targets \textbf{4.2a-d} in 49-85% yield over the two steps. The hydrogenation reaction with the sulfone derived from \textbf{4.7d} failed to go to completion over the course of two days, resulting in the low yield of \textbf{4.2d} (49%). The yield was improved after recovering the unreacted material and repeating the hydrogenation.

Scheme 4.2: Synthesis of targets \textbf{4.2a-d}.

\textbf{4.2.3 Biological screening of C-sulfones containing 9-16 carbon alkyl chains}

\textit{C}-Sulfones \textbf{4.2a-d} were tested against \textit{M. tuberculosis}, and all four compounds were found to be inactive. We were not too surprised or discouraged by these results. The \textit{C}-sulfones were tested at the same time as our \textit{C}-phosphonic acid series (Chapter 2). The only \textit{C}-phosphonic acid that was biologically active had a chain that was 22 atoms in length and the compounds with shorter alkyl groups were completely inactive. Our \textit{C}-sulfone with the
longest chain, **4.2d**, had only 16 atoms. Therefore, we cannot rule out that compounds **4.2a-d** were inactive due to the presence of the sulfone functionality. Our next endeavor was to synthesize compounds with longer alkyl chains.

### 4.2.4 Synthesis of long chain alcohols

The longest alkyl iodide that is commercially available is 1-iodohexadecane. Because longer alkyl halides were unavailable, we instead decided to search for reasonably priced long chain alcohols, which we could convert to compounds with better leaving groups. Our two choices were 1-eicosanol (C\(_{20}\)H\(_{42}\)O) and 1-triacontanol (C\(_{30}\)H\(_{62}\)O). One problem we did not anticipate is the low solubility of compounds with significantly long alkyl chains. 1-Eicosanol was soluble in THF only at room temperature and above. 1-Triacontanol was completely insoluble in many common organic solvents (*e.g.*, hexanes, THF, pyridine, glacial acetic acid, CHCl\(_3\)) and mixtures of these and other solvents. We later discovered that although this compound has not been explored in synthetic chemistry, it is well known in the agricultural community. 1-Triacontanol is an effective plant growth stimulator that originates from the epicuticular waxes of alfalfa. It is administered to plants in concentrations typically in the range of 2-20 µg/L of a solvent mixture containing CHCl\(_3\), water, and Tween 20 (polyethylene sorbitan monolaurate).²

Because decaprenolphosphoarabinose has a chain 40 atoms in length (50 carbons total), we wanted to synthesize a chain significantly longer than 20 atoms. Instead of pursuing alcohols like 1-triacontanol, we opted to synthesize a long chain alcohol containing an ether linkage, which would increase its solubility in organic solvents. The C-phosphonic
acid that was biologically active against *M. tuberculosis* also contained an ether linkage, and thus we thought this modification would not have deleterious effects on biological activity.

The synthesis of the long chain alcohol was more difficult than we expected. After exploring several routes, we finally found a successful route to an alcohol that was 27 atoms in length. The synthesis of this alcohol, 4.13, is described in Scheme 4.3.


Commercially available 1,10-decanediol, 4.10, was reacted with dihydropyran and a catalytic amount of *p*-toluenesulfonic acid at room temperature. The monoprotected alcohol, 4.11, was obtained in 53% yield along with diprotected compound and unreacted 4.10. Alcohol 4.11 was then alkylated with 1-iodohexadecane to afford 4.12 in low yield. We tried to improve the yield by increasing the reaction temperature, however this resulted in E2 elimination of the iodide to form 1-hexadecene. Deprotection of the tetrahydropyranyl ether was achieved by heating 4.12 in a mixture of AcOH/THF/H$_2$O at 65 °C for 3 h. Alcohol 4.13 was formed in 84% yield as a white solid.
4.2.5 Synthesis of C-sulfones with alkyl chains containing 20 and 27 atoms

The synthesis of sulfone 4.16 is illustrated in Scheme 4.4. Thiol 4.5 was treated with $n$-butyl lithium at 0 °C. 1-$p$-Toluenesulfonyloxydecadecane, 4.14 (prepared from 1-eicosanol and $p$-toluenesulfonyl chloride), was added and the reaction proceeded at room temperature to afford the thioether, which was impossible to separate from 4.14. Oxidation of the crude mixture with $m$-chloroperoxybenzoic acid afforded sulfone 4.15 in 30% yield from 4.5. Hydrogenolysis of 4.15 with 10% palladium on activated carbon afforded target 4.16 in 80% yield.

Scheme 4.4: Synthesis of C-sulfone 4.16.

The synthesis of 4.19 is shown in Scheme 4.5. Thiol 4.5 was treated with potassium hydride and 18-crown-6 at room temperature. 10-$O$-hexadecyl-$p$-toluenesulfonyloxydecane, 4.17 (prepared as described for 4.14), was added and the reaction proceeded at room temperature. This reaction provided the thioether, which could not be separated from unreacted 4.17. We also attempted other reaction conditions (e.g., $n$-
BuLi/THF, NaH/DMF), but the major product was disulfide 4.9. Oxidation of the crude mixture with \( m \)-chloroperoxybenzoic acid afforded sulfone 4.18 in 30% yield from 4.5. Hydrogenolysis of 4.18 with 10% palladium on activated carbon afforded target 4.19 in 60% yield.

![Scheme 4.5: Synthesis of C-sulfone 4.19.](image)

Characterization of sulfones 4.16 and 4.19 was challenging. As the length of the alkyl chains increased, the solubility of the sulfones decreased. For example, the sulfones with chains that were 9, 10, and 12 carbons in length all dissolved in chloroform. The sulfone with a chain of 16 carbons was insoluble in chloroform, but was soluble if warmed in methanol. Sulfone 4.16, with a 20-carbon chain, was soluble in warm methanol, but quickly crystallized out of the NMR tube (<1 h). In order to obtain our spectra, the NMR tube containing the sample had to be quickly transferred from a warm water bath to the instrument. Sulfone 4.19, with an ether linkage, was insoluble in methanol and partially soluble in chloroform at room temperature. The NMR spectra of 4.19 were recorded in
CDCl₃ immediately after transferring the solution to an NMR tube. When left in an NMR tube overnight, this compound formed a solid that was more gel-like than crystalline.

4.3 Synthesis of C-sulfones with unsaturated alkyl chains

In addition to synthesizing C-sulfones with long saturated chains, we also wanted to synthesize sulfones with polyunsaturated chains similar to the polyprenylated chain of decaprenol. We needed to modify our synthetic route by using a protecting group that would be compatible with olefins in the deprotection step. Our initial target, 4.20, has an unsaturated chain derived from the monoterpene, geraniol (Figure 4.4).

![Geranyl C-sulfone](image)

Figure 4.4: Geranyl C-sulfone.

4.3.1 Retrosynthetic analysis of 4.20

The retrosynthetic analysis of 4.20 is shown in Figure 4.5. This route is almost identical to our previous route to C-sulfones 4.2a-d. The main difference is that we changed the protecting groups from benzylic groups to p-methoxybenzyl groups. In order to switch protecting groups, we had to add a few additional steps to synthesize iodide 4.25. Lactol 4.23 could be prepared in 3 steps from D-arabinose. Wittig olefination of 4.23 and iodocyclization of 4.24 would generate iodide 4.25. In addition to changing our protecting group, we had to
explore alternatives to m-CPBA for the oxidation of sulfide 4.29. The terminal olefin of 4.29 is electron rich and susceptible to oxidation by this reagent.

Figure 4.5: Retrosynthetic analysis of 4.20.
4.3.2 Synthesis of C-sulfone with geranyl chain

The synthesis of iodide 4.25 is illustrated in Scheme 4.6. D-Arabinose reacted with allyl alcohol in the presence of acetyl chloride to form an inseparable mixture containing both allyl furanosides and allyl pyranosides. The allyl glycoside mixture reacted with p-methoxybenzyl chloride in DMF to afford allyl furanoside 4.22 in 50% yield over both steps. Compound 4.22 was heated at 80 °C with Wilkinson’s catalyst (7 mol%), and then reacted with mercury (II) chloride to afford lactol 4.23 in 97% yield. Wittig olefination of 4.23 with methylenetriphenylphosphorane afforded 4.24 in 72% yield. Iodocyclization of 4.24 afforded 4.25 in 99% yield.

![Scheme 4.6: Synthesis of 4.25.](image)

The synthesis of 4.20 from iodide 4.25 is illustrated in Scheme 4.7. Iodide 4.25 was reacted with potassium thioacetate to afford 4.26 in 92% yield. Reduction of 4.26 with lithium aluminum hydride at room temperature afforded thiol 4.27 in 92% yield. Alkylation
of 4.27 was achieved by addition of n-butyl lithium at 0 °C, followed by geranyl chloride 4.28\(^3\) (prepared from geraniol) to afford 4.29 in 57% yield. The yield of this step was low due to formation of a disulfide analogous to 4.9. Many oxidizing agents were explored for the conversion of 4.29 to 4.30 (e.g., 99% m-CPBA, KHSO\(_5\), (\(t\)-BuO\(_2\))\(_2\),\(^4\) Davis’s oxaziridine). However, very little sulfone was formed, and the sulfoxide was usually the major product. The only reagent we found to be somewhat successful was sodium periodate.\(^5\) This was a very tedious reaction requiring careful monitoring over the course of two days. The sulfide was converted to the sulfoxide in a matter of hours, but complete conversion to the sulfone was never achieved. Complicating matters further was the formation of aldehyde 4.31, presumably from oxidation of the terminal olefin to the epoxide, followed by oxidative cleavage. Aldehyde 4.31 was confirmed by mass spectroscopy and also from NMR data. The \(^1\)H-NMR spectrum showed an O=C-H peak at 9.65 ppm, in addition to the loss of one vinylic C-H peak and two CH\(_3\) peaks. The yield of this reaction was low (43%), but could be improved by recycling the sulfoxide. Deprotection of 4.30 with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) afforded 4.20 as an inseparable mixture of compounds. The product was confirmed by mass spectroscopy, but the \(^1\)H-NMR spectrum showed that there were four olefinic C-H peaks (instead of two), and multiple singlets (at least six) were observed that corresponded to hydrogens from CH\(_3\) groups in the geranyl chain.
After some consideration, we abandoned our methodology for synthesizing polyprenylated C-sulfones. The yield of the oxidation step was unsatisfactory, and 4.30 was sensitive to the conditions of the deprotection step. Our ultimate goal was to synthesize sulfones with longer terpene chains than geraniol, and this methodology was not amenable to their synthesis.

The final C-sulfone compound synthesized was 4.35 (Scheme 4.8). Decaprenol has branching methyl groups, and we made this compound in an effort to determine if branching would have any effect on the biological activity of the C-sulfone. Thiol 4.5 was alkylated
with 4.32, generated \textit{in situ}, to afford 4.33 in 76% yield. Oxidation of 4.33 provided 4.34 in 53% yield. Deprotection of sulfone 4.34 afforded 4.35 in 64% yield.

Scheme 4.8: Synthesis of 4.35.

C-Sulfones 4.16, 4.19, and 4.35 are currently being screened against \textit{M. tuberculosis}. It will be interesting to see if the sulfones with longer chains (4.16 and 4.19) show similar activity to our \textit{C}-phosphonic acid inhibitor (Chapter 2). If neither shows inhibition, it would suggest that the charge of the phosphate monoanion is necessary for enzyme recognition.

4.4 Experimental

\textbf{General.} Solvents were distilled from the appropriate drying agents before use. Unless stated otherwise, all reactions were carried out at room temperature under a positive pressure of argon and were monitored by TLC on silica gel 60 F$_{254}$. Spots were detected under UV light or by charring with 10\% H$_2$SO$_4$ in ethanol. Solvents were evaporated under reduced pressure and below 40 °C (bath). Organic solutions of crude products were dried
over anhydrous Na$_2$SO$_4$. Unless otherwise indicated, column chromatography was performed on silica gel 60 (40–60 µM). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). Optical rotations were measured at 21±2 °C. $^1$H NMR spectra were recorded at 250, 400, or 500 MHz, and chemical shifts are referenced to either TMS (0.0, CDCl$_3$) or CD$_3$OH (4.78, CD$_3$OD). $^{13}$C NMR spectra were recorded at 62.5, 100, or 125 MHz and chemical shifts are referenced to CDCl$_3$ (77.00, CDCl$_3$) or CH$_3$OH (49.00, CD$_3$OD). Electrospray mass spectra were recorded on samples suspended in mixtures of THF and CH$_3$OH with added trifluoroacetic acid or NaCl.

