UNIVERSITY OF ALBERTA

Synthesis of Modified Oligosaccharides and Sugar Nucleotides to Probe Galactofuranosyltransferase 2 from *Mycobacterium tuberculosis*

By

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Dedicated to My loving parents and Hongmei

Abstract

Tuberculosis (TB), caused by *Mycobacterium tuberculosis,* is one of the most lethal bacterial diseases in the world. Data from the WHO shows that approximately eight million people are infected by the pathogen every year and one quarter of them die of the disease. Although great efforts have been made to combat TB, the disease has not been eradicated. This is partially due to the ability of *M. tuberculosis* to persist in the host despite the use of antibiotics. The persistent nature is mainly attributed to the cell wall of *M. tuberculosis*, which protects the pathogen from the hostile environment produced by the human immune system and anti-tuberculosis agents.

The mycolyl-arabinogalactan-peptidoglycan (mAGP) complex, a lipidated polysaccharide, is the major structural component of the cell wall and is essential for bacterial viability. The complex consists of three components, mycolic acids, arabinogalactan and peptidoglycan. The biological synthesis of mAGP complex involves several enzymes including galactosyltransferases. Due to their vital significance to *M. tuberculosis* survival, the identification of inhibitors of these enzymes is an active research field. Among them, a galactofuranosyltransferase, termed GlfT2, is responsible for the preparation of the galactan component of the mAGP complex, and has been a research focus of our group. This bifunctional enzyme transfers D-galactofuranose (Gal*f*) residues from the donor species UDP-Gal*f*, to oligosaccharide acceptors in alternating β-(1→5) and β-(1→6) linkages, to achieve the full-length galactan. To date, none of the anti-tuberculosis drugs in clinical use are known to inhibit this enzyme and so it represents a novel drug target.

In my work, I have synthesized four trisaccharide acceptor analogs in which one or two hydroxyl groups have been deoxygenated (**10–13**). These compounds were intended to inhibit the enzyme. When screened against GlfT2, three of the trisaccharides, **10, 12** and **13**, inhibited the enzyme, but the fourth, **11**, does not, and instead surprisingly acts as a substrate. Two analogs of the donor substrate, UDP-Gal*f*, have also been synthesized. These compounds were those in which the hydroxyl group at C5 or C6 was deoxygenated. The 5-deoxy UDP-Gal*f* analog (**18**) displayed inhibition ability and was not a substrate, while the 6-deoxy-UDP-Gal*f* (**20**) was a substrate but not an inhibitor. In addition, precursors to other modified UDP-Gal*f* derivatives, in which single hydroxyl groups have been methylated or deoxygenated, have been synthesized.

Acknowledgements

I would like to express my heartfelt thanks to my supervisor, Prof. Todd L. Lowary, for his talented guidance, continued support, and encouragement throughout my studies, some of which will influence me throughout my life. In the past three years, I learned lots of things from him, not only chemistry, but also the positive and serious-minded attitude to research and life.

I prefer to express my appreciation to Jean Pearcy, Myles Poulin and Ruixiang Blake Zheng, Their professional knowledge and high collaboration are extremely important to me to successfully finish the projects.

I am very grateful to all members of the Lowary group for being so kind and helpful at all time. Special thanks go to Dr. Maju, Dr. Wenjie Peng, Wei Shi, Dianjie Hou and Yu Bai in that they taught me lots of skills and knowledge which are very helpful to my research.

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LIST OF ABBREVIATIONS

Abbreviation	Full Name of Compound
Ac	Acetyl
aq.	Aqueous
AgOTf	Silver trifluoromethane sulfonate
AIBN	2,2′-Azobis(2-methylpropionitrile)
Bn	Benzyl
Bz	Benzoyl
Cat.	Catalytic
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DIAD	Diisopropylazodicarboxylate
DMAP	4-Dimethylaminopyridine
DMF	N,N'-Dimethylformamide
Et	Ethyl
Me	Methyl
NIS	*N*-iodosuccinimide
Ph	Phenyl
Tf	Trifluoromethanesulfonyl
THF	Tetrahydrofuran
TMS	Trimethylsilyl
Tol	Tolyl
Tr	Triphenylmethyl or Trityl

Chapter 1: Introduction

1.1 Background on Bacteria

Bacteria can be defined as a widespread group of single-cell microorganisms without a nucleus. Some cause infectious diseases in animals and humans.[1] The Gram stain was a traditional method used to classify bacteria, which divides these organism into two groups (Gram-positive, Gram-negative).[2] Those called "Gram-positive" are the bacteria with a thick cell wall, which can be stained purple with the "Gram stain", while "Gram-negative" are those bacteria have thin cell walls that are stained pink by this reagent.[3] Today, this classic method is still used to designate bacteria and additional systems that use genetic techniques are also commonly used to further classify bacteria.[4]

The identification of bacteria has important significance to the correct treatment of diseases. Presently, the polymerase chain reaction (PCR) is widely used for this purpose.[5] This technology, which can amplify a tiny amount of the bacterial DNA in several hours, employs instruments (probe-base colorimetric, fluorescence emission technology, etc) to identify bacteria by their genetic information. To date, more than 5,000 species of bacteria have been identified.[6] Most bacteria are harmless and exist as commensal which are protected from the human immune system, and in some cases, they are beneficial. For example, lactobacilli have been used widely to produce cheese and other fermented foods. On the other hand, some pathogenic bacteria are the most formidable enemies of human beings. Among them, a classic example is the organism, *Mycobacterium tuberculosis*, which causes the deadly respiratory disease tuberculosis (TB). As my project is related to *Mycobacterium tuberculosis*, I will focus the rest of my discussion on this organism.

1.2. Background on *Mycobacterium tuberculosis*

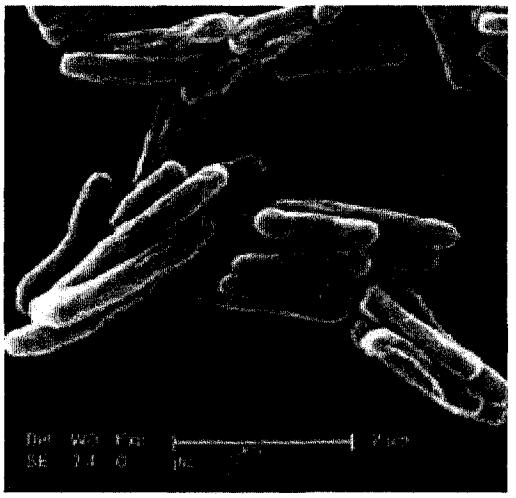


Figure 1.1 Scanning electron micrograph of *M. tuberculosis* (http://www.cbc.ca/health/story/2006/03/17/tb-who060317.html?ref=rss).

Mycobacteria belong to the genus Actinobacteria, which are classified as Gram-positive bacteria due to their lack of a typical outer membrane. In all mycobacterial species, a common characteristic is that they share a hydrophobic and thick cell wall, which significantly contributes to the hardiness of this family of bacteria. As mentioned above, *M. tuberculosis* (**Figure 1.1**),[7] one species of mycobacteria, is the cause of tuberculosis (TB). The following sections present a brief overview of tuberculosis.

1.2.1 History of Tuberculosis

Tuberculosis (TB) is one of the oldest diseases and has greatly influenced human history. Because TB causes skeletal deformation, evidence of the disease can be collected easily from preserved bodies. The earliest case of this disease was discovered from the remains of a bison that died about 18,000 ago,[8] while the skeletal remains of prehistoric humans indicated that people were afflicted by this disease at least 4,000 years ago.[9] Archaeologists have also found that the spines of many mummies were damaged by TB, which suggests the disease was prevalent in ancient Egypt. In 500 B.C.,[10] TB was introduced into Europe and was called "phthisis" in ancient Greece. Hippocrates, a great

physician of ancient Greece, diagnosed phthisis by chest pain and continuous fever, along with coughing up blood.[11] He believed that patients inherited the disease from their families, but Aristotle, the greatest Greek philosopher, disagreed with him and stressed that the disease was infectious.[12]

The industrial revolution brought a new chapter to human history. At that time, urban areas rapidly expanded with increased population. These highly crowded cities provided a very good place for the spread of TB. During the 16th century, TB was one of main reasons causing population decline in Europe.[13] By the first half of the 1800s, the morality of TB reached its peak. Data from Wilson indicates that more than 25% deaths in London were caused by TB.[14]

For thousands of years, people held two different viewpoints about the origin of TB. The first one was presented by doctors from the Mediterranean region, mainly in Italy where it was believed that TB was a contagious disease. Conversely, physicians in the northern Europe opposed this viewpoint because it did not explain why some people were immune to the disease, and supported Hippocrates's theory that the disease was inherited.[15] In the 19th century, the controversy was settled. In 1865, Jean-Antoine Villemin, a French military physician, successfully infected healthy rabbits with a culture of the TB bacteria, thus proving the contagious nature of the disease.[16] This report resulted in strong attacks from the French medical establishment, notably Herman Pidoux, who stressed that TB was caused by social problems, such as poverty, malnutrition, poor sanitation, and overwork. [17] Pidoux argued that the solution should be to improve the life of the poorer classes, a view that was strongly supported by workers at that time.

In 1882, Robert Koch successfully identified a bacterium, which he termed *Mycobacterium tuberculosis* and confirmed that it is the cause of TB a discovery for which he received the Nobel Prize.[18] Following this, Edward Trudeau, an American doctor, did a series of experiments to explain the causes of tuberculosis.[19,20,21] In the first part of his experiments, 10 rabbits treated with the tubercle bacilli were divided into two equal groups and raised in different environments. One group enjoyed a good environment with enough food, while the second group only had a crowded cage and little food. After 3 months, only one rabbit living in the good environment died of TB, and conversely, the mortality of the rabbits raised under poor conditions was 80%. In another comparison experiment, two groups of healthy rabbits were bred in the above environments. All rabbits from the good environment still were healthy after 3 months. Although the rabbits living in poor conditions looked unhealthy, none of them were infected by TB. These results further confirmed that TB was indeed caused by a bacterium, but it was very important to the survival of TB patients that they have a clean environment, enough nutrition, and a good relaxed living situation.

Stimulated by these experiments, many European countries improved their public sanitation systems to reduce the spread of TB. Since the 1850s, the mortality of tuberculosis declined in Europe from 500 out of 100,000 to 50 out of 100,000 in 100 years, which was largely attributed to these massive projects.[22]

The BCG (Bacillius Calmette and Guerin) vaccine was the first effective method to protect people from TB, and was invented by Albert Calmette and Camille Guerin in 1921.[23] The vaccine is an attenuated strain of *Mycobacterium bovis*, which causes TB in cattle. To date, the vaccine is still routinely used in many countries. In 1943, Albert Schatz and Selman Waksman isolated the first antibiotic used for the treatment of TB, Streptomycin.[24] With the development of antibiotics to treat TB, the disease was temporarily defeated, and from the 1950's to the 1980's, the morbidity caused by TB continuously declined in Europe and North America. A survey from the USA indicated that there were 84,304 people infected with tuberculosis in 1953, and the number was reduced to 22,201 in 1985. At the same time, 19,707 people died of tuberculosis in 1953, while the number was 1,752 in 1985.[25]

Figure 1.2 World TB incidence. Cases per 100,000; Red ≥300, orange = 200–300; yellow = 100–200; green 50–100; blue ≤50, grey = N/A (Adapted from Global tuberculosis control - surveillance, planning, financing WHO Report 2006).

However, the situation has changed since the mid 1980s. The morbidity due to TB has increased worldwide, especially in underdeveloped countries (**Figure 1.2**).[26] Currently, more than eight million people develop TB every year and two million of them will die due to the disease.[27] The problem has become more acute due to prevalence of HIV that produces immunocompromised populations susceptible to infection.[28] In addition, inconsistent use and over use of antibiotics have resulted in the emergence of multi-drug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB). Because the above events have happened primarily in underdeveloped countries, such as South Africa and India, it seems clear that poverty is a huge obstacle to the elimination of the disease. In other words, if we cannot overcome poverty worldwide, which usually leads to poor sanitation, malnutrition and overwork, it is hopeless that TB will be completely defeated naturally.[11]

1.2.2 Clinical Manifestations and Pathogenesis of TB

Besides pulmonary TB, a lung disease, *M. tuberculosis* also causes other forms of TB, including spinal tuberculosis, scrofula, meningitis, and other acquilae, which are grouped together and called extrapulmonary TB.[29] All of these forms are mainly transmitted through the respiratory route when people inhale the pathogens in the form of aerosols that are produced by people suffering from active pulmonary TB. After the bacteria enter human lungs, TB develops as illustrated in Figure 1.3.[30, 31]

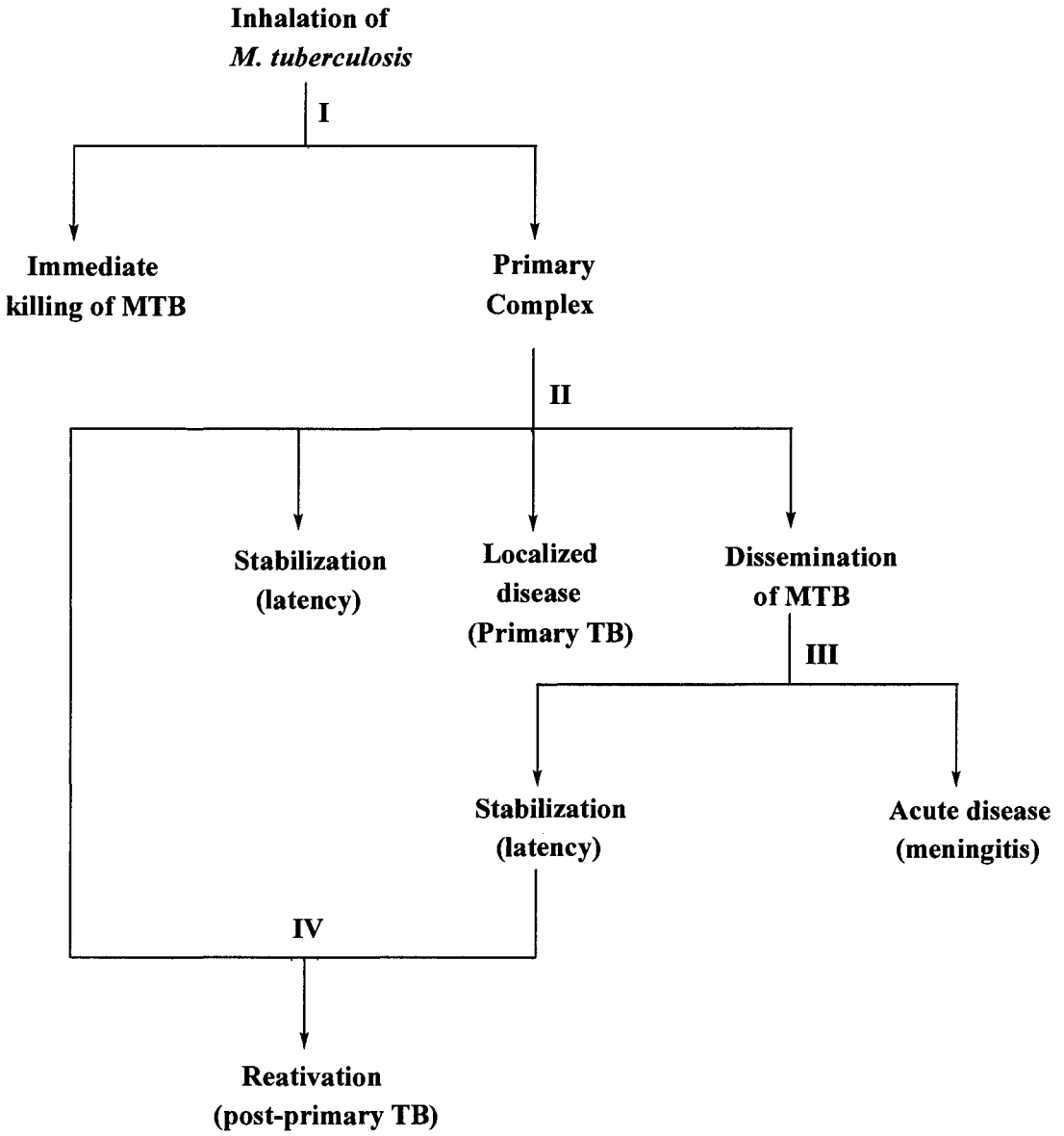


Figure 1.3 Clinical stages of tuberculosis.

At first, the inhaled pathogens are located in the lung alveoli, where they are phagocytosed by macrophages of the immune system. The bacteria are then enveloped by an endocytic vacuole called a phagosome. In some cases, macrophages can destroy these pathogens by phagosome-lysosome fusion, which forms a hostile microenvironment to the bacteria. However, if the immune system is not strong enough, *M. tuberculosis* will

use its persistent nature to adapt to the environment of host cells. For example, the pathogens can arrest the process of phagosome-lysosome fusion and inhibit the Ca^{2+} signaling boost response of the immune system to invaders.[32] Another postulate is that the pathogens possibly take advantage of the macrophages to dodge immunosurveillance by $CD4^+$ T cells.[33]

In the second stage, after the pathogens successfully escape destruction by macrophages, they will duplicate and cause an inflammatory response that induces mononuclear cells to congregate from neighboring blood vessels. The infected macrophages are surrounded by these giant cells and additional macrophages to form the tubercle complex **(Figure 1.4)** with lymphocytes.[34] The immune system uses the complex to prevent the bacteria from diffusing to other organs. Whether or not the infection develops to the next stage depends on the host's immune system. In immunocompetent individuals, their infection usually remains in a latent status, but to populations with immunodeficiencies, the enveloped bacteria can break from the tubercle and diffuse to other parts of the lung by circulation.

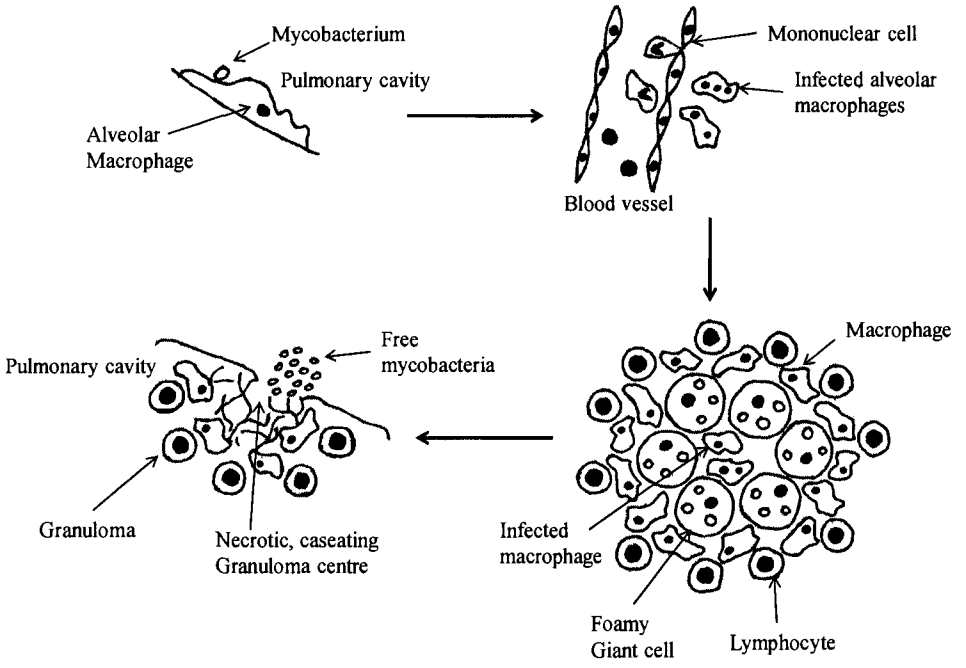


Figure 1.4 Formation and collapse of the tubercle complex
(Adapted and modified from Russell, D. G. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 569–577).

After patients develop to the third stage, many patients have acute symptoms, and even die of fatal diseases, like meningitis. Other patients may convert to disseminated TB, by which patients suffer severe chest pain with strong coughing and weight loss.

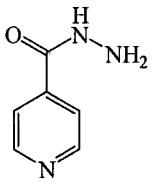
The final infection stage is reactivation of latent TB. Although the factors resulting in reactivation are not fully understood, it is believed that any factors causing the collapse of the host's immune system (such as HIV infection, malnutrition, or overwork) can induce this stage. The last stage due to the reactivated pathogens is called post-primary TB, which is primarily presented in the lungs. It is also frequent that extrapulmonary lesions

occur in bones and joints at this stage, which leads to one of the symptoms, chronic back pain. Without suitable treatments, patients at this stage will die of the disease.

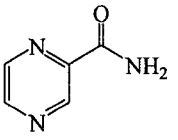
Although humans have made great efforts to combat TB in the past hundred years, the disease has not been eradicated to date. The problem is due, in part, to the uncertain pathogenesis of TB, including persistence and resistance. Persistence means the survival of *M. tuberculosis* despite the use of antibiotics, while resistance is described as genetic mutations of organisms so that they lose their susceptibility to drugs.[35] Because of the persistence of *M. tuberculosis*, the treatments usually require multiple antibiotics and last at least 6 months. In underdeveloped countries, many patients can not afford the lengthy treatment, or do not live in a situation conducive to long drug regimens. The problem is worse for drug-resistant strains. Many strains of *M. tuberculosis* are resistant to one antibiotic, and multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant (XDR-TB) strains have made the current regimens less effective. More expensive and less potent second generation drugs must be used in these cases. Therefore, the identification of new antibiotics for treating TB has become a research focus in the past decades. In the following section, I will briefly introduce the front-line antituberculosis drugs and the standard regimens.

1.2.3 Drugs and Regimens.

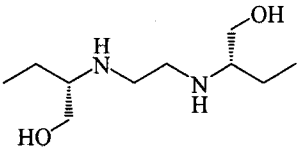
To date, there are five drugs listed as the front-line antituberculosis reagents. The structures of them are shown in **Figure 1.5** and their mechanisms of action are discussed below.[36]



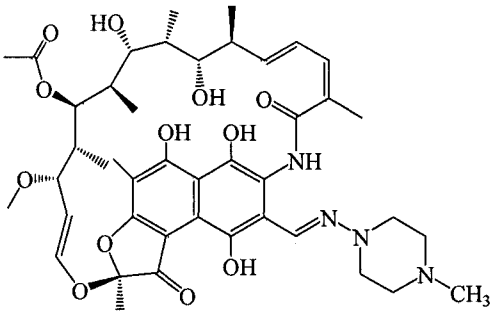
Isoniazid



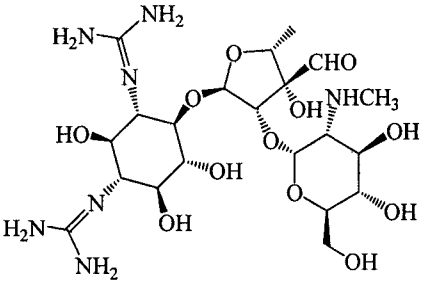
Pyrazinamide



Ethambutol



Rifampicin



Streptomycin

Figure 1.5 Structure of the first line antituberculosis drugs.

Isoniazid (INH) is a pro-drug, which generates isonicotinoyl radicals by activation of *katG*-encoded mycobacterial catalase peroxidase. These reactive reactants couple NAD+ and NADP+ to afford INH-NAD(P) complexes, which inhibit enoyl reductase (InhA). This enzyme is essential in the biosynthesis of mycolic acids that form part of the organism's cell wall (see discussion later).[37] INH can effectively destroy actively growing *M. tuberculosis* while the medicine is also used to treat the latent TB. *Rifampicin* (RMP) prevents the transcription of mRNA and the subsequent synthesis of protein by inhibiting RNA-polymerase.[38] *Ethambutol* (EMB) interferes with the biosynthesis of the arabinofuranose-containing portions of the mycobacterial cell wall (see discussion later) by inhibiting one or more arabinosyltransferase enzymes.[39,40,41] *Pyrazinamide* (PZA) was introduced as an antituberculosis drug in 1985. Although the structure is similar to

Isoniazid, its biological target is uncertain. Recently, it has been proposed that *pyrazinamide* is hydrolyzed by a pyrazinamidase of *M. tuberculosis* to afford pyrazinoic acid, and organelles of the bacterial cells die of the resulting low pH environment.[42] *Streptomycin* (STM or S) was the first antibiotic used to treat TB. The drug prevents the biosynthesis of proteins by inhibiting the 30S ribosome subunit of mycobacteria.[43]

In all drug regimens, the five drugs mentioned above must be used in combination, and the use of a single drug will fail to treat the disease due to the development of resistance.[44] A common disadvantage is that these antibiotics must be taken for a long time (at least 6 months and often longer) to be useful for treating TB, which leads to problems with compliance and in turn drug resistance. It has become a more and more urgent task to prepare highly effective anti-TB agents. By studying the mechanisms of action of these drugs, it has been revealed that many drugs target the biosynthesis of the cell wall in *M. tuberculosis*. Thus, the cell wall has attracted lots of attention for the development of new anti-TB drugs, and also been the focus of our group. In the following section, I will discuss the structure and biosynthesis of the mycobacterial cell wall, which will lead to the rationale behind my project.

1.3 Structure of the Cell Wall of *M. tuberculosis*

As discussed in the first section, mycobacteria are often classified as Gram-positive bacteria. These bacteria have a thick cell wall and usually can be stained purple by Gram stain. However, mycobacteria are different from other Gram-positive bacteria due to their extremely hydrophobic cell wall. Because the cell wall is responsible for substantial protection of mycobacteria from its environment, it is, not surprisingly, the target of

many clinically used antituberculosis agents. The focus of much research in this area is the identification of new compounds that act in this manner.[45]

There is now a good understanding of the structure of the mycobacterial cell wall, which is characterized by a double lipid bilayer structure **(Figure 1.6)**. The outermost layer is composed of lipids and fatty acids including glycolipids, phenolic glycolipids, and the lipidated polysaccharides, lipomannan (LM), lipoarabinomannan (LAM). These are non-covalently bound to a layer of lipids called mycolic acids and together these form one of the bilayers. In turn, the mycolic acids are attached to a large polysaccharide called arabinogalactan-peptidoglycan and this structure is known as the mycolic-arabinogalactan-peptidoglycan (mAGP) complex.[46] The mAGP complex is the largest structural component of the cell wall and located just outside of the second lipid bilayer, the plasma membrane. The mycolic acids of the cell wall are organized in a nearly crystal-like structure,[47] which provides the bacteria with significant protection from its environment. This structure is essential to the viability of mycobacteria tuberculosis. In the following section, I will discuss more about each of these cell wall components.

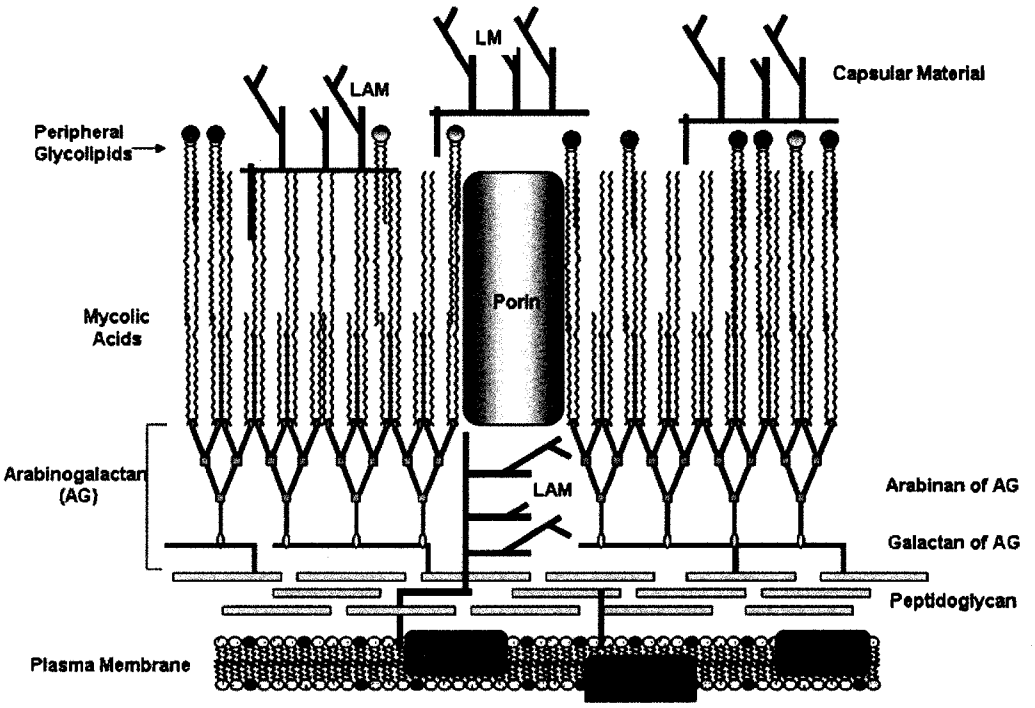


Figure 1.6 Structure of the cell wall of *M. tuberculosis*.
LAM: lipoarabinomannan, LM: lipomananan

1.3.1 Lipoarabinomannan (LAM) and Lipomannan (LM)

Lipoarabinomannan (LAM) and lipomannan (LM) are two glycolipids, located in the outermost layer of the cell wall of mycobacteria. They are composed of three components, a polysaccharide core, a phosphatidylinositol anchor and capping motifs.[48] The polysaccharide core of LAM has two types of domains, arabinan and mannan, while lipomannan (LM) has only the mannan.[49]

The generalized structure of LAM is shown in **Figure 1.7**.[50] The arabinan is connected with the phosphatidylinostol anchor via a linear D-mannan, made up of α-(1→6) linked manopyranose residues. About half of these mannose units have another mannopyranosyl residue attached to O-2. In contrast to the mannan domain, which has a relatively simple structure, the arabinan has a more complicated branched structure. The

backbone of the arabinan is a linear polymer of α-(1→5)-D- arabinofuranosyl (Ara*f*) groups, which has α-(1→3)-D-Ara*f* branch points, and the chains are terminated by β-(1→2)-D-Ara*f* residues. The terminal structures in this polysaccharide are either linear tetra-arabinofuranosides (Ara4) or biantennary hexa-arabinofuranosides (Ara6). The non-reducing ends of these side chains are terminated by the capping motifs, either one or more α-D-mannopyranose residues (ManLAM), inositol phosphate groups, or 5-deoxy-5-α-thiomethyl-α-D-xylofuranose (MTX) residues. LAM from *M. tuberculosis* is capped by mannose or MTX residues. In addition to its major function as an antigen of the cell wall, which recognizes the mannose receptor on macrophages, ManLAM also demonstrates a variety of biological activities such as suppression of T lymphocyte proliferation, [51] and inhibition of γ–interferon-mediated activation of murine macrophages.[52] In fast growing mycobacteria, like *M. smegmatis,* the LAMs are called PILAM due to their inositol phosphate caps.