1-(Nonyl)-2,5-anhydroglucityl sulfone (4.2a) Thioether 4.6a (91 mg, 0.16 mmol) was dissolved in CH$_2$Cl$_2$ (5 mL) and stirred at rt. 3-Chloroperoxybenzoic acid ($m$-CPBA, 117 mg, 0.47 mmol) was added and the mixture stirred for 20 min. The reaction mixture was neutralized with a saturated solution of sodium bicarbonate and diluted with CH$_2$Cl$_2$ (20 mL). The organic layer was washed with H$_2$O (2 x 10 mL) and then dried over Na$_2$SO$_4$. The solvent was evaporated to afford 4.8a as a crude white solid (200 mg) which was used in the next step without any further purification.

Sulfone 4.8a was dissolved in CH$_3$OH (5 mL) containing AcOH (10 µL). Palladium on carbon (10%, 25 mg) was added and the reaction mixture was stirred under hydrogen overnight at atmospheric pressure. After filtering the solid and evaporating the solvent, the residue was purified by chromatography (EtOAc) to afford 4.2a (42 mg, 79% over 2 steps) as a white solid: R$_f$ 0.21 (EtOAc); [α]$^2$D +6.7 (c 1.0, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) δ$_H$ 0.86 (t, 3 H, $J$ = 6.9 Hz), 1.33-1.19 (m, 10 H), 1.45-1.35 (m, 2 H), 1.83-1.75 (m, 2 H), 3.16-3.03 (m, 2 H), 3.32 (dd, 1 H, $J$ = 14.6, 3.6 Hz), 3.41 (dd, 1 H, $J$ = 14.8, 8.2 Hz), 3.78-3.68 (m, 2 H), 3.91-3.87 (m, 1 H), 4.06-4.01 (m, 1 H), 4.17-4.10 (m, 2 H), 4.36-4.32 (m, 1 H), 4.51-4.45 (m, 1 H), 4.74-4.69 (m, 1 H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ$_C$ 14.1, 21.6, 22.6, 28.5,
1-(Decyl)-2,5-anhydroglucityl sulfone (4.2b) Thioether 4.6b (133 mg, 0.23 mmol) was dissolved in CH$_2$Cl$_2$ (5 mL) and stirred at rt. 3-Chloroperoxybenzoic acid (172 mg, 0.69 mmol) was added and the mixture stirred for 20 min. The reaction mixture was neutralized with a saturated solution of sodium bicarbonate and diluted with CH$_2$Cl$_2$ (30 mL). The organic layer was washed with H$_2$O (2 X 20 mL) and then dried over Na$_2$SO$_4$. The solvent was evaporated to afford 4.8b as a crude white solid (251 mg) which was used in the next step without any further purification.

Sulfone 4.8b was dissolved in CH$_3$OH (5 mL) containing AcOH (10 µL). Palladium on carbon (10%, 40 mg) was added and the reaction mixture was stirred under hydrogen overnight at atmospheric pressure. After filtering the solid and evaporating the solvent, the residue was purified by chromatography (EtOAc) to afford 4.2b (92 mg, 85% over 2 steps) as a white solid: R$_f$ 0.25 (EtOAc); [α]$_{25}^D$ +8.7 (c 1.0, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 0.85 (t, 3 H, $J$ = 6.8 Hz), 1.34-1.18 (m, 12 H), 1.43-1.35 (m, 2 H), 1.83-1.73 (m, 2 H), 3.15-3.03 (m, 2 H), 3.32 (dd, 1 H, $J$ = 14.8, 4.6 Hz), 3.41 (dd, 1 H, $J$ = 14.8, 8.4 Hz), 3.79-3.68 (m, 2 H), 3.92-3.87 (m, 1 H), 4.07-3.99 (m, 2 H), 4.17-4.13 (m, 1 H), 4.27-4.22 (m, 1 H), 4.51-4.45 (m, 1 H), 4.71-4.67 (m, 1 H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 14.1, 21.7, 22.7, 28.5, 29.2, 29.3, 29.4, 29.5, 31.9, 52.8, 54.2, 62.1, 75.4, 78.0, 78.3, 86.1; HRMS calcd for C$_{16}$H$_{32}$O$_6$SNa$^+$: 375.1812. Found: 375.1804.

1-(Dodecyl)-2,5-anhydroglucityl sulfone (4.2c) Thioether 4.6c (140 mg, 0.23 mmol) was dissolved in CH$_2$Cl$_2$ (5 mL) and stirred at rt. 3-Chloroperoxybenzoic acid (167 mg, 0.68 mmol) was added and the mixture stirred for 20 min. The reaction mixture was neutralized with a saturated solution of sodium bicarbonate and diluted with CH$_2$Cl$_2$ (30 mL).
The organic layer was washed with H$_2$O (2 X 20 mL) and then dried over Na$_2$SO$_4$. The solvent was evaporated to afford 4.8c as a crude white solid (181 mg) which was used in the next step without any further purification.

Sulfone 4.8c was dissolved in CH$_3$OH (5 mL) containing AcOH (10 µL). Palladium on carbon (10%, 40 mg) was added and the reaction mixture was stirred under hydrogen overnight at atmospheric pressure. After filtering the solid and evaporating the solvent, the residue was purified by chromatography (EtOAc) to afford 4.2c (65 mg, 76% over 2 steps) as a white solid: R$_f$ 0.26 (EtOAc); $[\alpha]^{25}_D$ +10.3 (c 0.8, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$H 0.86 (t, 3 H, $J = 6.8$ Hz), 1.34-1.18 (m, 16 H), 1.46-1.35 (m, 2 H), 1.90-1.77 (m, 2 H), 2.68 (broad s, 3 H), 3.15-3.03 (m, 2 H), 3.30 (dd, 1 H, $J = 14.8$, 3.8 Hz), 3.41 (dd, 1 H, $J = 14.8$, 8.4 Hz), 3.87-3.75 (m, 2 H), 3.98-3.95 (m, 1 H), 4.04-4.00 (m, 1 H), 4.24 (s, 1 H), 4.57-4.52 (m, 1 H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 14.1, 21.7, 22.7, 28.5, 29.2, 29.4, 29.4, 29.6, 29.7, 29.7, 31.9, 52.8, 54.2, 62.2, 75.5, 78.1, 78.3, 86.1; HRMS calcd for C$_{18}$H$_{36}$O$_6$SNa$: 403.2125$. Found: 403.2152.

1-(Hexadecyl)-2,5-anhydroglucityl sulfone (4.2d) Thioether 4.6d (140 mg, 0.21 mmol) was dissolved in CH$_2$Cl$_2$ (5 mL) and stirred at rt. 3-Chloroperoxybenzoic acid (153 mg, 0.62 mmol) was added and the mixture stirred for 20 min. The reaction mixture was neutralized with a saturated solution of sodium bicarbonate and diluted with CH$_2$Cl$_2$ (30 mL). The organic layer was washed with H$_2$O (2 X 20 mL) and then dried over Na$_2$SO$_4$. The solvent was evaporated to afford the sulfone as a crude white solid (210 mg) which was used in the next step without any further purification.

The crude sulfone was dissolved in CH$_3$OH (5 mL) containing AcOH (10 µL). Palladium on carbon (10%, 40 mg) was added and the reaction mixture was stirred under hydrogen overnight at atmospheric pressure. After filtering the solid and evaporating the
solvent, the residue was purified by chromatography (EtOAc:hexane, 5:1) to afford 4.2d (37 mg, 49% over 2 steps) as a white solid: Rf, 0.21 (5:1, EtOAc:hexane); [α]$_D^{25}$ +3.6 (c 1.2, CH$_3$OH); $^1$H NMR (400 MHz, CD$_3$OD) δ$_H$ 0.82 (t, 3 H, $J = 6.9$ Hz), 1.31-1.17 (m, 24 H), 1.41-1.33 (m, 2 H), 1.78-1.68 (m, 2 H), 3.25-3.02 (m, 3 H), 3.35 (dd, 1 H, $J = 15.2$, 9.2 Hz), 3.65-3.55 (m, 2 H), 3.79-3.75 (m, 1 H), 3.89-3.87 (m, 1 H), 3.94-3.90 (m, 1 H), 4.39-4.34 (m, 1 H); $^{13}$C NMR (100 MHz, CD$_3$OD) δ$_C$ 14.4, 22.8, 23.7, 29.5, 30.2, 30.5, 30.7, 30.8, 30.8, 33.1, 54.4, 55.0, 63.4, 77.2, 79.4, 79.5, 88.1; HRMS calcd for C$_{22}$H$_{44}$O$_6$SNa$: 459.2751. Found: 459.2764.

3,4,6-Tri-O-benzyl-2,5-anhydroglucityl thioacetate (4.4) Iodide 4.3 (4.70 g, 8.63 mmol) was dissolved in dry DMF (30 mL) and stirred at rt. Potassium thioacetate (1.48 g, 12.9 mmol) was added and the reaction mixture stirred for 12 h. The mixture was diluted with ether (250 mL), washed with H$_2$O (3 X 100 mL), and dried over MgSO$_4$. Purification by chromatography (hexane:EtOAc, 5:1) afforded 4.4 (3.98 g, 94%) as a light yellow oil: Rf, 0.23 (hexane:EtOAc, 6:1); [α]$_D^{25}$ +25.1 (c 1.1, CHCl$_3$); $^1$H NMR (250 MHz, CDCl$_3$) δ$_H$ 2.31 (s, 3 H), 3.20 (d, 2 H, $J = 6.9$ Hz), 3.50 (dd, 1 H, $J = 9.9$, 6.8 Hz), 3.60 (dd, 1 H, $J = 9.9$, 5.6 Hz), 3.93-3.89 (m, 2 H), 3.98-3.95 (m, 2 H), 4.60-4.36 (m, 6 H), 7.38-7.22 (m, 15 H); $^{13}$C NMR (62.5 MHz, CDCl$_3$) δ$_C$ 27.9, 30.4, 70.2, 71.4, 71.5, 73.2, 79.9, 82.9, 82.9, 83.2, 127.6, 127.6, 127.7, 127.8, 127.8, 128.7, 128.8, 128.4, 137.5, 137.6, 138.1, 195.4; HRMS calcd for C$_{53}$H$_{82}$O$_7$SNa$: 515.1863. Found: 515.1867.

3,4,6-Tri-O-benzyl-2,5-anhydroglucityl thiol (4.5) Thioacetate 4.4 (170 mg, 0.35 mmol) was dissolved in anhydrous ether (7 mL) and stirred at rt. Lithium aluminum hydride (13 mg, 0.35 mmol) was added and the reaction mixture stirred for 1 h. The reaction mixture was diluted with ethyl acetate (30 mL), and the organic layer was washed with H$_2$O (10 mL), brine (10 mL), and dried over Na$_2$SO$_4$. Purification by chromatography (hexane:EtOAc, 6:1)
afforded 4.5 (151 mg, 97%) as a light colorless oil: R\textsubscript{f} 0.44 (hexane:EtOAc, 4:1); [\alpha]^{25}\textsubscript{D} +44.3 (c 1.1, CHCl\textsubscript{3}); \textsuperscript{1}H NMR (250 MHz, CDCl\textsubscript{3}) \textsuperscript{\delta}H 1.39 (t, 1 H, J = 8.8 Hz), 2.76 (d, 1 H, J = 7.2 Hz), 2.80 (d, 1 H, J = 7.2 Hz), 3.49 (dd, 1 H, J = 9.8, 6.7 Hz), 3.59 (dd, 1 H, J = 9.9, 5.9 Hz), 3.96-3.93 (m, 1 H), 4.01-3.98 (m, 1 H), 4.18-4.05 (m, 2 H), 4.39 (d, 1 H, J = 11.8 Hz), 4.60-4.47 (m, 5 H), 7.38-7.21 (m, 15 H); \textsuperscript{13}C NMR (62.5 MHz, CDCl\textsubscript{3}) \textsuperscript{\delta}C 22.7, 70.4, 71.4, 73.3, 83.1, 83.1, 83.2, 83.2, 127.6, 127.7, 127.8, 127.8, 127.9, 128.3, 128.5, 137.6, 137.7, 138.1.