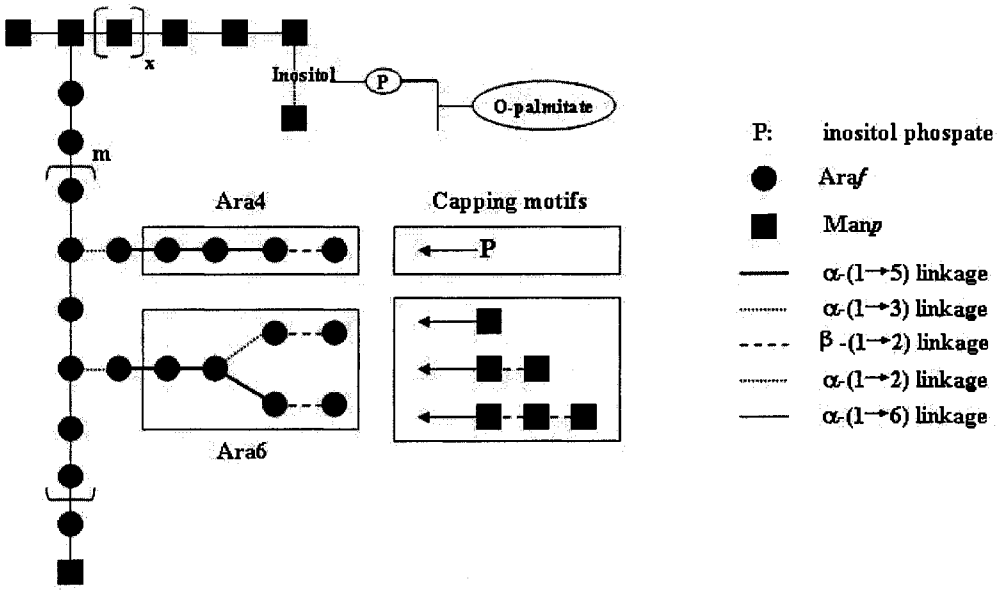


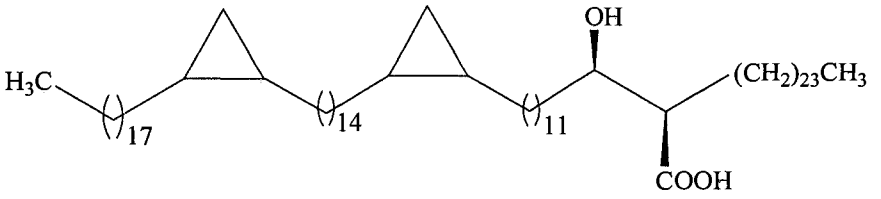
Figure 1.7 Schematic depiction of LAM (Adopted from Vercellone, A.; Nigou, J.; Puzo, G. *Frontier in Bioscience* **1998**, *3*, e149–163.).

1.3.2 Mycolic Arabinogalactan Pepetidoglycan (mAGP) Complex

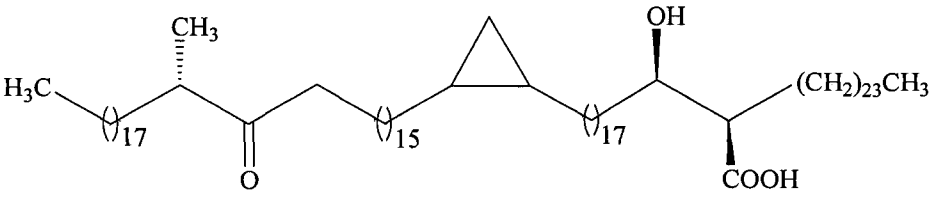
The mAGP complex is a lipidated polysaccharide consisting of three domains: mycolic acids, arabinogalactan and peptidoglycan.

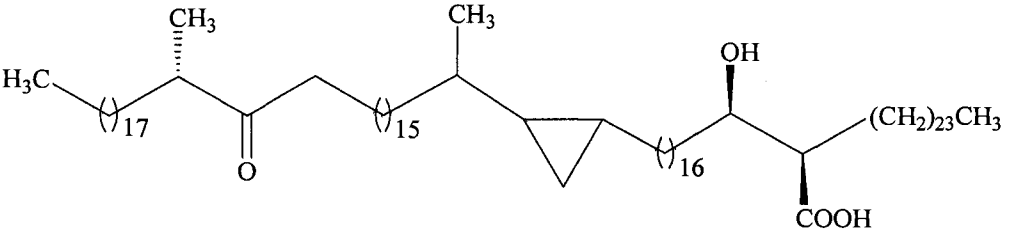
1.3.2.1 Mycolic Acids

As defined by Asselineau and Lederer, mycolic acids are β-hydroxy fatty acids with a long α-alkyl side chain. The family contains more than 500 branched hydroxyl acids with a large number of carbon atoms (60-90).[53] They can be divided into different groups, depending on their functional groups, for instance, methoxymycolates and ketomycolates. These compounds are present in the mAGP complex of mycobacteria and other actinomycetes. *M. tuberculosis* has three types of mycolic acids, α-mycolates, ketomycolates and methoxymycolates, which are shown in **Figure 1.8**. In total, the α-mycolates are the most abundant form (>70%) in *M. tuberculosis*, while the other two forms are present in minor amounts (10-15%).[54] The mycolic acids are covalently esterfied to the non-reducing termini of the arabinogalactan (AG). Due to strong van der Waals interactions among these tightly packed lipids, the mAGP complex presents a nearly crystalline structure with very low permeability to the environment. In addition, mycolic acids offer many other important functions to *M. tuberculosis*. Besides, the basic function in the cell wall skeleton, they are involved in the response to resistance against chemical injury, and dehydration.[55]

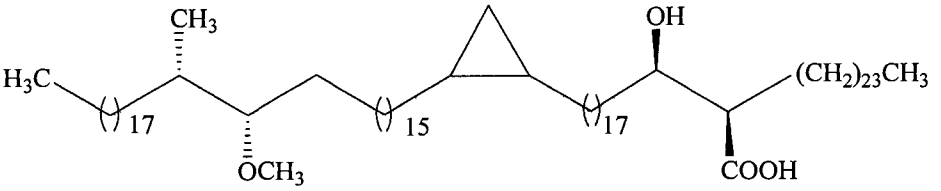


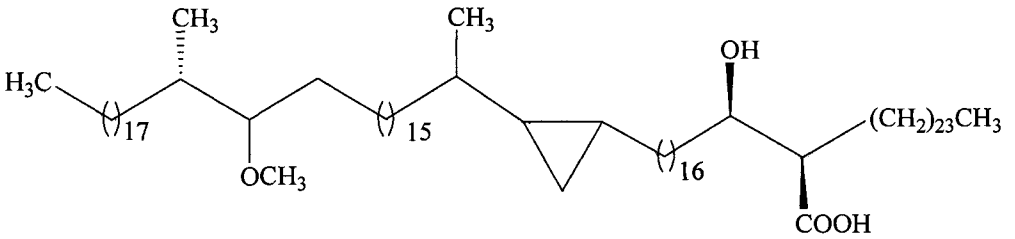
α-mycolates





ketomycolates





methoxymycolates

Figure 1.8 Representative structures of mycolic acids in the mAGP complex of *M. tuberculosis*.

1.3.2.2 Arabinogalactan (AG)

The arabinogalactan contains two kinds of carbohydrate residues, arabinofuranose (Ara*f*) and galactofuranose (Gal*f*). As shown in **Figure 1.9**,[43,56,57] the branched arabinans are attached to a linear galactan backbone, which is, in turn, connected to the peptidoglycan anchor. The galactan backbone contains approximately 30 D-Gal*f* residues, which are sequentially arranged via alternative β-(1→5) and β-(1→6) linkages. The branched arabinans consist of by ~90 D-Ara*f* residues attached through three different linkages, α-(1→5), α-(1→3) and β-(1→2).

To date, although the biological function of the arabinogalactan is still not well understood, but it has been postulated that it simply serves as a scaffold for attaching the mycolic acids to the cell wall complex.

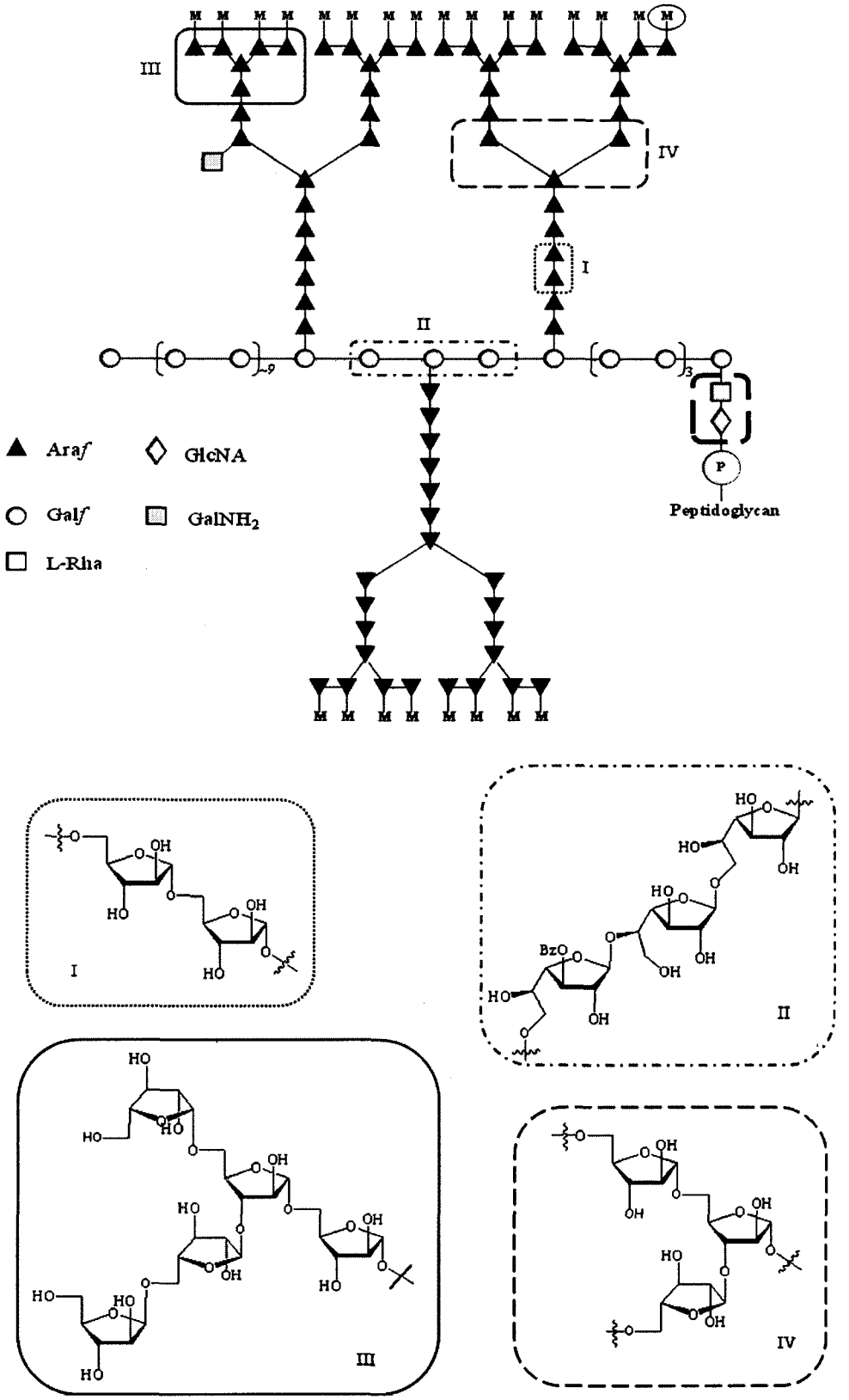


Figure 1.9 Structure of the arabinogalactan domain

1.3.2.3 Peptidoglycan

Peptidoglycan is a mesh-like polymer consisting of sugars and amino acids. Two sugar residues, β-N-acetylmuramic acid and β-N-acetylglucosamine are connected in alternating (1→4) linkages in the backbone of the molecule. In every disaccharide unit, a tetrapeptide chain (L-alanine, D-isoglutamic acid, L-lysine, D-alanine) is attached to the β-N-acetylmuranmic acid and crosslinked to a neighboring tetrapeptide chain through a peptide bridge **(Figure 1.10).**[58] The primary function of peptidoglycan is to maintain the cell shape and it also serves as a scaffold to anchor other cell wall components.

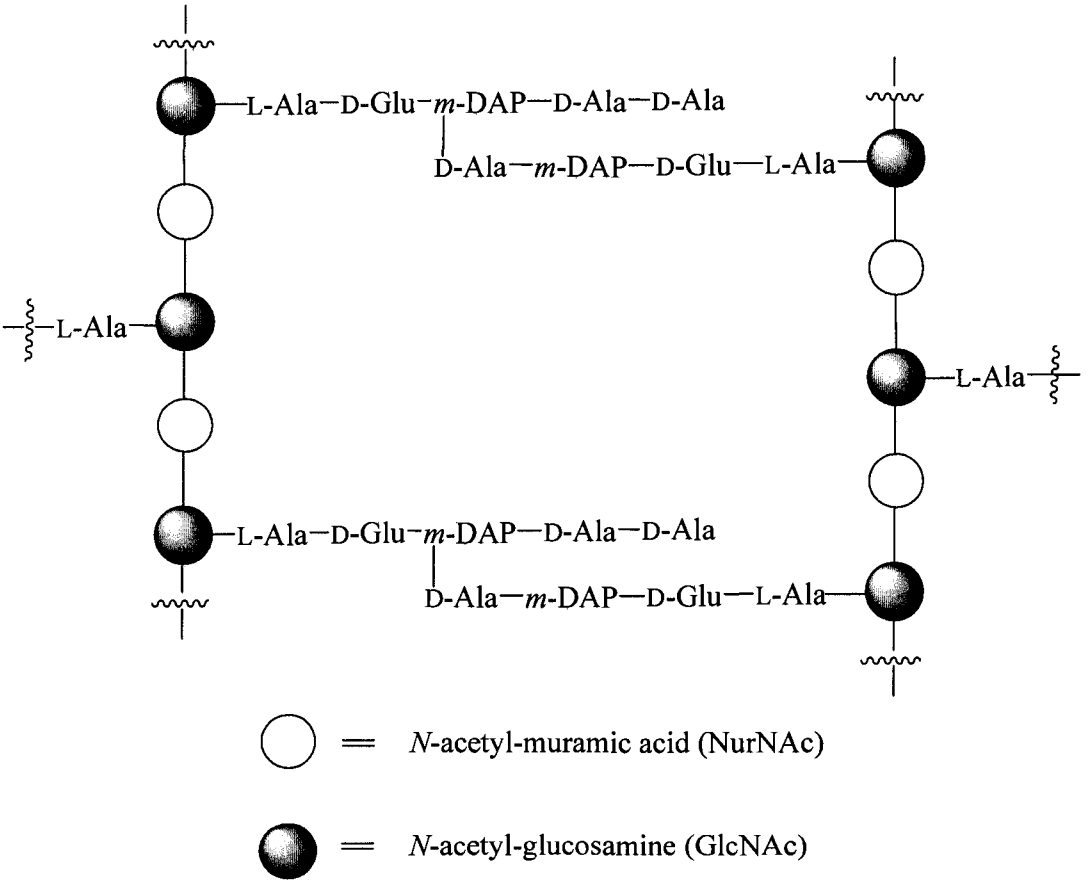


Figure 1.10 Structure of the peptidoglycan.

1.3.2.4 Biosynthesis of the mAGP

In 2000, Brennan and coworkers proposed a biosynthetic pathway for the mAGP complex **(Figure 1.11)**.[59] At the beginning, one 2-acetamido-2-deoxy-glucopyranose (Glc*p*NAc) and one rhamnopyranose (Rha*p*) residue are added from their sugar nucleotide donors (UDP-Glc*p*NAc and TDP-Rha*p*) to a polyprenol chain to form what is known as the "linker disaccharide". Following this, approximately 30 Gal*f* residues are transferred from UDP-Gal*f* by galactofuranosyltransferases to form a linear galactan backbone. Then, the Ara*f* residues are attached to this backbone using the donor Ara*f*-P-decaprenol and catalyzed by arabinosyltransferases. Next, the entire arabinogalactan (AG) domain is coupled to peptidoglycan, and the non-reducing terminus of the molecule is capped by the mycolic acids to afford the whole mAGP complex.

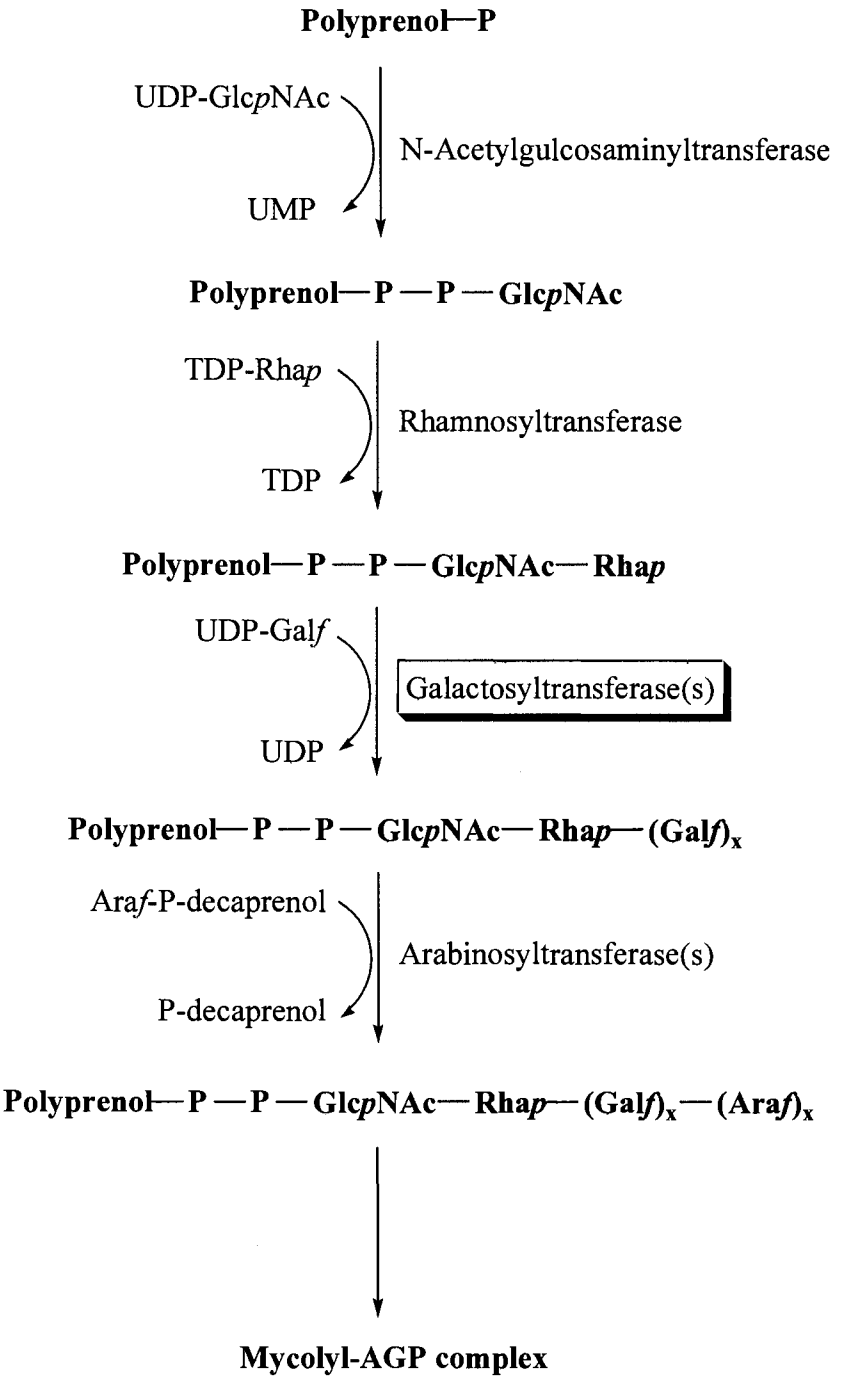


Figure 1.11 Proposed biosynthetic pathway of the mAGP complex (Adopted from Rose, et al, *J. Am. Chem. Soc.* **2006**, *128*, 6721–6729).

1.4. Research of Galactofuranosyltransferases

1.4.1 Current Progress

In the above section, I have given a brief introduction to the structure and biosynthesis of the mycobacterial cell wall. In the biosynthesis of the mAGP complex, several enzymes have been shown to be essential for the viability of the organism. The work in this thesis is related to the galactofuranosyltransferases that are involved in the assembly of the cell wall.

Although none of the drugs used to treat TB has been shown to inhibit these enzymes, it is known that galactan biosynthesis is essential for the organism to survive.[60] Furthermore, Gal*f*-containing glycoconjugates are absent in humans.[61] Hence, these enzymes are good targets for potential new antituberculoisis agents. In fact, research on the enzymes involved in galactan assembly has been studied for close to a decade and a more detailed picture of this process was proposed recently **(Figure 1.12)**.[62] Only two different galactofuranosyltransferases, termed UDP-galactofuranosyltransferase 1 (GlfT1) and UDP-galactofuranosyltransferase 2 (GlfT2), appear to be involved in the process. In 2000, Brennan and coworkers identified that the gene, *Rv*3808c from *M. tuberculosis*, encodes GlfT2, which is 68 kDa protein.[63,64,65] More recently, it has been shown that the gene *Rv*3782 encodes for GlfT1, a 34 KDa protein.[66] Both GlfT1 and GlfT2 are members of an increasing number of bifunctional glycosyltransferases[67] that disobey the "one-enzyme, one-linkage" rule.[68] GlfT1 produces both β-Gal*f*-(1→5)-β-Gal*f* and β-Gal*f*-(1→4)-α- Rha*p* linkages, while GlfT2 can synthesize both β-Gal*f*-(1→5)-β-Gal*f* and β-Gal*f*-(1→6)-β-Gal*f* glycosidic bonds.

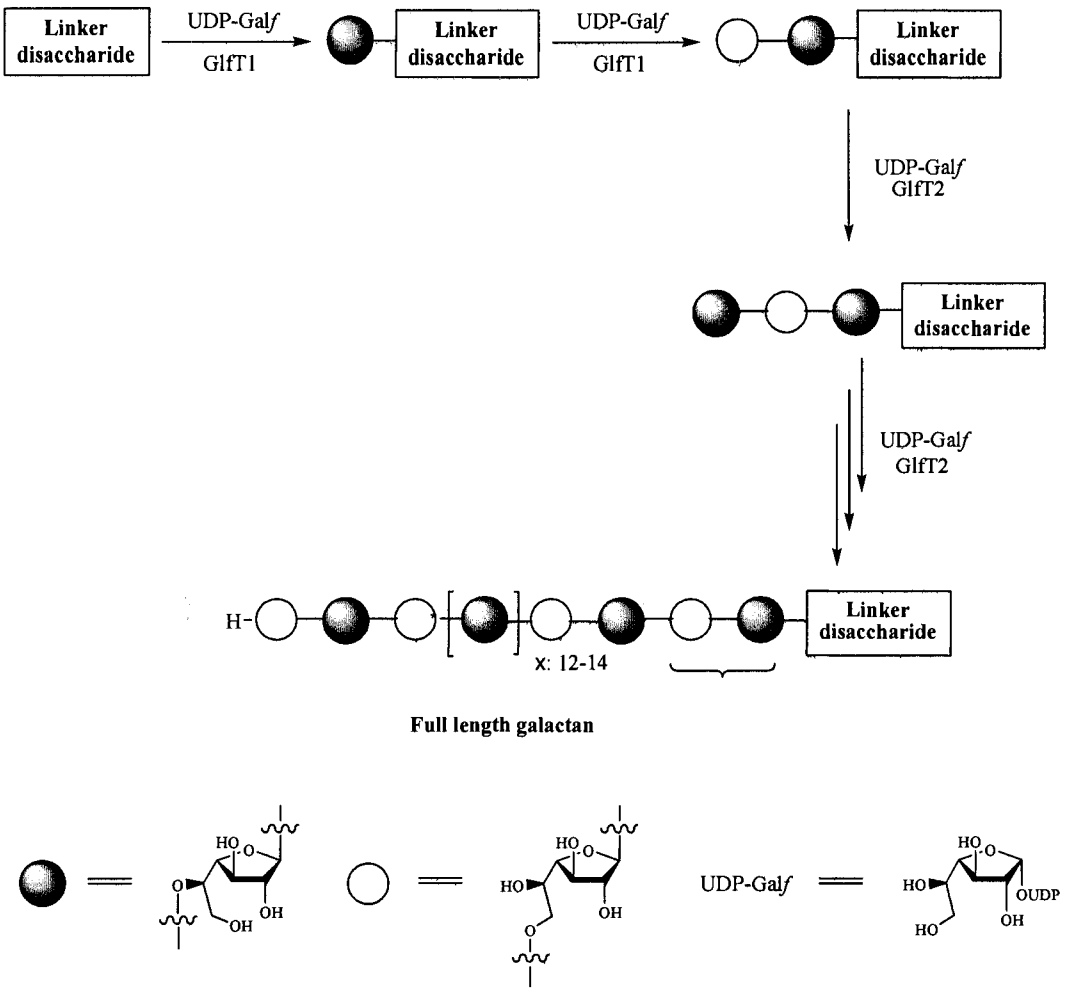


Figure 1.12 Proposed pathway for the assembly of the galactan domain of the mAGP complex (Modified and adapted from Rose et al, *Carbohydr. Res.* **2008**, *343*, 2130–2139.).

1.4.2 Function of Galactofuranosyltransferases (GlfT1 and GlfT2)

The formation of the galactan is divided into two stages each catalyzed by a single enzyme. In the first stage, GlfT1 attaches a β-Gal*f* residue from UDP-Gal*f* to the Rha*p* residue of the linker disaccharide via a β-(1→4) linkage.[69,70] The trisaccharide moiety is further elaborated by GlfT1 with the second Gal*f* residue donor (again from UDP-Gal*f*) through a β-(1→5) linkage to form a tetrasaccharide. Following this, the

galactofuranosyltransferase (GlfT2) substitutes the first enzyme (GlfT1) to finish the biosynthesis, in which more β-Gal*f* residues are sequentially added to the formed tetrasaccharide moiety in alternating β-(1→6) and β-(1→5) linkages to yield the fully elongated product (**Figure 1.13**).[62] It is presently unknown how the synthesis of the chain is terminated and it may be possible that a third enzyme is involved in this process.

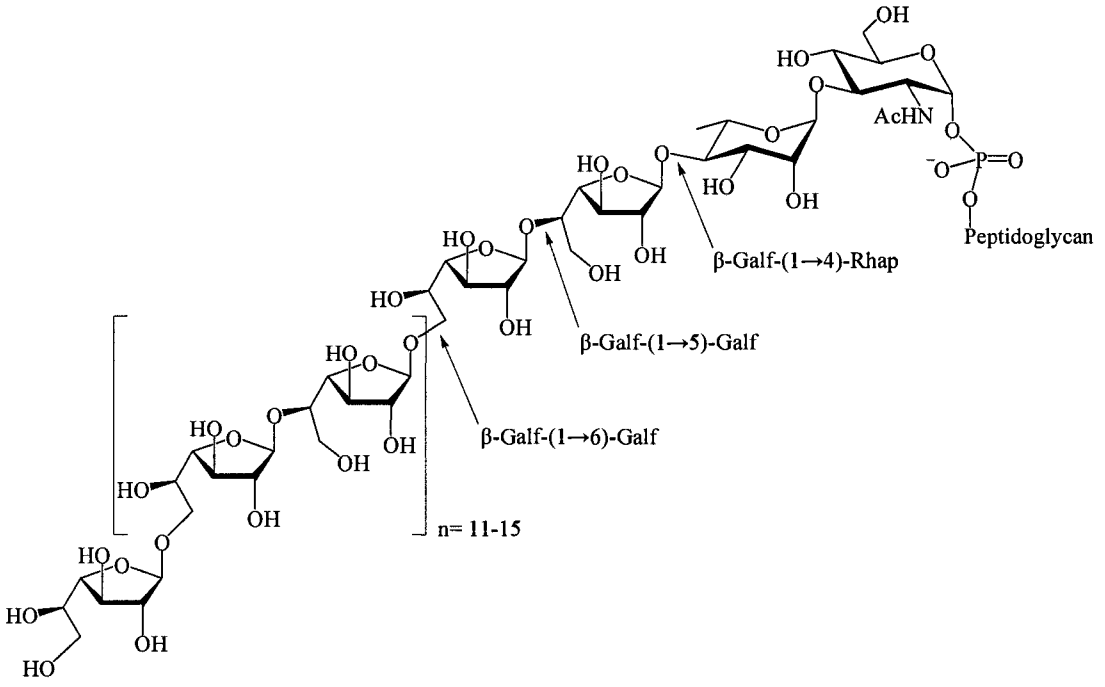


Figure 1.13 Linkages present in galactan.

1.4.3 Investigation of GlfT2 by Our Group

1.4.3.1 Previous Research

As part of a large program, my project developed from previous work in our group. To better present my thesis project, it is necessary to review this previous work first.

The ultimate aim of our program is to identify inhibitors of GlfT2. We expect that these inhibitors will be novel antituberculosis agents in the future. Our group was the first

to express and purify this protein and by using a panel of synthetic oligosaccharides **(Figure 1.14)**.[64] NMR spectroscopy and high resolution mass spectrometry of products produced by GlfT2 confirmed that the recombinant enzyme has the same activity as the wild-type one. Like the latter, it is bifunctional and can synthesize both β-(1→6) and β-(1→5) linkages.