1-(Nonyl)-3,4,6-tri-O-benzyl-2,5-anhydroglucityl sulfide (4.7a) Sodium hydride (19 mg, 0.80 mmol) was added to a solution of thiol 4.5 (120 mg, 0.27 mmol) in dry DMF (5 mL) stirring at rt. After 5 min, 1-iodononane (0.16 mL, 0.80 mmol) was added and the reaction mixture was stirred for 1 h. Methanol (1 mL) was added to this mixture, and after a few minutes ether (20 mL) was added. The organic layer was washed with H\textsubscript{2}O (2 X 40 mL), brine (15 mL), and then dried over Na\textsubscript{2}SO\textsubscript{4}. Purification by chromatography (hexane:EtOAc, 10:1) afforded 4.7a (111 mg, 72%) as a clear oil: R\textsubscript{f} 0.28 (hexane:EtOAc, 10:1); [\alpha]^{25}\textsubscript{D} +41.0 (c 1.0, CHCl\textsubscript{3}); \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \textsuperscript{\delta}H 0.88 (t, 3 H, J = 6.9 Hz), 1.39-1.22 (m, 12 H), 1.61-1.52 (m, 2 H), 2.53 (t, 2 H, J = 7.4 Hz), 2.91-2.73 (m, 2 H), 3.50 (dd, 1 H, J = 9.9, 6.8 Hz), 3.61 (dd, 1 H, J = 9.8, 5.8 Hz), 3.92-3.90 (m, 1 H), 3.98-3.96 (m, 1 H), 4.30-4.02 (m, 2 H), 4.59-4.40 (m, 6 H), 7.37-7.22 (m, 15 H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \textsuperscript{\delta}C 14.1, 22.6, 28.8, 29.2, 29.2, 29.5, 29.8, 30.1, 31.8, 32.9, 70.5, 71.4, 71.6, 73.3, 81.2, 82.5, 83.0, 83.6, 127.5, 127.6, 127.7, 127.7, 127.8, 127.8, 128.3, 128.3, 128.4, 137.8, 137.8, 138.2.

1-(Decyl)-3,4,6-tri-O-benzyl-2,5-anhydroglucityl sulfide (4.7b) n-Butyl lithium (0.1 mL, 0.16 mmol) was added to a solution of thiol 4.5 (66 mg, 0.15 mmol) in anhydrous ether (5 mL) stirring at 0 °C. After 5 min, 1-iododecane (38 µL, 0.18 mmol) was added and
the reaction mixture was stirred for 12 h. The mixture was then diluted with ether (20 mL), and the organic layer was washed with H₂O (10 mL), brine (10 mL), and then dried over MgSO₄. Purification by chromatography (hexane:EtOAc, 9:1) afforded 4.7b (56 mg, 65%) as a clear oil: Rₚ 0.35 (hexane:EtOAc, 10:1); [α]²⁵Dal +31.3 (c 1.0, CHCl₃); ¹H NMR (250 MHz, CDCl₃) δH 0.88 (t, 3 H, J = 6.6 Hz), 1.40-1.19 (m, 14 H), 1.62-1.50 (m, 2 H), 2.53 (t, 2 H, J = 7.4 Hz), 2.92-2.72 (m, 2 H), 3.49 (dd, 1 H, J = 10.0, 6.8 Hz), 3.61 (dd, 1 H, J = 9.8, 6.0 Hz), 3.94-3.90 (m, 1 H), 3.99-3.95 (m, 1 H), 4.29-4.00 (m, 2 H), 4.61-4.40 (m, 6 H), 7.39-7.18 (m, 15 H); ¹³C NMR (62.5 MHz, CDCl₃) δC 14.1, 22.6, 28.9, 29.2, 29.3, 29.5, 29.6, 30.1, 31.9, 32.9, 70.6, 71.4, 71.7, 73.3, 81.3, 82.6, 83.0, 83.6, 127.6, 127.6, 127.7, 127.7, 127.8, 128.3, 128.4, 137.8, 137.8, 138.2.

1-(Dodecyl)-3,4,6-tri-O-benzyl-2,5-anhydroglucityl sulfide (4.7c) Sodium hydride (40 mg, 1.00 mmol) was added to a solution of thiol 4.5 (150 mg, 0.33 mmol) in dry DMF (5 mL) stirring at rt. After 5 min, 1-iodododecane (0.25 mL, 1.00 mmol) was added and the reaction mixture was stirred for 1 h. Methanol (1 mL) was added to this mixture, and after a few minutes ether (20 mL) was added. The organic layer was washed with H₂O (2 X 40 mL), brine (15 mL), and then dried over Na₂SO₄. Purification by chromatography (hexane:EtOAc, 10:1) afforded 4.7c (168 mg, 82%) as a clear oil: Rₚ 0.45 (hexane:EtOAc, 9:1); [α]²⁵Dal +28.9 (c 1.0, CHCl₃); ¹H NMR (250 MHz, CDCl₃) δH 0.88 (t, 3 H, J = 6.6 Hz), 1.41-1.18 (m, 18 H), 1.62-1.50 (m, 2 H), 2.53 (t, 2 H, J = 7.4 Hz), 2.92-2.72 (m, 2 H), 3.49 (dd, 1 H, J = 9.9, 6.8 Hz), 3.61 (dd, 1 H, J = 9.9, 5.9 Hz), 3.94-3.90 (m, 1 H), 4.00-3.96 (m, 1 H), 4.30-4.01 (m, 2 H), 4.61-4.40 (m, 6 H), 7.39-7.20 (m, 15 H); ¹³C NMR (62.5 MHz, CDCl₃) δC 14.1, 22.7, 28.9, 29.2, 29.3, 29.5, 29.6, 29.7, 30.0, 70.5, 71.4, 71.6, 73.3, 81.2, 82.4, 83.0, 83.5, 127.6, 127.7, 127.8, 127.9, 128.3, 128.4, 137.7, 137.8, 138.1.
1-(Hexadecyl)-3,4,6-tri-O-benzyl-2,5-anhydroglucityl sulfide (4.7d)

Sodium hydride (40 mg, 1.00 mmol) was added to a solution of thiol 4.5 (150 mg, 0.33 mmol) in dry DMF (5 mL) stirring at rt. After 5 min, 1-iodohexadecane (350 mg, 1.00 mmol) was added and the reaction mixture stirred for 12 h. Methanol (1 mL) was added to this mixture, and after a few minutes ether (20 mL) was added. The organic layer was washed with H$_2$O (2 X 40 mL), brine (15 mL), and then dried over Na$_2$SO$_4$. Purification by chromatography (hexane:EtOAc, 10:1) afforded 4.7d (167 mg, 74%) as a clear oil: $R_f$ 0.56 (hexane:EtOAc, 9:1); $[\alpha]_{D}^{25}$ +28.2 (c 1.1, CHCl$_3$); $^1$H NMR (250 MHz, CDCl$_3$) $\delta$H 0.88 (t, 3 H, $J$ = 6.6 Hz), 1.39-1.20 (m, 26 H), 1.62-1.50 (m, 2 H), 2.53 (t, 2 H, $J$ = 7.4 Hz), 2.93-2.72 (m, 2 H), 3.49 (dd, 1 H, $J$ = 9.9, 6.8 Hz), 3.61 (dd, 1 H, $J$ = 9.9, 5.9 Hz), 3.94-3.90 (m, 1 H), 4.00-3.96 (m, 1 H), 4.30-4.01 (m, 2 H), 4.61-4.40 (m, 6 H), 7.38-7.22 (m, 15 H); $^{13}$C NMR (62.5 MHz, CDCl$_3$) $\delta$C 14.1, 22.6, 28.8, 29.2, 29.3, 29.5, 29.6, 29.6, 29.7, 30.0, 31.9, 70.5, 71.5, 71.6, 73.2, 81.2, 82.4, 83.0, 83.5, 127.5, 127.6, 127.6, 127.7, 127.7, 127.7, 128.3, 128.3, 128.4, 137.7, 137.7, 138.0.

10-Tetrahydropyranlyoxy-1-decanol (4.11) 1,10-decanediol (2.0 g, 11.5 mmol) was dissolved in THF (50 mL) and was stirred at rt. Dihydropyran (1.15 mL, 12.6 mmol) was added, followed by $p$-toluenesulfonic acid (328 mg, 1.73 mmol), and the reaction mixture was stirred for 2 h. Pyridine (2 mL) was added to the mixture, and the solvent was then evaporated. Purification by chromatography (hexane:EtOAc, 4:1) afforded 4.11 (1.56 g, 53%) as a clear oil, which was separated from 1,10-ditetrahydropyranlyoxydecane and unreacted 1,10-decanediol: $R_f$ 0.20 (4:1, hexane:EtOAc); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$H 1.33-1.20 (m, 12 H), 1.58-1.43 (m, 8 H), 1.69-1.63 (m, 1 H), 1.82-1.74 (m, 2 H), 3.33 (dt, 1 H, $J$ = 9.6, 6.7 Hz), 3.47-3.42 (m, 1 H), 3.57 (t, 2 H, $J$ = 6.7 Hz), 3.67 (dt, 1 H, $J$ = 9.6, 6.9 Hz), 3.85-3.79 (m, 1 H), 4.53-4.51 (m, 1 H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$C 19.6, 25.4, 135
25.7, 26.1, 29.3, 29.4, 29.4, 29.5, 29.7, 30.7, 32.7, 62.2, 62.8, 67.6, 98.8; HRMS calcd for C_{15}H_{30}O_{3}Na^{+}: 281.2087. Found: 281.2087.

10-O-Hexadecyl-1-tetrahydropyranlyoxydecane (4.12) 10-tetrahydropyranlyoxy-1-decanol, 4.11 (517 mg, 2.00 mmol), was dissolved in dry DMF (10 mL) and stirred at rt. Sodium hydride (160 mg, 4.00 mmol) was added, and after 10 min, 1-iodohexadecane (1.06 g, 3.01 mmol) in DMF (5 mL) was added. The reaction mixture was stirred for 12 h and then methanol (5 mL) was added. Et_{2}O (50 mL) was added and the organic layer was washed with H_{2}O (2 X 30 mL), brine (30 mL), and dried over Na_{2}SO_{4}. The solvent was evaporated and the crude residue was purified by chromatography (hexane:EtOAc, 7:1) to afford 4.12 (204 mg, 21%) as a colorless oil: R_{f} 0.58 (6:1, hexane:EtOAc); ^{1}H NMR (500 MHz, CDCl_{3}) \delta_{H} 0.86 (t, 3 H, J = 7.1 Hz), 1.39-1.19 (m, 38 H), 1.60-1.45 (m, 10 H), 1.72-1.66 (m, 1 H), 1.85-1.76 (m, 1 H), 3.38-3.33 (m, 5 H), 3.50-3.45 (m, 1 H), 3.70 (dt, 1 H, J = 9.6, 6.9 Hz), 3.87-3.82 (m, 1 H), 4.56-4.54 (m, 1 H); ^{13}C NMR (125 MHz, CDCl_{3}) \delta_{C} 14.1, 19.7, 22.7, 25.5, 26.2, 26.2, 29.3, 29.5, 29.5, 29.5, 29.5, 29.6, 29.6, 29.7, 29.8, 29.8, 30.8, 31.9, 62.3, 67.7, 71.0, 71.0, 98.8; HRMS calcd for C_{31}H_{62}O_{3}Na^{+}: 505.4591. Found: 505.4584.