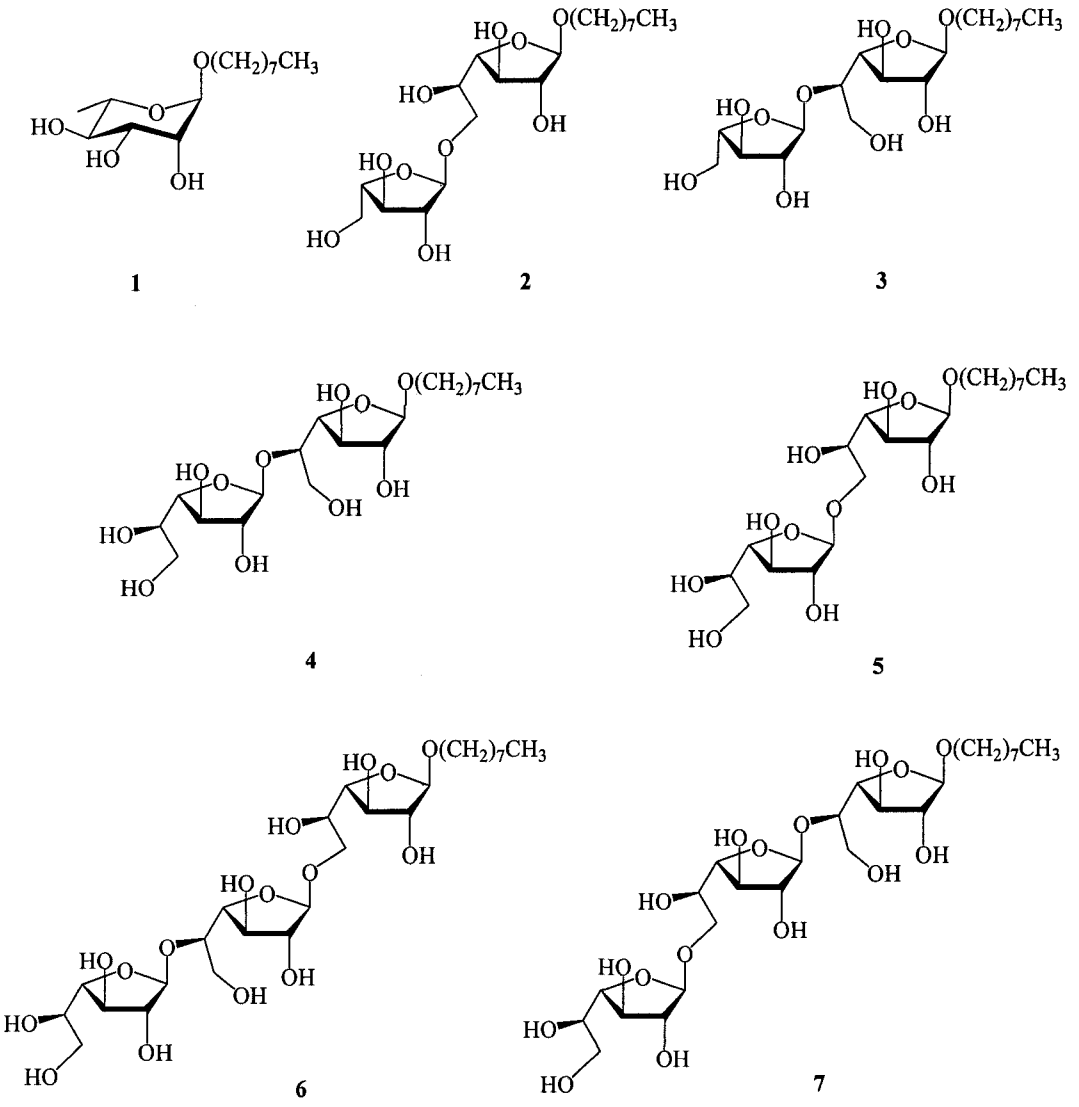


Figure 1.14 Acceptors used to probe specificity of GlfT2.

With the enzyme in hand, we characterized the substrate specificity of GlfT2 using the donor UDP-Gal*f* and saccharide acceptors. Whereas these saccharide acceptors were prepared with classic chemistry methods, our group chose a chemoenzymatic synthesis (**Figure 1.15**)[71] to prepare UDP-Gal*f* due to higher yield than the other reported methods, which rely on enzymatic synthesis, or chemical synthesis.

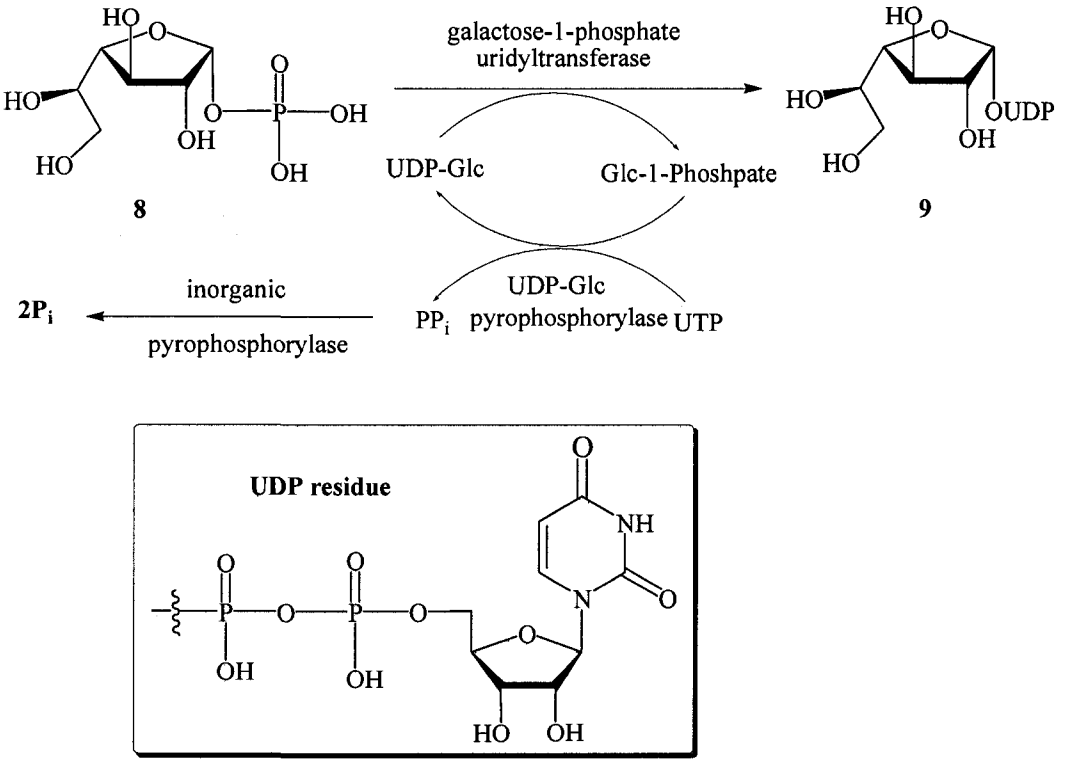


Figure 1.15 Chemoenymatic synthesis of UDP-Gal*f*.

Following the synthesis of these substrates, our group evaluated the biological activities of the saccharide acceptors with GlfT2.[64] The results indicated that trisaccharides **6** and **7** are better substrates than monosaccharide **1**, as well as two disaccharides **4** and **5**. Disaccharides **2** and **3**, in which the terminal D-Gal*f* residues are substituted by L-Ara*f* residues are weak substrates. The lack of activity of **1** supports the

proposed biosynthetic pathway of the galactan moiety, in which GlfT1 rather than GlfT2, attaches the first UDP-Gal*f* donor to the rhammonsyl moiety. This previous work built up a solid foundation for my thesis research.

1.4.3.2 Current Research

The primary goal of my project is to synthesize compounds that can be used to identify structure-activity relationships (SAR) between GlfT2 and a variety of modified substrates, including donors and acceptors. Such compounds may be potential inhibitors of the enzymes and useful biochemical tools.

In the previous studies, trisaccharides **6** and **7** (**Figure 1.14**) afforded a ladder of oligosaccharide products including the expected tetrasaccharides. The result is expected because the tetrasaccharides produced by the enzyme are also acceptors for further glycosylation reactions. GlfT2 is a bifuncational enzyme and one question that arose from previous work is if it possesses one or two active sites. To probe this, one goal of my project was to synthesize deoxygenated analogs of **6** and **7**, which were expected to be inhibitors of the enzyme. We designed the following two deoxygenated trisaccharides **10, 11** (**Figure 1.16**) and expected that they would serve as GlfT2 inhibitors as they lack the hydroxyl group necessary for glycosylation by the enzyme. However, because previous STD-NMR studies[72] had suggested that the GlfT2 only has a single active site, we postulated that the enzyme might glycosylate the adjacent hydroxyl group when it is faced with a mono-deoxygenated trisaccharide. Hence, we also synthesized trisaccharides **12** and **13 (Figure 1.16)**, in which both hydroxyl groups on the exocyclic fragment of the terminal Gal*f* residue are deoxygenated.

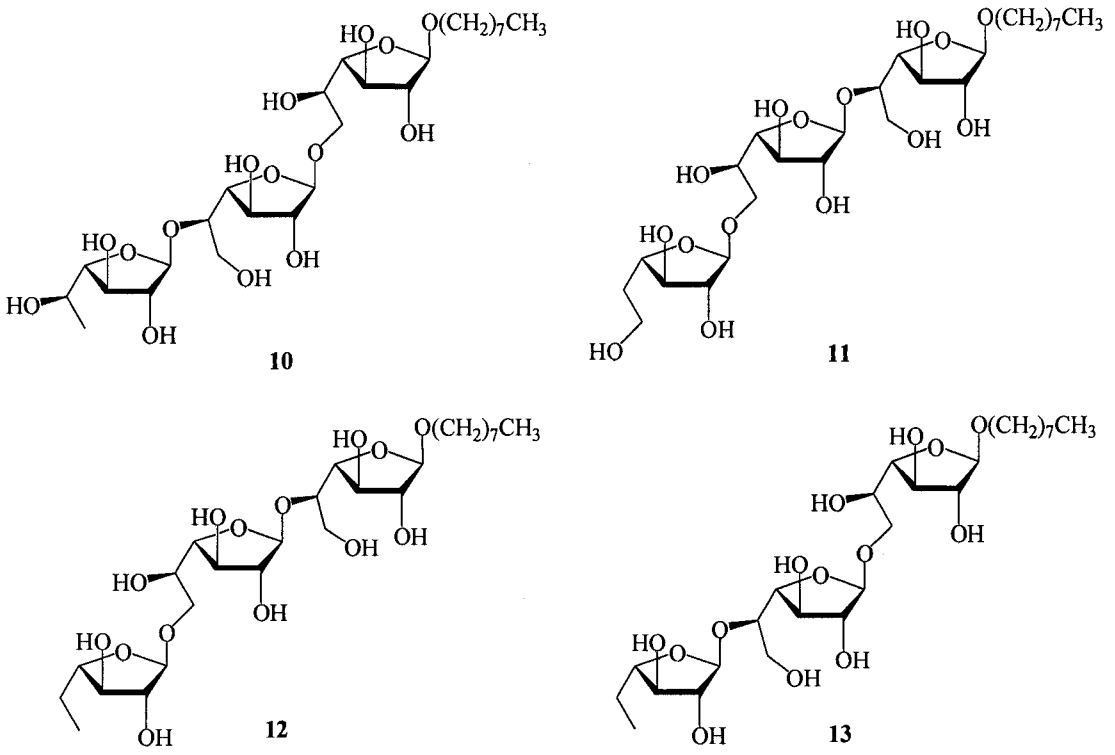


Figure 1.16 Structure of synthesized trisaccharides (**10-13**).

Along with the preparation of acceptors, we also want to probe the role of the UDP-Gal*f* donor in the enzyme reaction. We targeted for synthesis of a series of analogs for the further studies (**14-21**, **Figure 1.17**). To further probe the interaction between the donors and GlfT2, in addition to deoxygenated compounds, we also targeted methylated donors. We expected that these analogs could provide more detailed information for us to figure out the specificity between the GlfT2 and its substrates.

I completed the synthesis of the four trisaccharides (**10-13**) and their biological evaluation has been finished, while only two UDP donors (**18** and **20**) were synthesized; the others are still in the progress. In the next chapters, I will discuss the synthesis of these compounds.

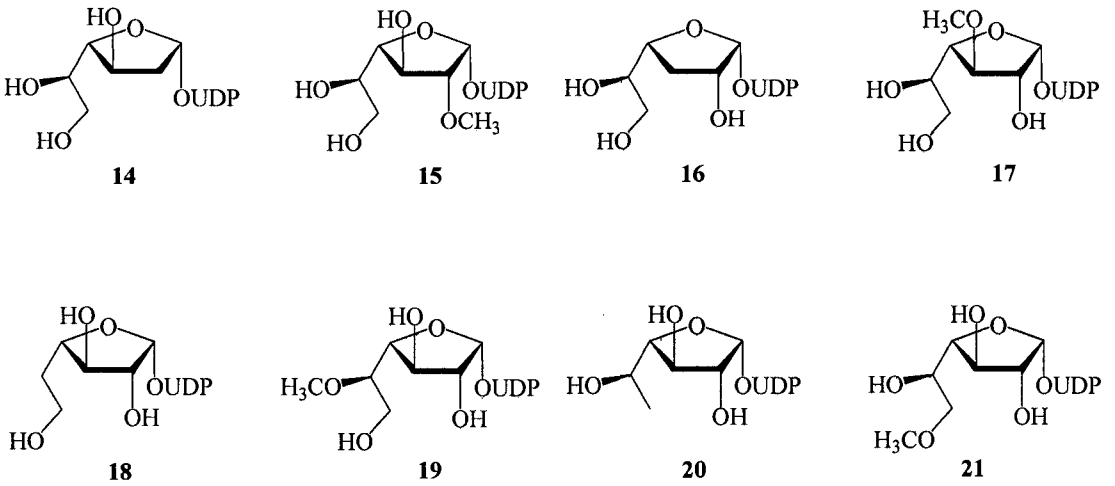


Figure 1.17 Structure of proposed UDP-Gal*f* donor analogs (**14-21**).

Chapter 2: Synthesis of Deoxygenated Analogs of Trisaccharide Acceptors for GlfT2

2.1 Project Aim

In previous studies, we[57, 59] and others[60,73] have probed the specificity of GlfT2 with a panel of di- and trisaccharides, which indicated that the trisaccharide **6** and **7** (**Figure 2.1**) are better substrates than other fragments of the mAGP tested. Based on these results, we proposed that if the two trisaccharides lose one or two exocyclic hydroxyl groups from their terminal residue, the resulting oligosaccharides may be the inhibitors to the GlfT2, not substrates any more. To prove the proposal, the first goal of my project was to synthesize four deoxygenated trisaccharides **(Figure 2.1)** to probe their ability to act as substrates or inhibitors of GlfT2.

To achieve our goals, a retrosynthetic analysis led us to seven monosaccharide building blocks **(Figure 2.1)**. Among them, compounds **22-25** have been reported already by our group, while compounds **26-28** were not known and their preparation was my first task. In the following sections, I will provide a detailed discussion of the pathways used for the synthesis of these three deoxygenated monosaccharide building blocks, and the formation of glycosidic linkages between them.