10-O-Hexadecyl-1-decanol (4.13) 10-O-hexadecyl-1-tetrahydropyranlyoxydecane, 4.12 (200 mg, 0.41 mmol), was dissolved in a 3:1:1 mixture of AcOH/THF/H_{2}O (10 mL) and heated at 65 °C for 3 h. The mixture was diluted with CH_{2}Cl_{2} (20 mL). The organic layer was washed with H_{2}O (2 X 10 mL), brine (10 mL), and dried over Na_{2}SO_{4}. The solvent was evaporated and the solid residue was purified by chromatography (hexane:EtOAc, 7:1) to afford 4.13 (139 mg, 84%) as a white solid: R_{f} 0.25 (6:1, hexane:EtOAc); ^{1}H NMR (250 MHz, CDCl_{3}) \delta_{H} 0.85 (t, 3 H, J = 6.9 Hz), 1.35-1.16 (m, 36 H), 3.40-3.30 (m, 8 H), 3.36 (t, 4 H, J = 6.7 Hz), 3.60 (t, 2 H, J = 6.6 Hz); ^{13}C NMR (62.5 MHz, CDCl_{3}) \delta_{C} 14.1, 22.7, 25.7,
26.2, 29.4, 29.4, 29.5, 29.5, 29.6, 29.7, 29.8, 31.9, 32.8, 63.0, 70.9, 71.0; HRMS calcd for C_{26}H_{54}O_{2}Na^+: 421.4016. Found: 421.4015.

1-(Decadecyl)-3,4,6-tri-O-benzyl-2,5-anhydroglucityl sulfone (**4.15**) Thiol 4.5 (291 mg, 0.65 mmol) was dissolved in THF (10 mL) and stirred at 0 °C. n-Butyl lithium (0.44 mL, 0.71 mmol) was added, followed by a solution of 1-p-toluenesulfonyloxydecadecane (400 mg, 0.88 mmol) in THF (15 mL). The reaction mixture was warmed to rt and stirred for 4 h and then neutralized with AcOH, and diluted with EtOAc (40 mL). The organic layer was washed with H₂O (15 mL), brine (15 mL), and dried over Na₂SO₄. The solvent was evaporated to afford an inseparable mixture of the product and alkylating agent (466 mg).

The crude sulfide (413 mg) was dissolved in CH₂Cl₂ (10 mL) and stirred at rt. m-CPBA (418 mg, 1.69 mmol) was added and the mixture stirred for 2 h. The reaction mixture was neutralized with a saturated solution of sodium bicarbonate (5 mL) and diluted with CH₂Cl₂ (50 mL). The organic layer was washed with H₂O (2 X 20 mL) and then dried over Na₂SO₄. The solvent was evaporated and the product was purified by chromatography (hexane:EtOAc, 4:1) to afford **4.15** (164 mg, 30% over 2 steps) as a white solid: Rₚ 0.32 (hexane:EtOAc, 4:1); [α]^{25}_D +5.2 (c 1.3, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δH 0.95 (t, 3 H, J = 6.8 Hz), 1.44-1.26 (m, 34 H), 1.92-1.82 (m, 2 H), 3.20-3.07 (m, 3 H), 3.67-3.48 (m, 3 H), 4.06-4.01 (m, 2 H), 4.21-4.18 (m, 1 H), 4.43-4.39 (m, 1 H), 4.65-4.52 (m, 6 H), 7.44-7.26 (m, 15 H); ¹³C NMR (100 MHz, CDCl₃) δC 14.1, 21.7, 22.6, 28.4, 29.0, 29.2, 29.3, 29.5, 29.6, 29.6, 29.6, 31.9, 53.2, 54.1, 70.1, 71.5, 71.6, 73.3, 75.5, 82.5, 83.1, 83.2, 127.6, 127.6, 127.7, 127.7, 127.8, 127.8, 127.9, 128.1, 128.3, 128.4, 128.5, 128.5, 137.1, 137.4, 137.9; HRMS calcd for C_{47}H_{70}O_{6}SNa^+: 785.4785. Found: 785.4781.
1-(Decadecyl)-2,5-anhydroglucityl sulfone (4.16) Sulfone 4.15 (120 mg, 0.16 mmol) was dissolved in methanol (5 mL) containing AcOH (10 µL). Palladium on carbon (10%, 50 mg) was added and the reaction mixture was stirred under hydrogen overnight at atmospheric pressure. After filtering the solid and evaporating the solvent, the residue was purified by chromatography (EtOAc:hexane, 5:1) to afford 4.16 (62 mg, 80%) as a white solid. The NMR was recorded in warm CD$_3$OD: R$_f$ 0.21 (5:1, EtOAc:hexane); $^1$H NMR (400 MHz, CD$_3$OD) δ$_H$ 0.82 (t, 3 H, $J = 6.8$ Hz), 1.30-1.10 (m, 32 H), 1.42-1.32 (m, 2 H), 1.79-1.68 (m, 2 H), 3.19-3.01 (m, 2 H), 3.35 (dd, 1 H, $J = 15.0$, 9.1 Hz), 3.65-3.51 (m, 2 H), 3.77-3.73 (m, 1 H), 3.84-3.78 (m, 1 H), 3.89-3.86 (m, 1 H), 4.39-4.34 (m, 1 H); $^{13}$C NMR (100 MHz, CD$_3$OD) δ$_C$ 14.5, 22.9, 23.7, 29.5, 30.2, 30.5, 30.7, 30.8, 33.1, 54.4, 55.0, 63.4, 77.2, 79.4, 79.6, 88.1; HRMS calcd for C$_{26}$H$_{52}$O$_6$SNa$: 515.3377.$ Found: 515.3348.

10-O-Hexadecyl-1-p-toluenesulfonyloxydecane (4.17) 10-O-hexadecyl-1-decanol, 4.13 (139 mg, 0.35 mmol), and 18-crown-6 (127 mg, 0.42 mmol) were added together and dissolved in THF (10 mL). Potassium hydride (17 mg, 0.42 mmol) was added to the mixture, and after 5 min, p-toluenesulfonyl chloride (200 mg, 1.05 mmol) was added. The reaction mixture was stirred for 12 h at rt and then methanol (1 mL) was added. The mixture was diluted with EtOAc (10 mL), and the organic layer was washed with H$_2$O (10 mL), brine (10 mL), and dried over Na$_2$SO$_4$. The solvent was evaporated and the solid residue was purified by chromatography (hexane:EtOAc, 9:1) to afford 4.17 (50 mg, 26%) as a white solid: R$_f$ 0.44 (6:1, hexane:EtOAc); $^1$H NMR (250 MHz, CDCl$_3$) δ$_H$ 0.85 (t, 3 H, $J = 6.8$ Hz), 1.38-1.14 (m, 38 H), 1.67-1.45 (m, 6 H), 2.42 (s, 3 H), 3.36 (t, 4 H, $J = 6.7$ Hz), 3.99 (t, 2 H, $J = 6.5$ Hz), 7.31 (d, 2 H, $J = 8.0$ Hz), 7.76 (d, 2 H, $J = 8.3$ Hz); $^{13}$C NMR (62.5 MHz, CDCl$_3$) δ$_C$ 14.1, 22.7, 25.7, 26.2, 26.2, 29.4, 29.4, 29.5, 29.5, 29.5, 29.6, 29.7, 29.8, 31.9, 32.8, 63.0, 70.6, 138
Thiol 4.5 (117 mg, 0.26 mmol) was dissolved in THF (5 mL) and stirred at rt. 18-crown-6 (95 mg, 0.31 mmol) was added, followed by potassium hydride (12 mg, 0.31 mmol). A solution of O-hexadecyl-1-p-toluenesulfonyloxydecane (59 mg, 0.11 mmol) in THF (15 mL) was added dropwise, and the reaction stirred for 45 min until the thiol could not be detected by TLC. The reaction mixture was neutralized with AcOH, diluted with EtOAc (20 mL), washed with H₂O (10 mL), brine (10 mL), and dried over Na₂SO₄. The solvent was evaporated to give the product (50 mg) that was inseparable from the alkylating agent.

The crude sulfone (46 mg) was dissolved in CH₂Cl₂ (3 mL) and stirred at rt. m-CPBA (41 mg, 0.17 mmol) was added and the reaction mixture stirred for 30 min. The reaction mixture was neutralized with a saturated solution of sodium bicarbonate (1 mL) and diluted with CH₂Cl₂ (20 mL). The organic layer was washed with H₂O (2 × 5 mL) and then dried over Na₂SO₄. The solvent was evaporated and the product was purified by chromatography (hexane:EtOAc, 6:1) to afford 4.18 (41 mg, 45% over 2 steps) as a white solid: Rf 0.24 (hexane:EtOAc, 5:1); [α]²⁵ +4.9 (c 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δH 0.81 (t, 3 H, J = 7.0 Hz), 1.37-1.10 (m, 38 H), 1.53-1.43 (m, 4 H), 1.80-1.67 (m, 2 H), 3.08-2.91 (m, 3 H), 3.31 (overlapping t, 4 H, J = 6.8 Hz), 3.53-3.35 (m, 3 H), 3.91-3.88 (m, 2 H), 4.08-4.03 (m, 1 H), 4.27 (d, 1 H, J = 11.7 Hz), 4.53-4.38 (m, 6 H), 7.30-7.11 (m, 15 H); ¹³C NMR (100 MHz, CDCl₃) δC 14.1, 21.7, 22.7, 26.2, 26.2, 28.4, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 29.8, 31.9, 53.3, 54.2, 70.1, 70.9, 71.0, 71.6, 73.3, 75.5, 82.5, 83.2, 83.2, 127.7, 127.7, 127.7, 127.8, 127.8, 127.9, 128.1, 128.4, 128.5, 128.5, 128.6, 137.1, 137.5, 137.9; HRMS calcd for C₅₃H₈₂O₇SNa⁺: 885.5673. Found: 885.5601.
1-(Decyl-10′-O-hexadecyl)-2,5-anhydroglucityl sulfone (4.19) Sulfone 4.18 (40 mg, 0.048 mmol) was dissolved in a 5:1 mixture of methanol/AcOH (2 mL). Palladium on carbon (10%, 20 mg) was added and the reaction mixture was stirred under hydrogen overnight at atmospheric pressure. After filtering the solid and evaporating the solvent, the residue was purified by chromatography (EtOAc:hexane, 3:1) to afford 4.19 (17 mg, 60%) as a white solid: \( R_f \) 0.12 (3:1, EtOAc:hexane); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) H 0.86 (t, 3 H, \( J = 6.8 \) Hz), 1.35-1.18 (m, 36 H), 1.45-1.36 (m, 2 H), 1.59-1.49 (m, 4 H), 1.86-1.77 (m, 2 H), 3.17-3.01 (m, 2 H), 3.43-3.25 (m, 9 H), 3.82-3.72 (m, 2 H), 4.03-3.92 (m, 2 H), 4.21 (s, 1 H), 4.56-4.48 (m, 1 H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) C 14.1, 21.8, 22.7, 26.1, 26.2, 28.4, 29.0, 29.1, 29.4, 29.5, 29.6, 29.7, 29.7, 31.9, 52.7, 54.3, 62.3, 71.0, 71.0, 75.8, 77.2, 78.2, 78.6, 86.2; HRMS calcd for C\(_{32}\)H\(_{64}\)O\(_7\)SNa\(^+\): 615.4265. Found: 615.4259

Allyl 3,4,6-tri-O-p-methoxybenzyl-D-arabinofuranoside (4.22) Acetyl chloride (3.8 mL, 50.0 mmol) was added dropwise to a mixture of D-arabinose (5.0 g, 33.0 mmol) and allyl alcohol (100 mL). After 20 min., the reaction mixture was neutralized with pyridine. The solvent was evaporated and the crude oil was purified by chromatography (CH\(_2\)Cl\(_2\):CH\(_3\)OH, 7:1) to afford the product (6.17 g) as a mixture of diastereomers.

The crude mixture (1.0 g, 5.3 mmol) was dissolved in dry DMF (30 mL) and stirred at rt. Sodium hydride (1.3 g, 31.6 mmol) was added, followed by p-methoxybenzyl chloride (3.1 mL, 21.0 mmol) and the reaction mixture was stirred for 12 h. Methanol (5 mL) was added, and the mixture was diluted with EtOAc (50 mL). The organic layer was washed with H\(_2\)O (3 X 50 mL), brine (30 mL), and dried over Na\(_2\)SO\(_4\). Purification by chromatography (hexane:EtOAc, 3:1) afforded 4.22 (1.42 g, 50% over 2 steps) as a 2:1 \( \alpha/\beta \) mixture, and also allyl pyranoside anomers (700 mg): \( R_f \) 0.36 (3:1, hexane:EtOAc); NMR data for allyl 3,4,6-tri-O-p-methoxybenzyl-\( \alpha \)-D-arabinofuranoside: \(^1\)H NMR (250 MHz, CDCl\(_3\)) \( \delta \) H 3.64-3.48 (m,
2 H), 3.83-3.74 (m, 9 H), 3.90-3.85 (m, 1 H), 4.05-3.94 (m, 2 H), 4.29-4.15 (m, 2 H), 4.55-4.36 (m, 6 H), 5.07 (s, 1 H), 5.37-5.13 (m, 2 H), 6.00-5.82 (m, 1 H), 6.90-6.76 (m, 6 H), 7.27-7.10 (m, 6 H); $^{13}$C NMR (62.5 MHz, CDCl$_3$) $\delta$C 55.1, 55.2, 68.0, 69.3, 71.6, 71.7, 72.9, 80.6, 83.1, 88.0, 105.3, 113.6, 113.7, 117.1, 129.4, 129.4, 129.5, 129.6, 129.9, 130.1, 134.2, 159.1, 159.2, 159.3; HRMS calcd for C$_{32}$H$_{38}$O$_8$Na$: 573.2794$. Found: 573.2789.

3,4,6-Tri-$O$-$p$-methoxybenzyl-$D$-arabinofuranose (4.23) Allyl glycoside 4.22 (1.02 g, 1.86 mmol) was dissolved in a 7:3:1 mixture of ethanol/benzene/H$_2$O (32 mL). Diazabicyclo[2.2.2]octane (92 mg, 0.82 mmol) was added, followed by Wilkinson’s catalyst (Ph$_3$P)$_3$RhCl (127 mg, 0.14 mmol) and the reaction mixture was heated at 80 °C for 12 h. The solid was then filtered and the solvent evaporated to a light orange oil. This oil was dissolved in a 10:1 mixture of acetone/H$_2$O (22 mL) and stirred at rt. Mercury (II) oxide (505 mg, 2.33 mmol) was added, followed by mercury (II) chloride (505 mg, 1.86 mmol) and the reaction mixture was stirred for 12 h. The solvent was then evaporated and the crude mixture diluted with ether (40 mL). The organic layer was washed with a saturated solution of potassium iodide (2 X 10 mL), H$_2$O (10 mL), brine (10 mL), and then dried over MgSO$_4$. The solvent was evaporated and the residual oil purified by chromatography (hexane:EtOAc, 1:1) to afford 4.23 (916 mg, 97%) as a colorless oil: R$_f$ 0.17 (1:1, hexane:EtOAc); $^1$H NMR (250 MHz, CDCl$_3$) $\delta$H 3.59-3.41 (m, 3 H), 3.85-3.74 (m, 9 H), 4.20-3.86 (m, 3 H), 4.60-4.35 (m, 6 H), 5.39-5.25 (m, 1 H), 6.90-6.81 (m, 6 H), 7.27-7.14 (m, 6 H); Selected NMR data: $^{13}$C NMR (62.5 MHz, CDCl$_3$) $\delta$C 55.2, 69.8, 71.3, 71.5, 72.9, 81.8, 82.2, 86.2, 96.1, 101.0, 113.7, 113.6, 159.1, 159.3, 159.3, 159.4; HRMS calcd for C$_{29}$H$_{34}$O$_8$Na$: 533.2146$. Found: 533.2125.

3,4,6-Tri-$O$-$p$-methoxybenzyl-1,2-dideoxy-$D$-arabino-hex-1-enitol (4.24) $n$-Butyl lithium (1.4 mL, 2.22 mmol) was added dropwise to a solution of methyl
triphenylphosphonium bromide (793 mg, 2.22 mmol) in THF (5 mL) stirring at rt. After 10 min, lactol 4.23 (189 mg, 0.37 mmol) in THF (5 mL) was added. After 1.5 h the reaction mixture was diluted with EtOAc (20 mL) and the organic layer was washed with H₂O (15 mL), brine (15 mL), and dried over Na₂SO₄. The solvent was then evaporated to a light yellow oil which was purified by chromatography (hexane:EtOAc, 2:1) to afford 4.24 (135 mg, 72%) as a colorless oil: R_f 0.29 (2:1, hexane:EtOAc); [α]²⁺D −15.0 (c 1.1, CHCl₃); ¹H NMR (250 MHz, CDCl₃) δH 2.87 (d, 1 H, J = 4.9 Hz), 3.65-3.55 (m, 3 H), 3.83 (s, 9 H), 4.13-3.94 (m, 2 H), 4.33 (d, 1 H, J = 11.6 Hz), 4.64-4.46 (m, 5 H), 5.35-5.29 (m, 1 H), 5.37 (s, 1 H), 6.07-5.90 (m, 1 H), 6.93-6.82 (m, 6 H), 7.30-7.18 (m, 6 H); ¹³C NMR (62.5 MHz, CDCl₃) δC 55.2, 55.2, 55.2, 70.2, 70.3, 70.6, 72.9, 73.6, 79.7, 80.0, 113.6, 113.7, 113.7, 113.7, 118.6, 129.4, 129.6, 129.7, 129.9, 130.2, 130.3, 135.2, 159.2, 159.2, 159.2; HRMS calcd for C₃₀H₃₆O₇Na⁺: 531.2353. Found: 531.2319.

2,5-Anhydro-1-deoxy-1-iodo-3,4,6-tri-O-p-methoxybenzyl-D-glucitol (4.25)

Alkene 4.24 (135 mg, 0.27 mmol) was dissolved in THF (2 mL) and stirred at rt. To this solution was added sodium bicarbonate (223 mg, 2.65 mmol) in H₂O (5 mL), followed by iodine (470 mg, 1.86 mmol) in Et₂O (10 mL). After 1 h, sodium bisulfite was added until the organic layer was a light yellow color. The mixture was then diluted with EtOAc (30 mL) and the organic layer was washed with H₂O (15 mL), brine (15 mL), and dried over Na₂SO₄. The solvent was then evaporated to a light yellow oil which was purified by chromatography (hexane:EtOAc, 3:1) to afford 4.25 (167 mg, 99%) as a colorless oil: R_f 0.31 (3:1, hexane:EtOAc); [α]²⁺D +36.9 (c 1.1, CHCl₃); ¹H NMR (250 MHz, CDCl₃) δH 3.28-3.12 (m, 2 H), 3.33 (dd, 1 H, J = 9.9, 6.9 Hz), 3.44 (dd, 1 H, J = 9.9, 6.0 Hz), 3.68 (s, 3 H), 3.70 (s, 6 H), 3.81 (d, 1 H, J = 2.3 Hz), 3.89 (d, 1 H, J = 3.7 Hz), 4.13-4.04 (m, 1 H), 4.26-4.16 (m, 1 H), 4.45-4.28 (m, 6 H), 6.79-6.72 (m 6 H), 7.15-7.07 (m, 6 H); ¹³C NMR (62.5 MHz, CDCl₃) δC 142.
3,4,6-Tri-\textit{O}-\textit{p}-methoxybenzyl-2,5-anhydroglucityl thioacetate (4.26) Iodide 4.25

(166 mg, 0.26 mmol) was dissolved in dry DMF (2 mL) and stirred at rt. Potassium thioacetate (120 mg, 1.05 mmol) was added and the reaction mixture stirred for 12 h. The mixture was then diluted with ether (20 mL), washed with \( \text{H}_2\text{O} \) (3 X 10 mL), and dried over \( \text{MgSO}_4 \). Purification by chromatography (hexane:EtOAc, 2:1) afforded 4.27 (141 mg, 92%) as a light yellow oil: \( R_f \) 0.19 (hexane:EtOAc, 3:1); \([\alpha]^{25}_D \ +30.6 \) (c 1.0, CHCl\(_3\)); \(^1\)H NMR (250 MHz, CDCl\(_3\)) \( \delta \) 2.31 (s, 3 H), 3.17 (d, 2 H, \( J = 6.9 \) Hz), 3.46 (dd, 1 H, \( J = 10.0, 6.8 \) Hz), 3.56 (dd, 1 H, \( J = 9.9, 5.6 \) Hz), 3.82-3.75 (m, 9 H), 3.94-3.86 (m, 2 H), 4.11-4.01 (m, 2 H), 4.52-4.29 (m, 6 H), 6.90-6.80 (m, 6 H), 7.26-7.13 (m, 6 H); \(^{13}\)C NMR (62.5 MHz, CDCl\(_3\)) \( \delta \) 27.9, 30.4, 55.1, 55.1, 69.9, 71.0, 71.1, 72.8, 79.8, 82.5, 82.8, 113.6, 113.7, 129.2, 129.2, 129.3, 129.6, 130.2, 159.0, 159.2, 195.3; HRMS calcd for \( \text{C}_{32}\text{H}_{38}\text{O}_8\text{SNa}^+ \): 605.2180. Found: 605.2155.

3,4,6-Tri-\textit{O}-\textit{p}-methoxybenzyl-2,5-anhydroglucityl thiol (4.27) Thioacetate 4.26

(180 mg, 0.31 mmol) was dissolved in anhydrous ether (20 mL) and stirred at rt. Lithium aluminum hydride (13 mg, 0.34 mmol) was added and the reaction mixture stirred for 1.5 h. The reaction mixture was diluted with ethyl acetate (30 mL), and the organic layer was washed with \( \text{H}_2\text{O} \) (20 mL), brine (20 mL), and dried over \( \text{Na}_2\text{SO}_4 \). Purification by chromatography (hexane:EtOAc, 3:1) afforded 4.27 (152 mg, 92%) as a light colorless oil: \( R_f \) 0.29 (hexane:EtOAc, 3:1); \([\alpha]^{25}_D \ +41.7 \) (c 1.0, CHCl\(_3\)); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 1.37 (t, 1 H, \( J = 8.8 \) Hz), 2.74 (dd, 2 H, \( J = 8.6, 7.2 \) Hz), 3.46 (dd, 1 H, \( J = 9.9, 6.6 \) Hz), 3.54 (dd, 1 H, \( J = 9.9, 5.9 \) Hz), 3.78 (s, 3 H), 3.79 (s, 3 H), 3.79 (s, 3 H), 3.97-3.87 (m, 2 H), 4.10-4.04.
(m, 2 H), 4.29 (d, 1 H, J = 11.4 Hz), 4.53-4.40 (m, 5 H), 6.88-6.82 (m, 6 H), 7.25-7.14 (m, 6 H); $^{13}$C NMR (100 MHz, CDCl$_3$) δC 22.7, 55.2, 55.2, 55.2, 70.2, 71.1, 71.1, 72.9, 81.7, 82.8, 83.0, 83.0, 113.7, 113.8, 129.3, 129.3, 129.4, 129.7, 129.8, 130.2, 159.1, 159.3, 159.3.