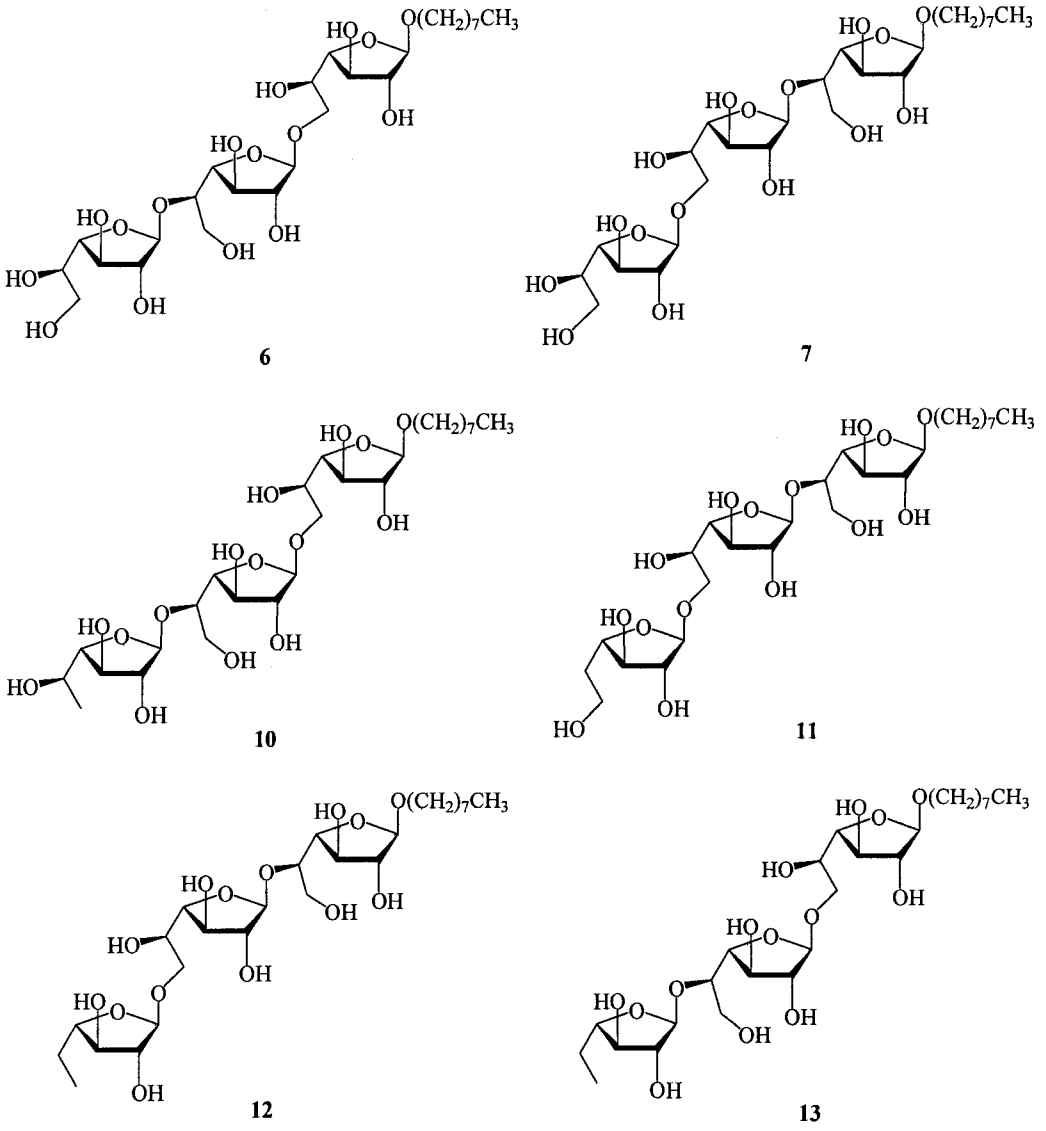


Figure 2.1 Trisaccharide substrates for Glf2 (**6-7**) and deoxygenated analogs (**10-13**).

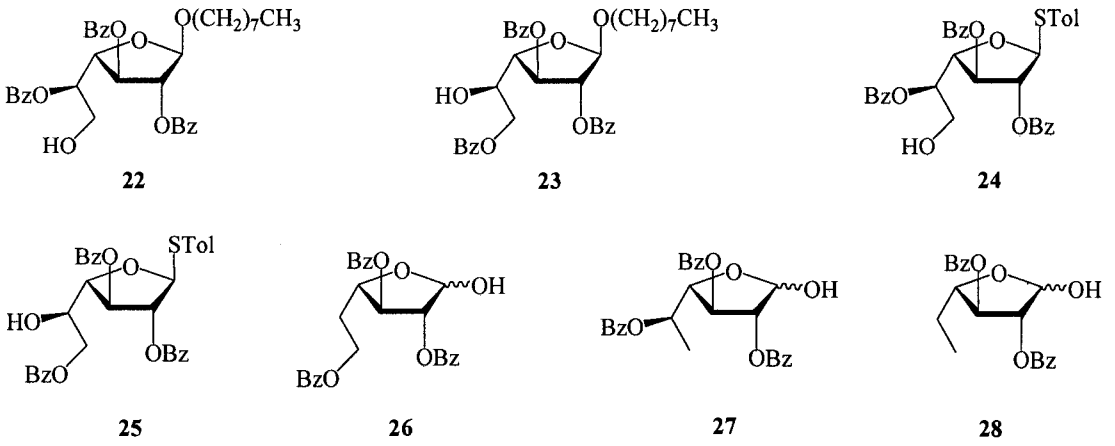


Figure 2.2 Structure of target building blocks for deoxygenated analogs.

In this thesis, NMR spectroscopy and HRMS were employed to characterize all target products and their intermediates. Furthermore, to identify anomeric stereochemistry of these glycosidic linkages, the magnitude of the $^{3}J_{H\text{-}1,H\text{-}2}$ and the chemical shift of the anomeric carbon from NMR spectroscopy provided firm evidence to me. In my projects, all $^{3}J_{H\text{-}1,H\text{-}2}$ of the trisaccharides and chemical shift of anomeric carbons of the final compounds (including the UDP-Gal*f* analogs described in Chapter 3) are listed in the **Table 2.1**. The data exactly match the previous trends that correlate anomic stereochemistry in galactofuranosides with NMR parameters.[74]

Table 2.1 Coupling constants of H-1's and chemical shift of C-1's in targets

	$^{3}J_{H\text{-}1,H\text{-}2}$ (Hz)			$\delta_{C\text{-}1}$ (ppm)		
	H-1	H-1'	H-1"	C-1	C-1'	C-1"
6"-deoxy-(1→5)-(1→6)-trisaccharide (**10**)	0	0	0	107.8	108.6	107.9
5"-deoxy-(1→6)-(1→5)-trisaccharide (**11**)	0	0	0	108.0	108.2	108.6
5",6"-dideoxy-(1→5)-(1→6)-Trisaccharide (**12**)	0	0	0	107.9	107.6	107.2
5",6"-dideoxy-(1→6)-(1→5)-Trisaccharide (**13**)	0	0	0	108.2	108.3	108.5
6-deoxy UDP-Gal*f* (**20**)	4.3			100.1		
5-deoxy UDP-Gal*f* (**21**)	4.2			98.8		

2.2 Overall Synthetic Approach

2.2.1 Synthesis of 6"-Deoxy-trisaccharide 10.

Galactose is an abundant monosaccharide, which exists mainly in the pyranose form in nature. To convert the galactopyranose into galactofuranose, a variety of approaches have been reported in the past. Among them, Fischer glycosylation[75,76] is the most popular method. The reaction is carried out by reacting galactopyranose with an alcohol in the presence of an acidic catalyst. Under kinetic conditions, this process affords an unprotected galactofuranoside isomer. Other synthetic routes involve the benzoylation of D-galactopyranose at high temperature,[77] the reduction of galactonolactones,[78] and the cyclization of dithioacetals catalyzed by mercuric salts.[79] These methodologies have the following limitations: (1) Fischer glycosylation often gives a mixture of galactofuranosides and galactopyranosides, which is difficult to purify; (2) The benzoylation of D-galactopyranose at high temperature is hard to control. (3) The reduction of galactonolactone involves expensive reagents (disiamylborane) and (4) mercuric salts are unfriendly to human health and the environment.

In 1986, Szarek and co-workers developed a variation of the dithioacetal cyclization method.[80] In this alternative approach, iodine (0.5% by weight) was added into an alcoholic solution of a series of dithioacetals (e.g., **30**, **Figure 2.3**), which afforded the corresponding furanosides. Compared to the previous conditions (involving mercuric salts), iodine is less toxic and the reaction is easier to work up; however, the reported procedure usually takes more than 12 h and frequently even a whole day. Recently, our group reported a modification of these conditions.[81] When the concentration of iodine was increased to 5% (by weight) in the alcohol, the dithioacetal **30** can be rapidly and

efficiently converted into the galactofuranosides without contamination by the galactopyranosides. This procedure is relatively simple and efficient so our group chose it to prepare methyl 2,3,5,6-tetra-*O*-acetyl-α/β-D- galactofuranoside **31**, as shown in **Figure 2.3**.

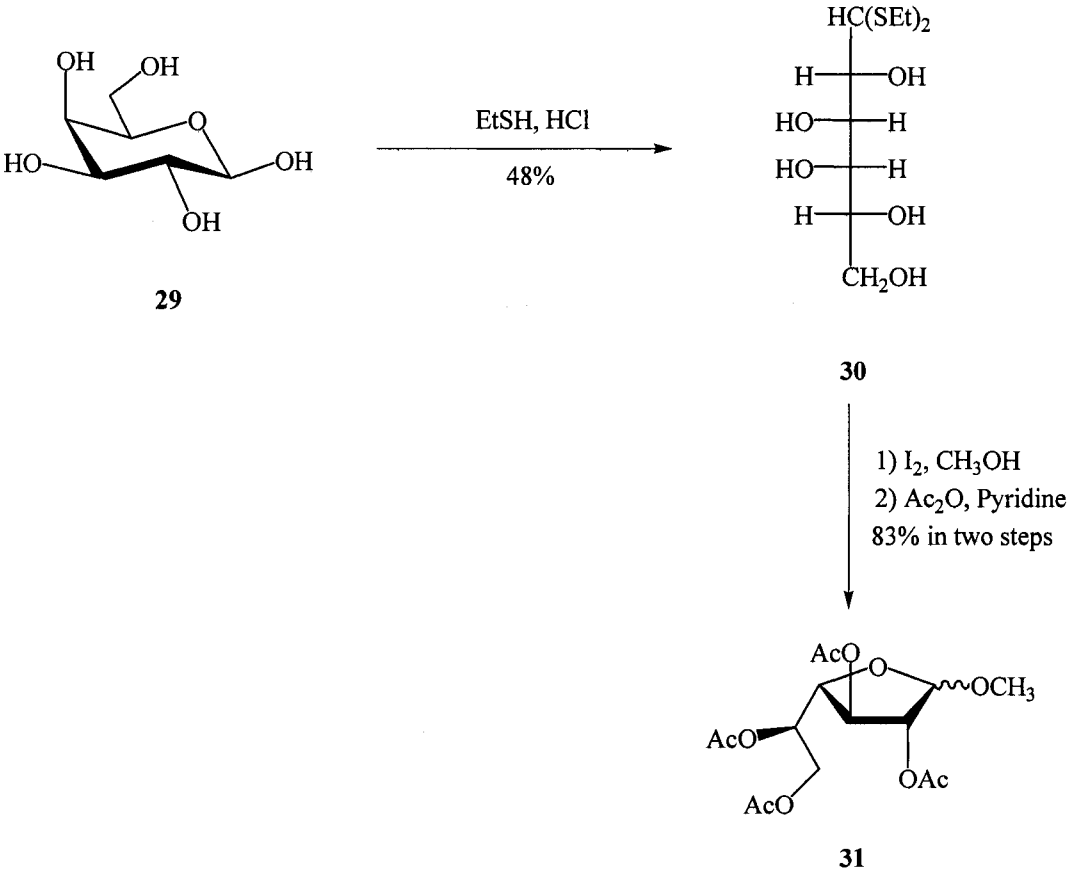
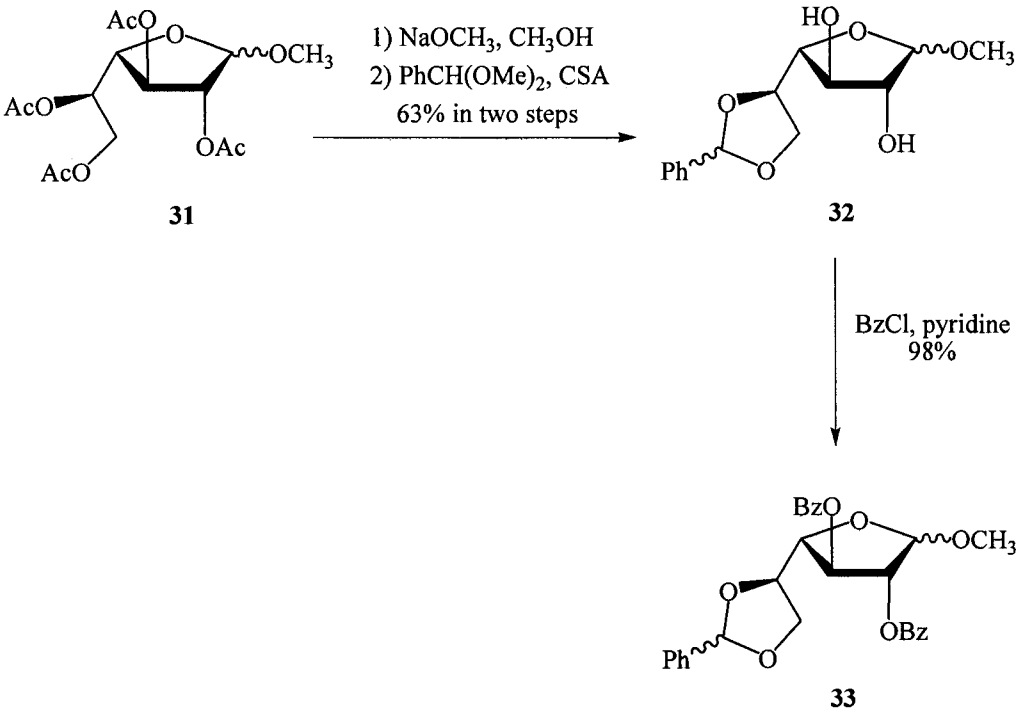


Figure 2.3 Synthesis of a mixture of isomers **31**.

First, D-galactopyranose **29** was treated with ethanethiol in the presence of hydrochloric acid to afford the dithioacetal **30**, which can be purified by recrystallization from ethanol as a white solid in 48% yield.[82] Ideally, this compound should be stored open in the fume hood for several days to remove trace amounts of ethanethiol. This white dithioacetal was dissolved in methanol with 5% iodine to form a pair of isomers,

methyl α/β-D-galactofuranosides. At the end of the reaction, solid sodium thiosulfate and solid sodium bicarbonate were added to quench the excess iodine and to neutralize the reaction mixture before concentration. The presence of these salts complicated purification at this stage and thus the crude products were acetylated[83] to provide an organic-soluble product that could be readily separated from these salts. Overall, the cyclization afforded compound **31** in 83% overall yield.



31 **32** **33**

Figure 2.4 Synthesis of acetal **33**.

Methyl glycoside **31** was treated with sodium methoxide in methanol[84] to remove the acetate esters and after deacetylation, the excyclic diol fragment was selectively protected with benzaldehyde dimethyl acetal and camphorsulfonic acid (**Figure 2.4**).[85] This two-step sequence produced **32** as a mixture of isomers in 63% overall yield.

Because **32** contained four isomers, which were impossible to separate by column chromatography, the assignment of NMR spectra was very difficult and only partial NMR spectra could be assigned. Fortunately, the HRMS spectra of **32** showed that molecular weight exactly matched the expected value, so we proceeded to the next step. Benzoylation of the remaining hydroxyl groups in **32** afforded **33** in 98% yield. As was the case for **32**, the product **33** was also a mixture of four stereoisomers and thus it was hard to prove their structures by NMR spectroscopy; however, HRMS provided the evidence that allowed us to continue to the next steps. In my project, compound **33** is a key intermediate, which was used to synthesize two distinct deoxygenated building blocks for use in the preparation of the targets.