**Geranyl chloride (4.28)** Lithium chloride (824 mg, 19.5 mmol) and 2,6-lutidine (3.0 mL, 25.9 mmol) were added to a solution of geraniol (1.0 g, 6.5 mmol) in dry DMF (25 mL) stirring at 0 °C. After 15 min., methanesulfonyl chloride (1.5 mL, 19.5 mmol) was added dropwise and the reaction stirred for 3 h at this cool temperature. The mixture was warmed to room temperature and diluted with Et$_2$O (100 mL). The organic layer was washed with H$_2$O (5 X 100 mL) and then dried over MgSO$_4$. The solvent was evaporated to afford 4.28 (1.08 g, 96%) as a light yellow liquid. The NMR data were the same as previously reported.³

**1-Geranyl-3,4,6-tri-O-p-methoxybenzyl-2,5-anhydroglucityl sulfide (4.29)** Thiol 4.27 (152 mg, 0.28 mmol) was dissolved in THF (10 mL) and was stirred at 0 °C. n-Butyl lithium (81 µL, 0.13 mmol) was added, and after 5 min, geranyl chloride, 4.28 (41 mg, 0.24 mmol), was added. After approximately 30 min, the reaction mixture was warmed to rt and stirred for 12 h. The mixture was diluted with EtOAc (20 mL), and the organic layer was washed with H$_2$O (10 mL), brine (10 mL), and dried over Na$_2$SO$_4$. The solvent was evaporated and the solid residue was purified by chromatography (hexane:EtOAc, 5:1) to afford 4.29 (108 mg, 57%) as a colorless oil: R$_f$ 0.35 (3:1, hexane:EtOAc); [α]$^25_D$ +19.3 (c 0.9, CHCl$_3$); $^1$H NMR (250 MHz, CDCl$_3$) δH 1.59 (s, 3 H), 1.63 (s, 3 H), 1.67 (s, 3 H), 1.67 (s, 3 H), 2.13-1.97 (m, 4 H), 2.84-2.67 (m, 1 H), 3.28-3.09 (m, 2 H), 3.45 (dd, 1 H, J = 9.9, 6.9 Hz), 3.56 (dd, 1 H, J = 9.9, 5.9 Hz), 3.83-3.76 (m, 9 H), 3.89-3.85 (m, 1 H), 3.95-3.90 (m, 1 H), 4.20-4.00 (m, 3 H), 4.55-4.33 (m, 6 H), 5.13-5.02 (m, 1 H), 5.29-5.18 (m, 1 H), 6.90-6.78 (m, 6 H), 7.27-7.13 (m, 6 H); $^{13}$C NMR (62.5 MHz, CDCl$_3$) δC 16.1, 17.7, 25.7, 26.5, 29.0, 30.0, 39.6, 55.2, 70.2, 71.0, 71.3, 72.9, 81.1, 82.3, 83.0, 83.3, 113.6, 113.7, 113.7, 113.8, 120.3,
123.9, 129.2, 129.3, 129.8, 129.9, 130.3, 131.6, 139.0, 159.1, 159.2, 159.2; HRMS calcd for C_{40}H_{52}O_{7}Na^+: 699.3326. Found: 699.3323.

1-Geranyl-3,4,6-tri-O-p-methoxybenzyl-2,5-anhydroglucityl sulfone (4.30) and aldehyde (4.31) Sulfide 4.29 (93 mg, 0.14 mmol) was dissolved in a mixture of acetonitrile (8 mL) and H_{2}O (0.3 mL). Sodium periodate (88 mg, 0.41 mmol) was added and the reaction mixture was heated at 70 °C. The reaction proceeded for 2 days and, during this time, sodium periodate (88 mg, 0.41 mmol) was added 3 more times for a total of 12 equivalents. The reaction mixture was cooled to rt overnight over the 2 days to avoid decomposition. After cooling to rt and evaporating the solvent, the crude mixture was purified by chromatography (hexane:EtOAc, 3:1) to afford 4.30 (42 mg, 43%) as a colorless oil, and also aldehyde 4.31 (9 mg): data for 4.30: R_f 0.25 (3:1, hexane:EtOAc); [α]^{25}_{D} +9.0 (c 0.9, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ_H 1.56 (s, 3 H), 1.65 (s, 3 H), 1.68 (s, 3 H), 2.06 (s, 4 H), 3.04-2.99 (m, 1 H), 3.47-3.37 (m, 2 H), 3.52 (dd, 1 H, J = 9.9, 5.6 Hz), 3.73 (dd, 1 H, J = 14.4, 7.6 Hz), 3.77 (s, 3 H), 3.78 (s, 3 H), 3.79 (s, 3 H), 3.83 (dd, 1 H, J = 14.2, 8.2 Hz), 3.92-3.88 (m, 2 H), 4.09-4.05 (m, 1 H), 4.27 (d, 1 H, J = 11.4 Hz), 4.51-4.38 (m, 6 H), 5.08-5.00 (m, 1 H), 5.30-5.25 (m, 1 H), 6.87-6.81 (m, 6 H), 7.20-7.01 (m, 6 H), ¹³C NMR (100 MHz, CDCl₃) δ_C 16.9, 17.7, 25.7, 26.3, 39.8, 52.0, 54.2, 55.2, 55.3, 55.3, 69.9, 71.2, 71.2, 72.9, 75.5, 82.5, 82.9, 83.2, 110.1, 113.8, 113.9, 123.5, 123.5, 129.3, 129.3, 129.4, 129.5, 129.5, 129.6, 130.1, 132.0, 146.3; HRMS calcd for C_{40}H_{52}O_{9}Na^+: 731.3224. Found: 731.3213; data for 4.31: R_f 0.32 (2:1, hexane:EtOAc); ¹H NMR (250 MHz, CDCl₃) δ_H 1.64 (s, 3 H), 2.50-2.25 (m, 4 H), 2.99-2.88 (m, 1 H), 3.51-3.29 (m, 3 H), 3.79-3.64 (m, 11 H), 3.88-3.80 (m, 2 H), 4.07-3.98 (m, 1 H), 4.20 (d, 1 H, 11.3 Hz), 4.49-4.30 (m, 6 H), 5.30-5.15 (m, 1 H), 6.85-6.70 (m, 6 H), 7.25-7.00 (m, 6 H), 9.65 (s, 1 H); HRMS calcd for C_{37}H_{46}O_{10}Na^+: 705.2704. Found: 705.2737.
1-Geranyl-3,4,6-tri-O-benzyl-2,5-anhydroglucityl sulfide (4.33)  

*n*-Butyl lithium (0.8 mL, 1.26 mmol) was added dropwise to a solution of 4.5 (515 mg, 1.14 mmol) dissolved in THF (10 mL) stirring at -78 °C. To this solution was added geranyl tosylate (4.32), which was prepared *in situ* by the addition of *n*-butyl lithium (3.9 mL, 6.20 mmol) to geraniol (870 mg, 5.64 mmol) in THF (15 mL) at -78 °C, followed by the addition of *p*-toluenesulfonyl chloride (1.40 g, 7.33 mmol). The reaction mixture was slowly warmed to rt over 2 h. Water (20 mL) and ether (30 mL) were added, and the organic layer was separated from the aqueous layer, washed with brine (10 mL), and dried over Na$_2$SO$_4$. The solvent was evaporated and the residual oil was purified by chromatography (hexane:EtOAc, 9:1) to afford 4.33 (509 mg, 76%) as a colorless oil: R$_f$ 0.42 (6:1, hexane:EtOAc); [$\alpha$]$^{25}$D +22.4 (c 1.0, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 1.58 (s, 3 H), 1.62 (s, 3 H), 1.67 (s, 3 H), 2.10-1.97 (m, 4 H), 2.85-2.75 (m, 2 H), 3.26-3.11 (m, 2 H), 3.49 (dd, 1 H, $J = 9.8$, 7.0 Hz), 3.60 (dd, 1 H, $J = 9.8$, 5.8 Hz), 4.18-3.91 (m, 2 H), 4.20-4.09 (m, 2 H), 4.59-4.39 (m, 6 H), 5.10-5.03 (m, 1 H), 5.27-5.20 (m, 1 H), 7.35-7.20 (m, 15 H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 16.0, 17.5, 25.5, 26.4, 29.0, 29.9, 39.5, 70.4, 71.2, 71.5, 73.1, 81.1, 82.5, 82.9, 83.4, 120.3, 123.8, 127.4, 127.5, 127.5, 127.5, 127.6, 127.7, 128.1, 128.2, 128.3, 129.6, 131.4, 137.7, 137.7, 138.1, 138.8; HRMS calcd for C$_{37}$H$_{46}$O$_4$Na$: 609.2919$. Found: 609.2914.

1-Geranyl-3,4,6-tri-O-benzyl-2,5-anhydroglucityl sulfone (4.34)  

Sulfide 4.33 (116 mg, 0.23 mmol) was dissolved in a mixture of acetonitrile (8 mL) and H$_2$O (0.3 mL). Sodium periodate (145 mg, 0.68 mmol) was added and the reaction mixture was heated at 70 °C. The reaction proceeded for 2 days and, during this time, sodium periodate (145 mg, 0.68 mmol) was added 3 more times for a total of 12 equivalents. The reaction mixture was cooled to rt overnight over the 2 days to avoid decomposition. After cooling to rt and evaporating the solvent, the crude mixture was purified by chromatography (hexane:EtOAc, 4:1) to afford
4.34 (65 mg, 53%) as a colorless oil: Rf 0.33 (3:1, hexane:EtOAc); [α]$_{D}^{25}$ +10.0 (c 1.1, CHCl$_3$); $^1$H NMR (250 MHz, CDCl$_3$) δ$_{H}$ 1.58 (s, 3 H), 1.67 (s, 6 H), 2.08 (s, 4 H), 2.86 (dd, 1 H, J = 13.3, 3.4 Hz), 3.05 (dd, 1 H, J = 13.2, 9.3 Hz), 3.66-3.44 (m, 4 H), 3.94-3.91 (m, 1 H), 4.01-3.97 (m, 1 H), 4.17-4.07 (m, 1 H), 4.36 (t, 1 H, J = 11.8 Hz), 4.60-4.45 (m, 6 H), 5.10-5.00 (m, 1 H), 5.35-5.24 (m, 1 H), 7.40-7.19 (m, 15 H); $^{13}$C NMR (62.5 MHz, CDCl$_3$) δ$_{C}$ 16.9, 17.6, 25.6, 26.2, 39.7, 51.8, 52.4, 70.1, 71.4, 71.4, 73.2, 75.1, 82.9, 83.4, 83.6, 111.1, 123.5, 127.6, 127.7, 127.7, 127.9, 128.3, 128.4, 128.4, 131.8, 137.4, 137.6, 138.0, 145.0; HRMS calcd for C$_{37}$H$_{46}$O$_6$SNa$: 641.2907. Found: 641.2874.

1-(3,7-Dimethyloctyl)-2,5-anhydroglucityl sulfone (4.35) Sulfone 4.34 (66 mg, 0.12 mmol) was dissolved in CH$_3$OH (5 mL) containing AcOH (10 µL). Palladium on carbon (10%, 30 mg) was added and the reaction mixture was stirred under hydrogen overnight at atmospheric pressure. After filtering the solid and evaporating the solvent, the residue was purified by chromatography (EtOAc:hexane, 3:1) to afford 4.35 (24 mg, 64%) as a white solid that was a 3:1 mixture of diastereomers: Rf 0.13 (5:1, EtOAc:hexane); [α]$_{D}^{25}$ +13.7 (c 1.0, CHCl$_3$); Selected NMR data: $^1$H NMR (400 MHz, CD$_3$OD) δ$_{H}$ 0.84 (s, 3 H), 0.85 (s, 3 H), 0.90-0.87 (m, 3 H), 1.19-1.08 (m, 3 H), 1.35-1.22 (m, 3 H), 1.63-1.45 (m, 3 H), 1.84-1.73 (m, 1 H), 3.28-3.04 (m, 3 H), 3.39 (td, 1 H, J = 15.0, 9.1 Hz), 3.67-3.55 (m, 2 H), 3.81-3.77 (m, 1 H), 3.93-3.90 (m, 1 H), 3.98-3.96 (m, 1 H), 4.42-4.38 (m, 1 H); Selected NMR data: $^{13}$C NMR (100 MHz, CDCl$_3$) δ$_{C}$ 20.0, 20.0, 23.4, 23.5, 26.1, 26.2, 29.5, 29.8, 29.9, 33.6, 33.7, 38.2, 38.3, 40.7, 53.6, 53.7, 54.7, 54.7, 57.2, 63.6, 63.8, 77.6, 77.6, 78.5, 79.8, 80.0, 82.2, 88.6; HRMS calcd for C$_{16}$H$_{32}$O$_6$SNa$: 375.1818. Found: 375.1823.
References


CHAPTER 5

SYNTHESIS OF NUCLEOTIDE DIPHOSPHATE ANALOGS OF D-ARABINOSE

5.1 Introduction

It is believed that decaprenolphosphoarabinose (DPA, 5.1, Figure 5.1) is the major glycosyl donor for the incorporation of arabinofuranose residues in the mycobacterial cell wall. As discussed in Chapter 1, it remains unknown if DPA is the sole donor of these residues or if there is an arabinosyl nucleotide donor (e.g., 5.4) involved as well. The presence of UDP-arabinose in mycobacteria has been reported, although attempts made by another group to isolate nucleotide diphosphate (NDP) arabinose from Mycobacterium smegmatis incubated with [1-3H]glucose were unsuccessful. As mentioned earlier, furanose-containing sugar nucleotides are extremely labile. The inability to isolate these species does not rule out their involvement in arabinan biosynthesis.
The main reason sugar nucleotides are unstable is because the glycosidic C-O-P bond is susceptible to hydrolysis. Our goal was to synthesize NDP-C-D-arabinose analogs (5.5, Figure 5.2), which are metabolically more stable. The rationale for synthesizing these compounds is that if one or more of them inhibit the AraT’s, then this provides indirect evidence for the importance of sugar nucleotide donors in arabinan biosynthesis. The retrosynthesis for the preparation of these compounds is shown in Figure 5.3. Phosphonate 5.6 could be deprotected with a Lewis acid to form 5.7. Phosphonic acid 5.7 could then be coupled with a nucleoside-5’-phosphomorpholine-\(N,N’\)-dicyclohexylcarboxamidine 5.8 (commercially available from Sigma) to form 5.5.
Figure 5.2: NDP-C-D-arabinose analogs.

Figure 5.3: Retrosynthesis of 5.5.

The synthesis of phosphonate 5.6 was described in Chapter 3. We prepared 5.7 by two different methods (A & B, Scheme 5.1). In one, we reacted 5.6 with iodonitromethane at 0 °C in dichloromethane and obtained 5.7 as a crude oil (method A). This reaction was not clean and the dark red oil was difficult to purify. Method B was more successful. We first removed the benzyl groups of 5.6 with 10% palladium on carbon to afford diethyl-2,5-
anhydroglucityl phosphonate. The removal of the ethyl esters proceeded cleanly with bromotrimethylsilane in dichloromethane at room temperature to afford the product as a light orange oil. The crude oil was purified by ion-exchange chromatography, and was eluted with a 0.2 M solution of triethylammonium bicarbonate. The triethylammonium salt was removed with Amberlite H⁺ resin in methanol. Phosphonic acid 5.7 was obtained in 75% overall yield as a clear and colorless oil.

Scheme 5.1: Synthesis of 5.7.

5.2 Attempts to synthesize sugar nucleotide analogs 5.5

The coupling reaction of phosphonic acid 5.7 with nucleotide morpholidates 5.8 proved to be especially challenging. The reaction conditions used to couple 5.7 with morpholidates 5.8a or 5.8b are shown in Table 5.1. Attempts to react the triethylammonium salt of 5.7 with 5.8a in pyridine were unsuccessful (A1). This reaction was also attempted using tetrazole (A2 & C). Because both the free phosphonic acid 5.7 and the
triethylammonium salt of 5.7 were not very soluble in pyridine (dissolution required extended stirring or gentle heating), we also tried running the reaction in a mixture of DMF/pyridine (A3), and then in 100% DMF, with or without the presence of tetrazole (B & D, respectively). These attempts were also unsuccessful.

Table 5.1: Attempts to synthesize 5.5.

The procedure we used was modified from a procedure to make UDP-C-Galβ (5.10, Figure 5.4). The general procedure for the coupling is the following: The free phosphonic acid or phosphonic acid salt of 5.7 (100 mg, 0.5 mmol) was dissolved in anhydrous pyridine
(2 mL) and evaporated to dryness using argon to bring the pressure back to normal. This process was repeated two times, and then the oil was redissolved in pyridine (1 mL) and placed under argon. The morpholidate 5.8 (0.5 mmol) was also co-evaporated with pyridine and added to 5.7 in pyridine (1 mL). The reaction was monitored by TLC (4:1, CH\textsubscript{3}OH:0.1 M NH\textsubscript{4}OAc or 1:1, CH\textsubscript{2}Cl\textsubscript{2}:MeOH), MS, and \textsuperscript{31}P NMR over the course of 2-5 days. A reaction occurred slowly over a 24 h period and there appeared to be no further change when the reaction proceeded for longer times. Upon completion of the reaction, the solvent was evaporated and NMR data and mass spectral data were recorded.

![Figure 5.4](image)

Figure 5.4: Uridine diphosphate C-\(\alpha\)-D-galactofuranose (UDP-C-Gal\(f\)) 5.10.

There were two indicators that we were looking for to determine if the coupling was occurring. We expected to see two doublets in the \textsuperscript{31}P-NMR spectrum at approximately -10 and -11 ppm (\(J_{P,P}\) 20.9 Hz) corresponding to the 2 phosphoryl groups, similar to that reported for a similar sugar nucleotide.\textsuperscript{4} Also, we expected that the R\(_f\) of the coupled product would be greater than that of 5.7. The \textsuperscript{31}P-NMR showed 4 singlets (\(\delta\) 45, 21, 2, -10 ppm), but no doublets were detected. The R\(_f\)’s of the products from the reaction were lower than the R\(_f\) for 5.7, indicating a more polar product. An example of a TLC plate after 2 days is shown in Figure 5.5. We were unable to completely separate the two more polar products by ion-
exchange chromatography, Iatrobeads, or other methods. However, based on mass spectral and $^1$H and $^{31}$P-NMR data, we concluded that the reaction was unsuccessful.

![Thin layer chromatography plate from sugar nucleotide coupling reaction.](image)

The fact that we could not obtain the product using this common procedure for sugar nucleotide coupling was discouraging. One possible cause is that the morpholidate was hydrolyzing during the reaction due to the possible presence of a small amount of water (Scheme 5.2). If hydrolysis was occurring, we would have expected to see two products that contain phosphorous, uridine 5'-monophosphate (5.11) and unreacted 5.7. This is not what we observed.
Scheme 5.2: Hydrolysis of UDP-morpholidate 5.8 to form uridine 5’-monophosphate 5.11 (UMP).

The $^{31}$P-NMR in D$_2$O (proton decoupled) showed 4 phosphorous signals ($\delta$P 45, 21, 2, -10 ppm). These peaks usually varied by 1-2 ppm. The small peak at 21 ppm is unreacted 5.7. The typical range for phosphonic acids is between 21 and 28 ppm, depending on the quantity of ammonium counterion present. The more pyridine or triethylamine in the sample, the more upfield the peak shifts. The $^{31}$P-NMR spectra taken in D$_2$O of 5.7 as the free diacid shows a peak at 28 ppm and a sample with pyridine shows a peak at 21.5 ppm. A few sample spectra are located in Appendix D of this dissertation. The peak at 2 ppm is likely hydrolyzed 5.8. Spectra were taken of uridine 5’-monophosphate 5.11 (Figure 5.6) as the triethylammonium salt. The $^{31}$P-NMR spectra taken in D$_2$O showed a peak at 1.3 ppm, which is very close to the chemical shift of the peak we observed. The corresponding morpholidate showed a phosphorous peak at 8.6 ppm (spectrum taken in D$_2$O). We cannot explain the major peaks at 45 and –10 ppm. The peak at 45 ppm is too far downfield to be a phosphonic acid. One possibility is that phostone 5.14 was formed as described previously (Chapter 2 and 3), and a possible mechanism for its formation is illustrated in Scheme 5.3. A similar α-D-galactofuranose 1,2-cyclophosphate has been reported, but the chemical shift is much lower ($\delta$P 17), and we could not detect this peak by MS.
Figure 5.6: Uridine 5'-monophosphate 5.11.

Scheme 5.3: Possible mechanism for formation of phostone 5.14.

It was difficult to interpret the $^1$H NMR spectra because we could not separate any individual products. The crude mixture was chromatographed on an Iatrobead column (CH$_2$Cl$_2$:CH$_3$OH, 1:1), and one of the fractions collected contained a mixture of nucleotides, with one that is likely UMP 5.11. In the $^1$H-NMR spectrum of 5.11 recorded in D$_2$O, the signal for the anomeric C-H peak appeared as a doublet at 5.86 ppm with $J = 4.9$ Hz, and the signals for the vinylic C-H peaks (two doublets) in the uridine moiety appeared at 7.86 ppm and 5.83 ppm with $J = 8.1$ Hz. The $^1$H-NMR spectrum (recorded in D$_2$O) of the nucleotide-containing fraction from the attempted coupling reaction showed the presence of two...
doublets at approximately 8 ppm ($J = 8$ Hz for each doublet), and also two sets of multiplets at 5.9 ppm and 5.7 ppm were present. The $^{31}$P-NMR spectrum of this fraction showed peaks at 1.3 ppm and –10 ppm. Another fraction showed a mixture of compounds in which no nucleotide was present in the $^1$H-NMR spectrum (recorded in D$_2$O), and in the $^{31}$P-NMR spectrum for this compound, peaks at 45 and 21 ppm were present. The $^1$H-NMR spectrum showed mostly unreacted 5.7, and a multiplet at 2.2 ppm was observed for the C-1 protons adjacent to the phosphorous (CH$_2$-P). Other proton signals were observed that are not related to protons in 5.7. One of the signals was a multiplet at 1.68 ppm, which could originate from protons on a carbon adjacent to a phosphorous. Another signal observed was a multiplet at 4.6 ppm, and this proton signal could originate from the proton at C-2. For 5.7, this proton signal appears at approximately 4.3 ppm.

A major complication of characterizing our compounds using mass spectral data was the great amount of fragmentation that was occurring. Even with C-phosphonic acid 5.7, a major parent peak could not be detected. There were many peaks corresponding to the many different associations of sodium and protons (Table 5.2). The sodiated free diacid form of 5.7 was seen at 251 amu. The protonated monotriethylammonium salt of 5.7 was observed at 330 amu. Also, clusters of molecules were seen at 479 and 501 amu.
Table 5.2: Mass spectral data for 5.7.

Some of the mass spectral data for uridine morpholidate 5.8b and uridine 5’-monophosphate 5.11 are shown in Table 5.3 and Table 5.4, respectively. In both samples a large amount of fragmentation had occurred. The sodiated ion of 5.8b was seen at 416 amu and the protonated triethylammonium salt of 5.11 was observed at 527 amu.
Table 5.3: Mass spectral data for 5.8b.

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<tr>
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<tr>
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</tr>
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Table 5.4: Mass spectral data for 5.11.

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<th>Fragment</th>
<th>Exact mass (amu)</th>
<th>Observed mass (amu)</th>
</tr>
</thead>
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<td>327.0844</td>
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<tr>
<td>347</td>
<td>C_{9}H_{13}N_{2}O_{3}P + Na^{+}</td>
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<td>347.0255</td>
</tr>
<tr>
<td>527</td>
<td>C_{9}H_{11}N_{2}O_{3}P + C_{12}H_{22}N_{2} + H^{+}</td>
<td>527.2840</td>
<td>527.2896</td>
</tr>
</tbody>
</table>
Some of the mass spectral data we obtained from our attempt at reacting 5.7 with 5.8b is presented in Table 5.5. A large amount of fragmentation was seen in the mass spectrum and only a few peaks are presented here. The peak at 251 amu corresponds to phosphonic acid 5.7. The peak at 265 amu is within 8.4 ppm of a mass that corresponds with C₆H₁₁O₆P + CH₃OH + Na⁺. We did not observe a peak that would correspond with phostone 5.14 (Figure 5.3). However, the peak at 265 amu would be M + CH₃OH + Na⁺ for 5.14. The peak at 294 amu corresponds to the carboxamidine salt 5.9. The other peaks seen at 327 amu and 623 amu are the same fragments seen in the mass spectrum of morpholidate 5.8b.