With compound **33** in hand, *N*-bromosuccimide (NBS) was employed to cleave the benzylidene acetal and to install a bromine atom at C-6; $BaCO_3$ is used in the reaction as a scavenger of acid formed in the process. The mechanism of this transformation, known as the Hanessian-Hullar reaction (**Figure 2.5**),[86] is believed to follow the pathway shown in **Figure 2.6**. Due to the existence of the trace HBr, NBS generates a tiny amount of bromine, which homolytically cleaves at high temperature to release bromine radicals. Upon hydrogen abstraction by the bromine radical, **33** afforded a bromo-acetal intermediate **35** via the radical **34**. Following this, loss of bromide ion from **35** with the assistance of the neighboring oxygen atom produces an oxacarbenium ion **36**. Finally, the bromide anion attacks C-6 of the cyclic acetal **36** to provide the 6-bromo substituted **37**.

Compound **37** (**Figure 2.5**) was the precursor for the following hydrogenation reaction in which it was expected to be reduced to afford the 6-deoxy building block. Initially, the hydrogenation catalyzed by 10% Pd–C was attempted under 15 PSI (1 atm)

H_2. Unfortunately, no product was obtained from the reaction. After ruling out other possibilities, such as a poisoned palladium catalyst, or the choice of solvents, we focused our attention on the hydrogen pressure. Fortunately, when the catalytic hydrogenation was performed at 45 PSI (3 atm) H_2,[87] compound **37** was successfully reduced to afford the 6-deoxy derivative **38** in 98% yield.

To convert compound **38** into a donor, its anomeric methoxyl group had to be removed first. Usually, due to the strength of the C–O bond, this transformation is carried out with strong acids, including HBr[88] or $BF_3 \cdot OEt_2$.[89] In my case, when **38** was reacted with 44% HBr in HOAc, the methoxyl group was substituted by a bromine atom to yield a very unstable intermediate. During the work up with H_2O, the bromine atom quickly exchanged with a hydroxyl group to afford **27** in excellent yield (**Figure 2.5**).

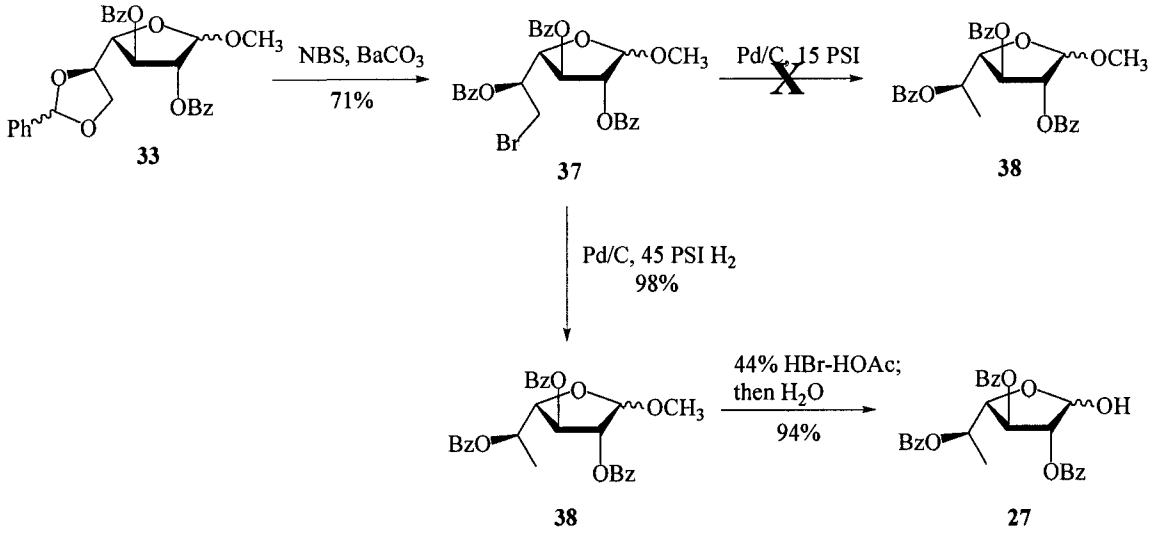


Figure 2.5 Synthesis of 6-deoxygenated building block **27**.

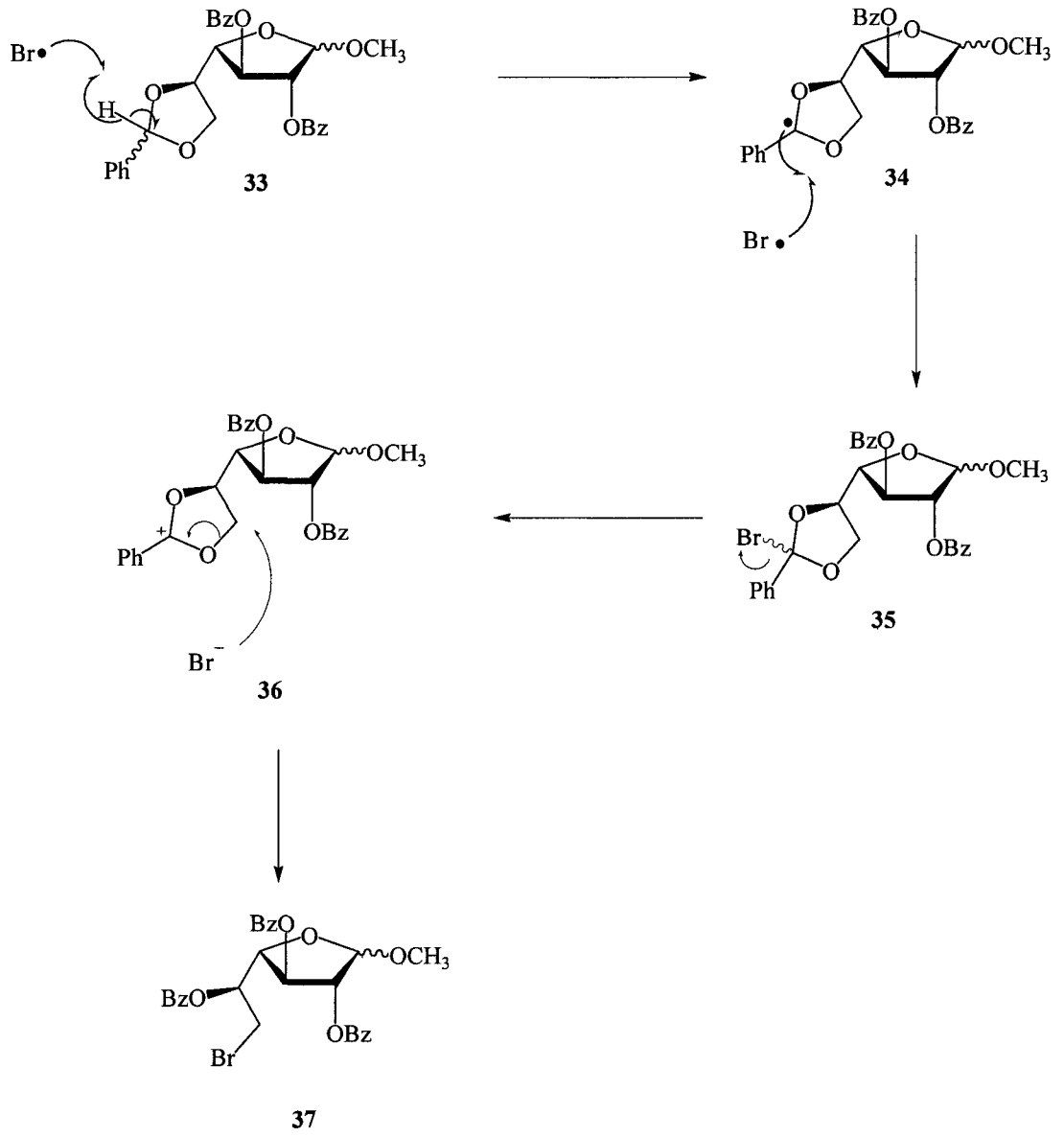


Figure 2.6 Mechanism of preparation of **37.**

Trichloroacetimidates were introduced by Sinaÿ [90] and well established by Schmidt.[91] Depending on the type of base used, either α- or β-trichloroacetimidates may be easily prepared from reducing sugars. Several Lewis acids can activate these donors, such as TMSOTf or $BF_3.OEt_2$. The donors are highly active and can be used to prepare glycosidic linkages in good yields. Here, compound **27** was coupled with

trichloroacetonitrile in the presence of DBU to provide a mixture of imidates. Due to the instability of this donor to silica gel chromatography, it was purifed by rapid passage through a silica column. At the same time, the acceptor tolyl 2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside **25** was prepared using known procedures.[75] The glycosylation reaction was performed to give the 6'-deoxy disaccharide **39** in 72% overall yield (**Figure 2.7**).

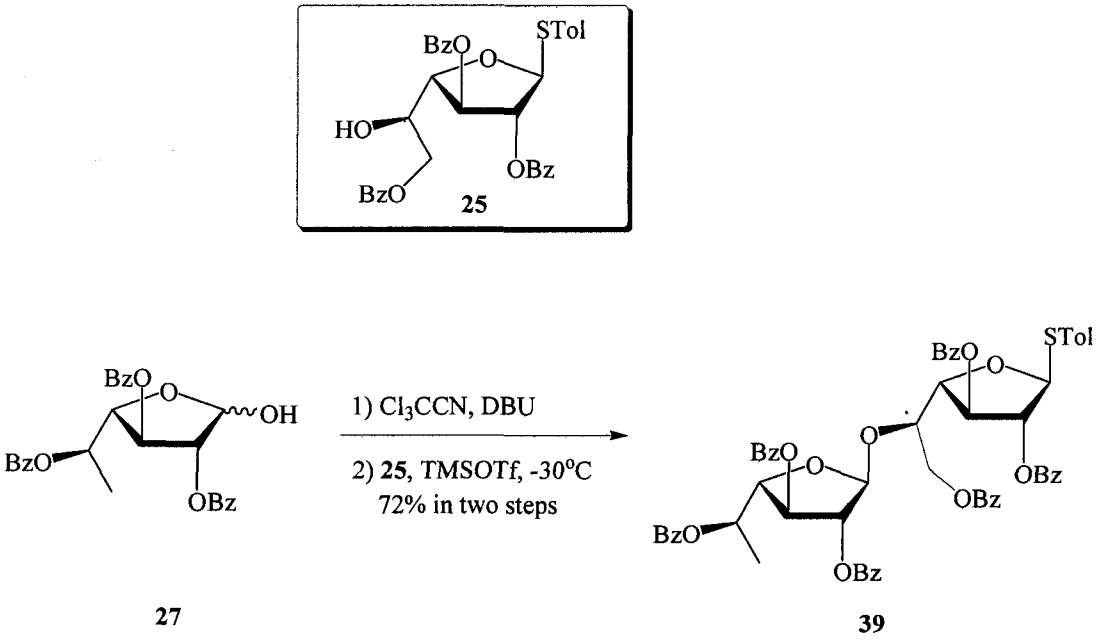


Figure 2.7 Synthesis of thioglycoside disaccharide **39**.

Thioglycoside **39** is a hydrolytically stable donor, which is only activated under specific reaction conditions. The common promoters include N-iodosuccinimide (NIS) and silver triflate, [92] iodine dicollidine perchlorate (IDCP), [93] and dimethy-(methylthio)-sulfonium triflate (DMTST).[94] In my case, NIS and AgOTf were employed, by which the donor **39** was attached to the acceptor **22** to form the trisaccharide **40** in

93% yield. After debenzoylation, the target product **10** was obtained in excellent yield of 96% (**Figure 2.8**). For trisaccharide **10**, all anomeric protons appeare as singlets in the ^{1}H NMR spectrum and the anomeric carbons have chemical shift from 108.6 to 107.8 ppm in the ^{13}C NMR spectrum. Based on these data, the structure of trisaccharide **10** is confirmed (see **Table 2.1** also).

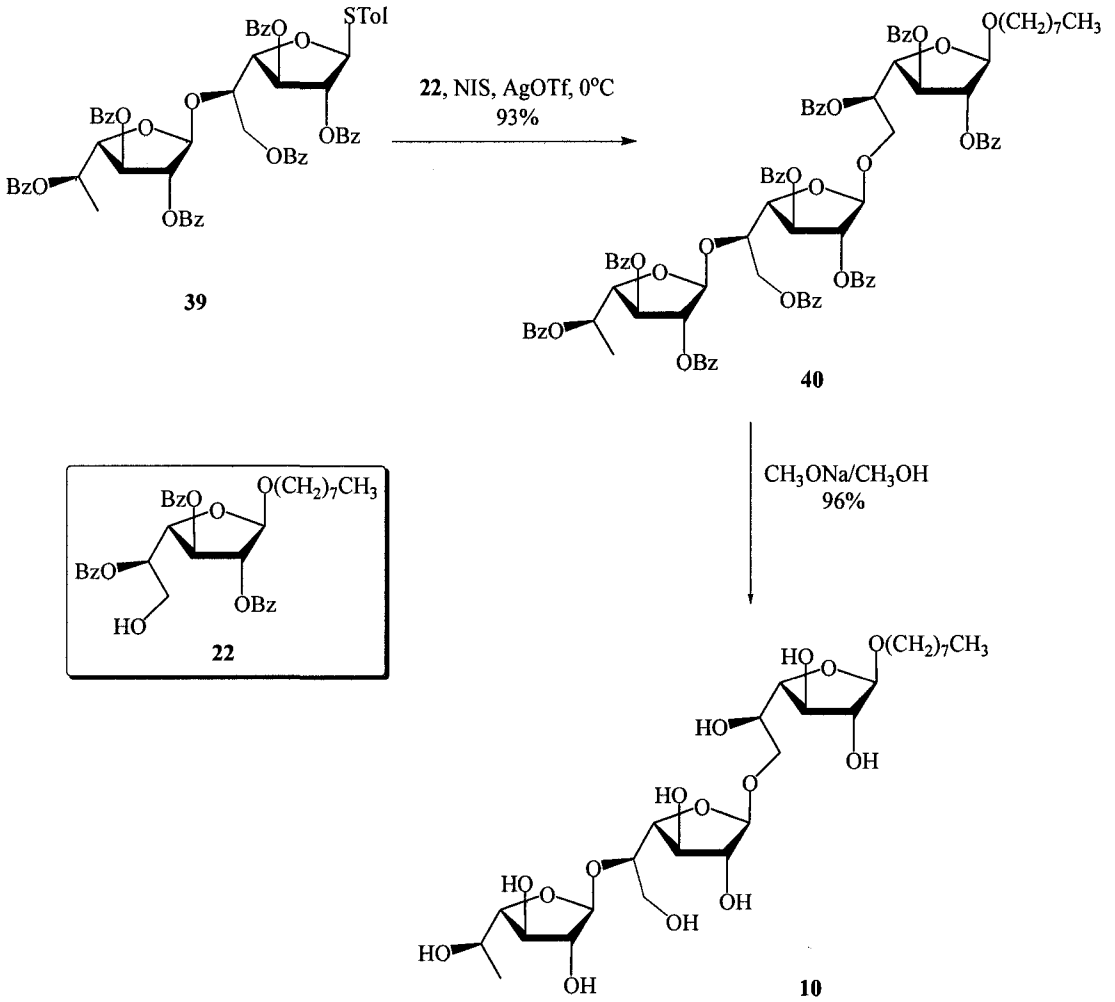


Figure 2.8 Synthesis of 6-deoxy-β-D-Gal*f*-(1→5)-β-D-Gal*f*-(1→6)-β-D-Gal*f*-octyl **10**.