![Diagram](image)

Table 5.5: Mass spectral data for coupling reaction of 5.7 and 5.8b.
5.3 Synthesis of sugar nucleotide with protected C-phosphonic acid

As an attempt to determine if the hydroxyl group at C-2 was responsible for the failure of the coupling reaction, we tried the coupling reaction with the benzylated phosphonic acid 5.16 (Figure 5.7).

![Figure 5.7: 3,4,6-Tri-O-benzyl-2,5-anhydroglucityl phosphonic acid 5.16.](image)

Our first attempt to synthesize 5.16 involved the reaction of diethyl phosphonate 5.6 (Chapter 3) with either iodo(trimethyl)silane or bromo(trimethyl)silane in dichloromethane at room temperature or at 0 °C (Scheme 5.4). However, we always observed loss of one or more benzyl groups and we could not separate 5.16 from this mixture.

![Scheme 5.4: Initial attempts to synthesize 5.16.](image)
We next attempted to synthesize 5.16 via our reduction/oxidation methodology that we developed for the synthesis of our C-phosphinic acid series (Chapter 3). The synthesis of 5.16 is shown in Scheme 5.5. Phosphonate 5.6 was reduced with lithium aluminum hydride to afford the phosphine, which was oxidized with a 98:2 mixture of pyridine/H$_2$O. After 3 days of monitoring this reaction by $^{31}$P-NMR, phosphonic acid 5.16 was formed in 42% overall yield from 5.6.

![Scheme 5.5: Synthesis of 5.16.](image)

We next attempted to synthesize 5.17 by reacting 5.16 with 5.8b (Scheme 5.6). We used the same procedure as for our initial attempts to synthesize 5.5. Both 5.16 and 5.8b were co-evaporated with pyridine and then mixed together and stirred at room temperature for 2 days under argon. The solvent was then evaporated and NMR and mass spectral data were taken of the crude product. Both a negative and positive ion mass spectrum were obtained. In the negative ion mass spectrum, a peak at 803 amu (M-H) was seen with a difference of 6.8 ppm. In the positive ion mass spectrum, a peak at 806 amu (M+2H) was observed with a difference of 10 ppm. Upon chromatography of this crude mixture, there were no doublets detected by $^{31}$P-NMR spectroscopy (there were 2 singlets at 33.9 and 22.3
ppm), and 5.17 was not observed in the $^1$H-NMR spectrum. The small quantity of product that was formed had likely decomposed during the purification process.

Scheme 5.6: Synthesis of 5.17.

5.4 Future work

It is not clear why we had difficulty synthesizing these sugar nucleotides. It is possible that arabinofuranose sugar nucleotides are inherently unstable, or a more likely reason is that we were not using the best reaction conditions for this coupling to take place. Future work should entail the exploration of other routes for making these compounds. For example, Kiessling and co-workers reported the synthesis of UDP-$\alpha$-D-galactofuranose 5.20 by reacting activated UMP-N-methylimidazole 5.18 (prepared from 5.11) with 5.19 (Scheme 5.7). After 2 hours at 0 °C, 5.19 was completely consumed and $^{31}$P-NMR spectrum showed that the proportion of 5.20 to be 83%, with a relatively small amount of phostone formation (17%). Further degradation of 5.20 to form the $\alpha$-D-galf phostone and 5'-UMP occurred during the purification process (ion-exchange HPLC with an ammonium acetate buffer). The
yield after purification (35%) is very good for such a labile sugar nucleotide. The instability of UDP-gal\(\text{f}\) has also been reported by another group who observed that 5.20 decomposed rapidly during a coupling reaction with UMP-imidazole and formed α-D-gal\(\text{f}\) phostone and 5’-UMP.\(^4\) In addition to degradation which occurred during the reaction, 5.20 decomposed upon standing at room temperature. After 24 hour at 22 °C in a 0.015 mol dm\(^{-3}\) aqueous solution, UDP-gal\(\text{f}\) 5.20 hydrolyzed (~95% extent) to form D-galactose and uridine 5’-diphosphate.

Scheme 5.7: Synthesis of UDP-α-D-galactofuranose 5.20 reported by Kiessling and co-workers.

A huge benefit of this method is that the reaction is completed in only two hours compared with several days. A faster reaction time would hopefully reduce any byproduct formation and make monitoring the progress of the reaction much easier. Furthermore, our
C-sugar nucleotides should be much more stable than 5.20, and thus this reaction should work even better for our compounds.

5.5 Experimental

**General.** Solvents were distilled from the appropriate drying agents before use. All reactions were carried out at room temperature under a positive pressure of argon and were monitored by TLC on silica gel 60 F254. Spots were detected under UV light or by charring with 10% H2SO4 in ethanol. Solvents were evaporated under reduced pressure and below 40 °C (bath). Organic solutions of crude products were dried over anhydrous Na2SO4. Unless otherwise indicated, column chromatography was performed on silica gel 60 (40–60 μM). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). 1H NMR spectra were recorded at 250 or 400 MHz and chemical shifts are referenced to TMS (0.0, CDCl3). 13C NMR spectra were recorded at 62.5 or 100 MHz and chemical shifts are referenced to CDCl3 (77.00, CDCl3). 31P NMR spectra were recorded at 101 or 162 MHz and chemical shifts are referenced to external phosphoric acid (0.0, CDCl3, CD3OD). Electrospray mass spectra were recorded on samples suspended in mixtures of THF and CH3OH with added trifluoroacetic acid or NaCl.

**2,5-Anhydro-D-glucityl phosphonic acid (5.7).** Phosphonate 5.6 (2.7 g, 4.87 mmol) was dissolved in glacial HOAc (10 mL). Palladium (10% on activated carbon, 500 mg) was added and the reaction mixture was stirred under hydrogen overnight at atmospheric pressure. The solid was then filtered off and the filtrate was concentrated to a clear residue that was purified by chromatography (9:1, CH2Cl2:CH3OH) affording 1-diethyl-2,5-anhydroglucityl phosphonate (1.13 g, 83%): Rf 0.33 (9:1, CH2Cl2:CH3OH). The diethyl phosphonate from above was dissolved in CH2Cl2 (5 mL). Bromotrimethylsilane (1.7 mL, 166...
12.9 mmol) was added and the reaction mixture stirred overnight. Methanol (1 mL) was added to the orange reaction mixture and the solvent was evaporated to give a crude oil. The oil was purified by ion-exchange chromatography (AG 1-X8 resin) and was eluted with a 0.2 M solution of triethylammonium bicarbonate. After evaporation of the eluant, the clear oil was dissolved in CH$_3$OH (5 mL) and treated with Amberlite H$^+$ resin. The resin was then filtered to afford 5.7 (853 mg, 91%) as a clear oil: $^1$H NMR (400 MHz, D$_2$O) $\delta$H 2.10–1.90 (m, 2 H), 3.66–3.47 (m, 3 H), 3.93–3.83 (m, 2 H), 4.15-4.08 (m, 1 H); $^{13}$C NMR (100 MHz, D$_2$O) $\delta$C 25.8 (d, $J_0 = 135.3$ Hz), 61.0, 75.6, 76.7 (d, $J = 8.8$ Hz), 77.4, 84.3; $^{31}$P NMR (162 MHz, D$_2$O) $\delta$P 28.0; HRMS calcd for [C$_6$H$_{13}$O$_7$P]Na$: 251.0291. Found: 251.0425.

**3,4,6-Tri-O-benzyl-2,5-anhydroglucityl phosphonic acid (5.16)** Lithium aluminum hydride (35 mg, 0.916 mmol) was added to a solution of phosphonate 5.6 (203 mg, 0.366 mmol) in anhydrous ether (5 mL). After 10 min, EtOAc (2 mL) was added to the reaction mixture, and after a few minutes H$_2$O (1 mL) was added. After all gas evolution had subsided, the mixture was filtered though Celite and the solvent was concentrated to give a clear residual oil. Purification by chromatography (hexane:EtOAc, 6:1) afforded the phosphine (85 mg, 52%) as a colorless oil. For NMR data, see Chapter 3.

This phosphine (85 mg, 0.19 mmol) was dissolved in a 98:2 mixture of pyridine/H$_2$O (5 mL). Iodine (190 mg, 0.76 mmol) was added and the reaction mixture stirred for 3 d at rt. The mixture was then diluted with CH$_2$Cl$_2$ (30 mL) and the organic layer was washed with a 5% solution of sodium bisulfite (20 mL), and was dried over Na$_2$SO$_4$. The product was purified by chromatography (CH$_2$Cl$_2$:CH$_3$OH, 10:1) to afford 5.16 (75 mg, 42% over 2 steps) as the pyridinium salt (0.23 eq pyridine: 1 eq diacid), which was a clear oil: R$_f$ 0.28 (3:1, CH$_2$Cl$_2$:CH$_3$OH); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 2.38-2.07 (m, 2 H), 4.31 (dd, 1 H, $J = 9.9$, 6.5 Hz), 4.40 (dd, 1 H, $J = 10.0$, 5.6 Hz), 3.95-3.86 (m, 2 H), 4.10-4.04 (m, 1 H), 4.55-4.33
(m, 7 H), 7.45-7.13 (m, 15 H), 10.68 (broad s, 1 H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 29.8 (d, $J = 93.9$ Hz), 70.2, 71.4, 71.4, 73.2, 75.5, 82.8, 83.1 (d, $J = 8.9$ Hz), 83.1, 127.6, 127.6, 127.7, 127.7, 128.3, 128.4, 128.4, 137.4, 137.6, 138.0; $^{31}$P NMR (101 MHz, CDCl$_3$) $\delta$P 28.2; HRMS calcd for C$_{27}$H$_{31}$O$_7$PNa$^+$: 521.1700. Found: 521.1699.

References


APPENDIX A

NMR SPECTRA OF C-PHOSPHONIC ACID DPA ANALOGS

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APPENDIX B

NMR SPECTRA OF C-PHOSPHINIC ACID DPA ANALOGS
APPENDIX C

NMR SPECTRA OF C-SULFONE DPA ANALOGS
APPENDIX D

NMR SPECTRA OF C-PHOSPHONIC ACID 5.7
SOLVENT: D2O
250 MHz
LIST OF REFERENCES

Chapter 1


Chapter 2


8. Incorporation of arabinose into AG has been shown through the use of radiolabeled 3 and mycobacterial membrane extracts. In addition, incubation of small oligosaccharide substrates with 3, in the presence of a mycobacterial membrane preparation, led to the formation of an oligosaccharide with additional β-(1→2) and α-(1→5) linkages (Lee, R. E.; Brennan, P. J.; Besra, G. S. *Glycobiology* **1997**, *7*, 1121). The lack of α-(1→3) linkages was attributed to either instability or absence of α-(1→3) AraT activity in the membrane preparation, or the possibility that this enzyme recognizes oligosaccharide substrates larger than those investigated. It is also conceivable that another activated donor (*e.g.*, a sugar nucleotide) is used by this AraT. The presence of UDP-Araf in mycobacteria has been reported (Singh, S.; Hogan S. E. *Microbios* **1994**, *77*, 217), but incorporation of this donor into arabinan has not been demonstrated.


22. The stereochemistry was proven by comparison of the $^1$H, $^{13}$C and $^{31}$P spectral data previously reported for 14: McClard, R. W.; Tsimikas, S.; Schriver, K. E. *Arch. Biochem. Biophys.* 1986, 245, 282.


29. We attempted simultaneous reduction of the alkene and hydrogenation of the benzyl ethers upon reaction with H₂, Pd/C, but were unsuccessful. Under these conditions, decomposition of the substrate was observed, which we attribute to the formation of a palladium-π-allyl complex from the allyl phosphonate.

30. Iatrobeads refers to a beaded silica gel 6RS-8060, which is manufactured by Iatron Laboratories (Tokyo).


**Chapter 3**


Chapter 4


Chapter 5