2.2.2 Synthesis of 5"-Deoxy-trisaccharide 11

It has been mentioned that compound **33** is a branch point that allowed us to prepare not only 6"-deoxy trisaccharide **10**, but also 5"-deoxy derivative **11**. Thus **33** was treated with a solution of 70% HOAc in H_2O to remove the benzylidene acetal, by which acylic diol **41** was prepared in 67% yield (**Figure 2.9**). Both of the two acyclic hydroxyl groups of **41** were protected as toluenesulfonate esters by reaction with *p*-toluenesulfonyl chloride in pyridine along with the presence of the nucleophilic catalyst 4-(dimethylamino) pyridine.[95] This reaction afforded **42** in 87% yield. After heating at reflux with solid NaI for 4 hours, the two tosylate groups were eliminated to afford alkene **44** in 93% yield. [96]

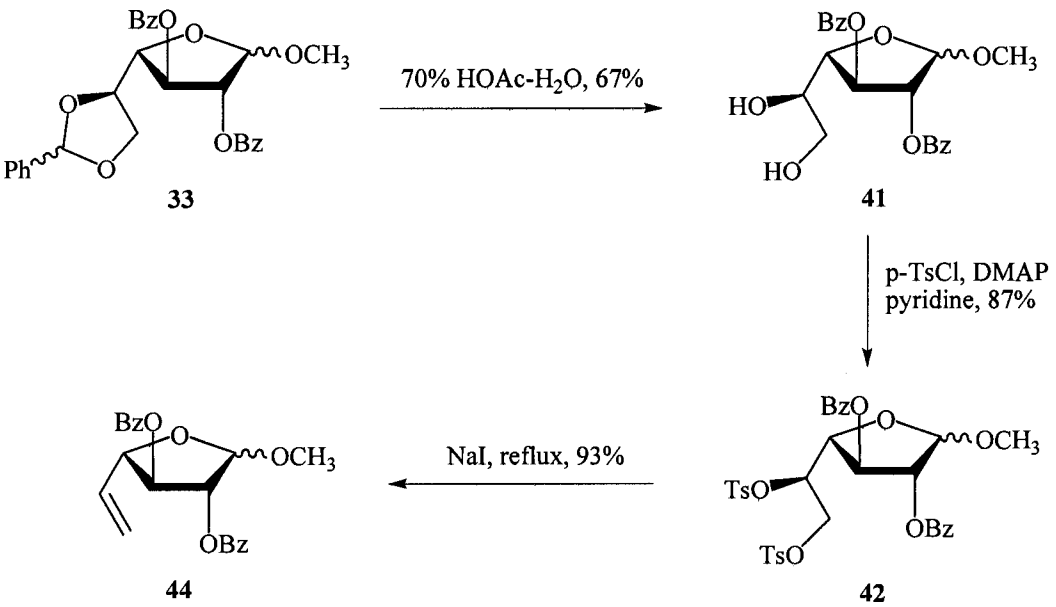


Figure 2.9 Synthesis of alkene **44**.

A possible mechanism is proposed for the elimination reaction (**Figure 2.10**). Although both of the two TsO groups are good leaving groups, the iodide anion prefers to

substitute the group on the C-6 due to less steric hindrance. The reaction produces a 6-iodo-5-tosylate intermediate **43**. Following this, another iodide anion attacks the 6-iodo substituent in **43**, which results in a sequential elimination reaction to afford alkene **44**.

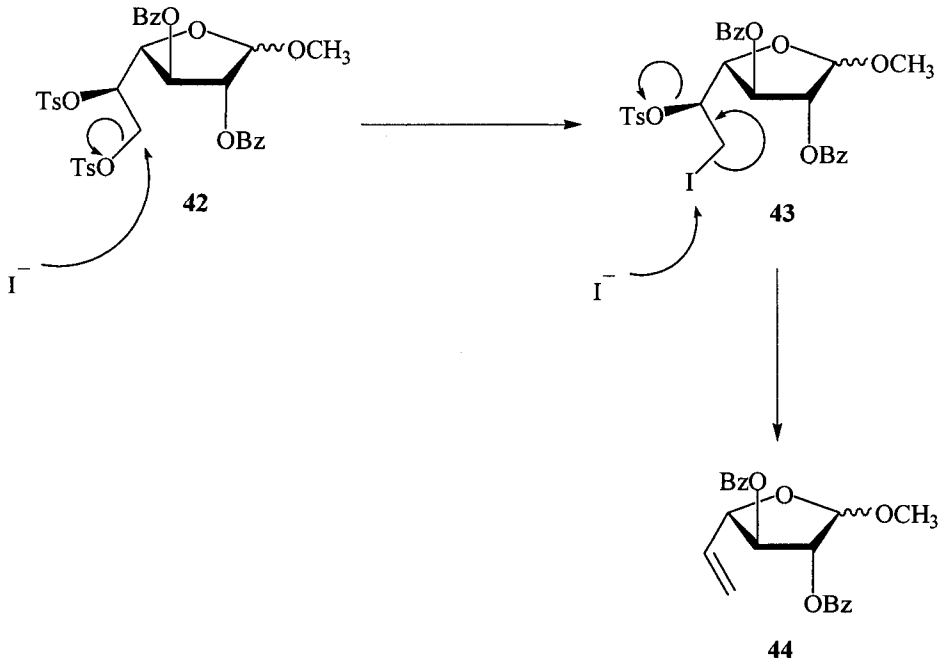


Figure 2.10 Mechanism of preparation **44**.

With **44** in hand, it was expected that the compound could be converted into the anti-Markovnikov product by the regioselective hydration via a hydroboration- oxidation sequence, the most common procedure to achieve this transformation. Unfortunately, although we attempted several kinds of borane reagents, including 9-BBN and BH_3, all of them failed to afford the expected hydroboration product for reasons that are unknown. To address this issue, another procedure was adopted (**Figure 2.11**). This procedure, reported by Szarek and co-workers,[97] uses iodine and silver trifluoroacetate to first produce a 5-iodo-6-*O*-trifluoroacetate species, which is the reduced with Raney Nickel and hydrogen to afford the desired product. When applied to **44**, this sequence afforded

intermediate **48**. The hydroxyl group in compound **48** was sequentially benzoylated to afford the fully protected 5-deoxy derivative **49** (**Figure 2.11**).

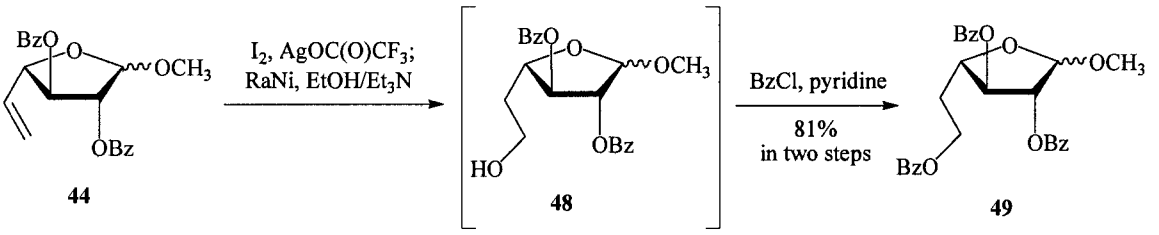


Figure 2.11 Synthesis of methyl 2,3,6-tri-*O*-benzoyl-5-deoxy-α/β-L-*arabino*-hexofuranoside **49**.

The proposed mechanism for the reaction is shown in **Figure 2.12**. Initially, the iodine bond is polarized by interaction with the silver cation. This increases the electrophilicity of I_2, by which the electron pair of the alkene attacks iodine to result in cleavage of the I–I bond. In turn, the lone pair of iodine moves to the alkene in concert leading to iodonium ion **45**. Following this, a trifluoroacetate anion opens the iodonium to afford 5-iodo-6-*O*-trifluoroacetate species **46**. The regioselectivity of the opening of iodonium ion **45** is presumably due to steric effects, which direct the nucleophile to the least hindered primary position. Subsequent hydrogenation of **46** followed by hydrolysis under basic condition leads to the reduction of the C–I bond and cleavage of the trifluoroacetate ester. Because the trifluoroacetate group is a strong electron-withdrawing substituent, this group can be easily hydrolyzed in the weakly basic solution to form compound **48**.

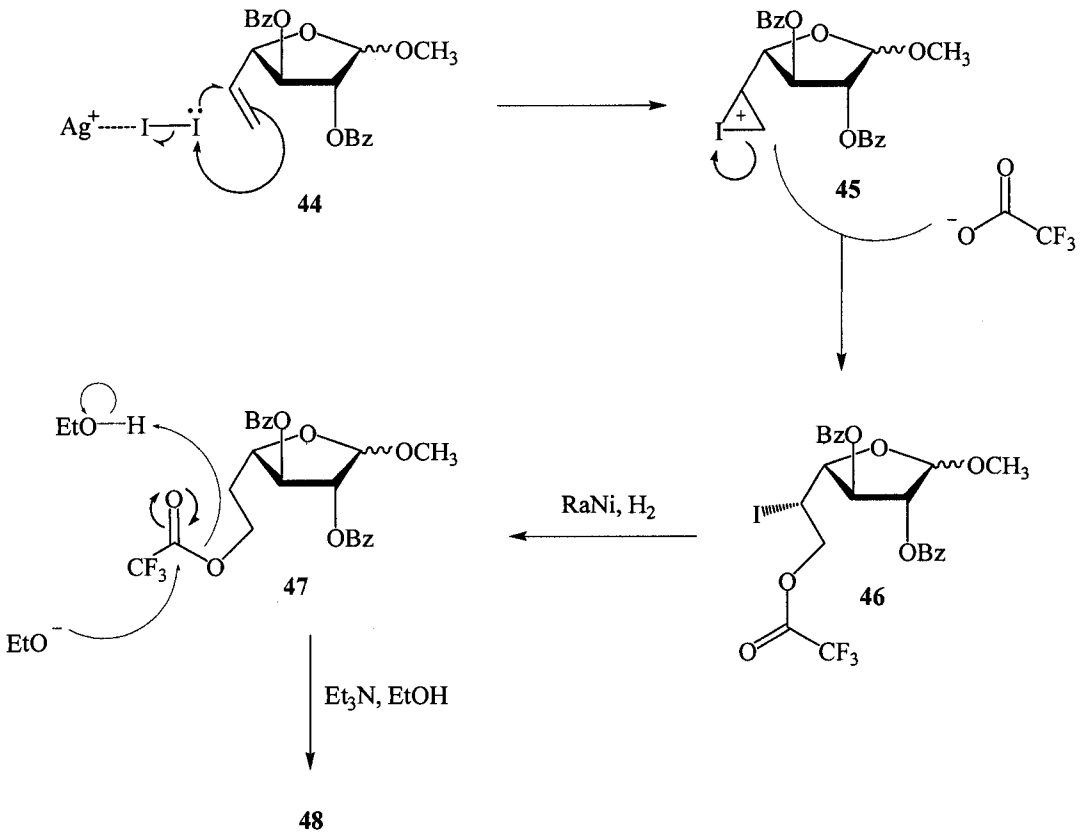


Figure 2.12 Mechanism for the formation of **48** from **44**.

This compound **49** was treated with the concentrated HBr–HOAc and then water to generate hemiacetal **26** in 93% yield. Compound **26** was reacted with trichloroacetonitrile and DBU to produce the trichloroactimidate donor intermediate. After quick passage through a silica gel column, the donor was mixed with the acceptor, tolyl 2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside **24** and dried overnight in the presence of P_2O_5. The glycosylation of (**24**) and imidate derived from **26** was promoted by TMSOTf in dichloromethane at -30 °C to give disaccharide **50** in 75% overall yield.

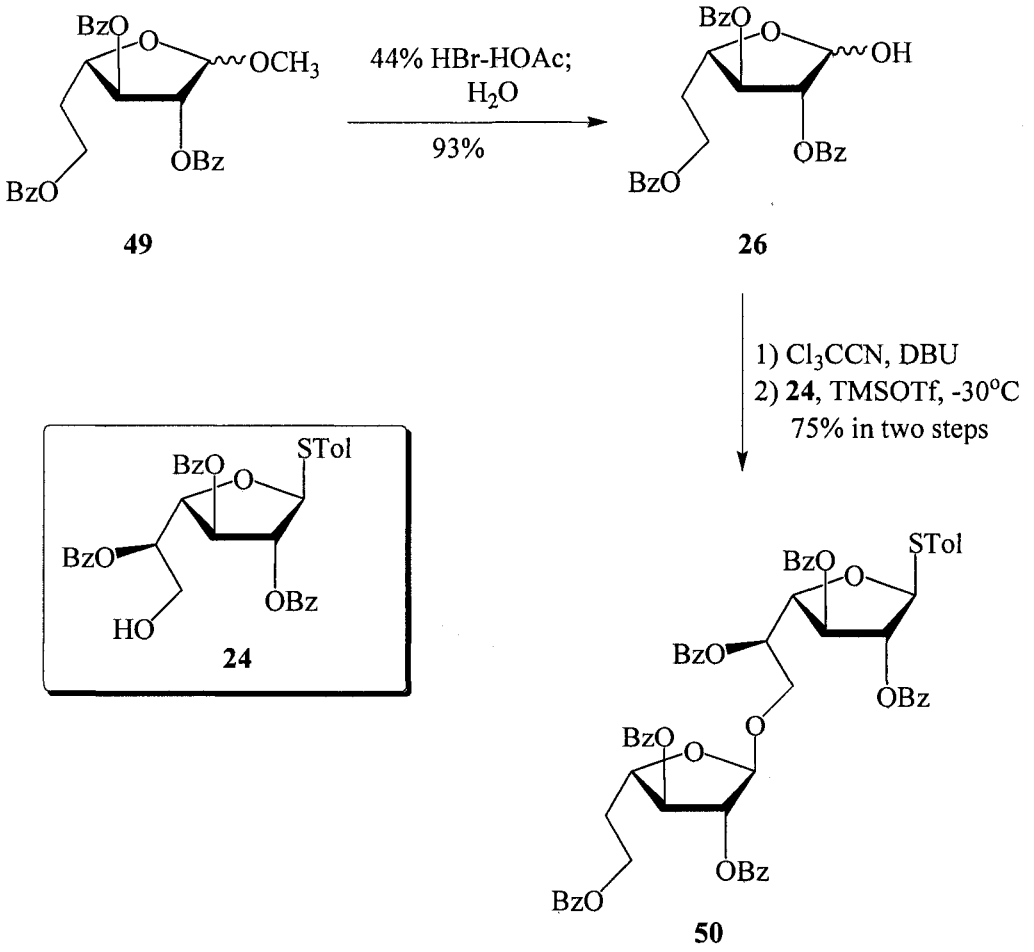


Figure 2.13 Synthesis of thioglycoside disaccharide **50**.

After obtaining disaccharide **50**, it was converted to trisaccharide **51** as shown in **Figure 2.14**. Octylglycoside **23** was added to disaccharide **50**, which was promoted by NIS and silver triflate to give the trisaccharide **51** in 75% yield. Following this, the benzoyl groups of trisaccharide **51** were removed by sodium methoxide in methanol to offer the expected trisaccharide **11** in 97% yield **(Figure 2.14)**. The conformation of **11** was determined by the same method used to evaluate compound **10**. There are three singlets for the anomeric protons in the ^{1}H NMR spectrum and the chemical shifts of all

anomeric carbons in the ^{13}C NMR spectrum are from 108.0 to 108.6 ppm, all of which confirm the stereochemistry of the 5"-deoxy- trisaccharide **11**.

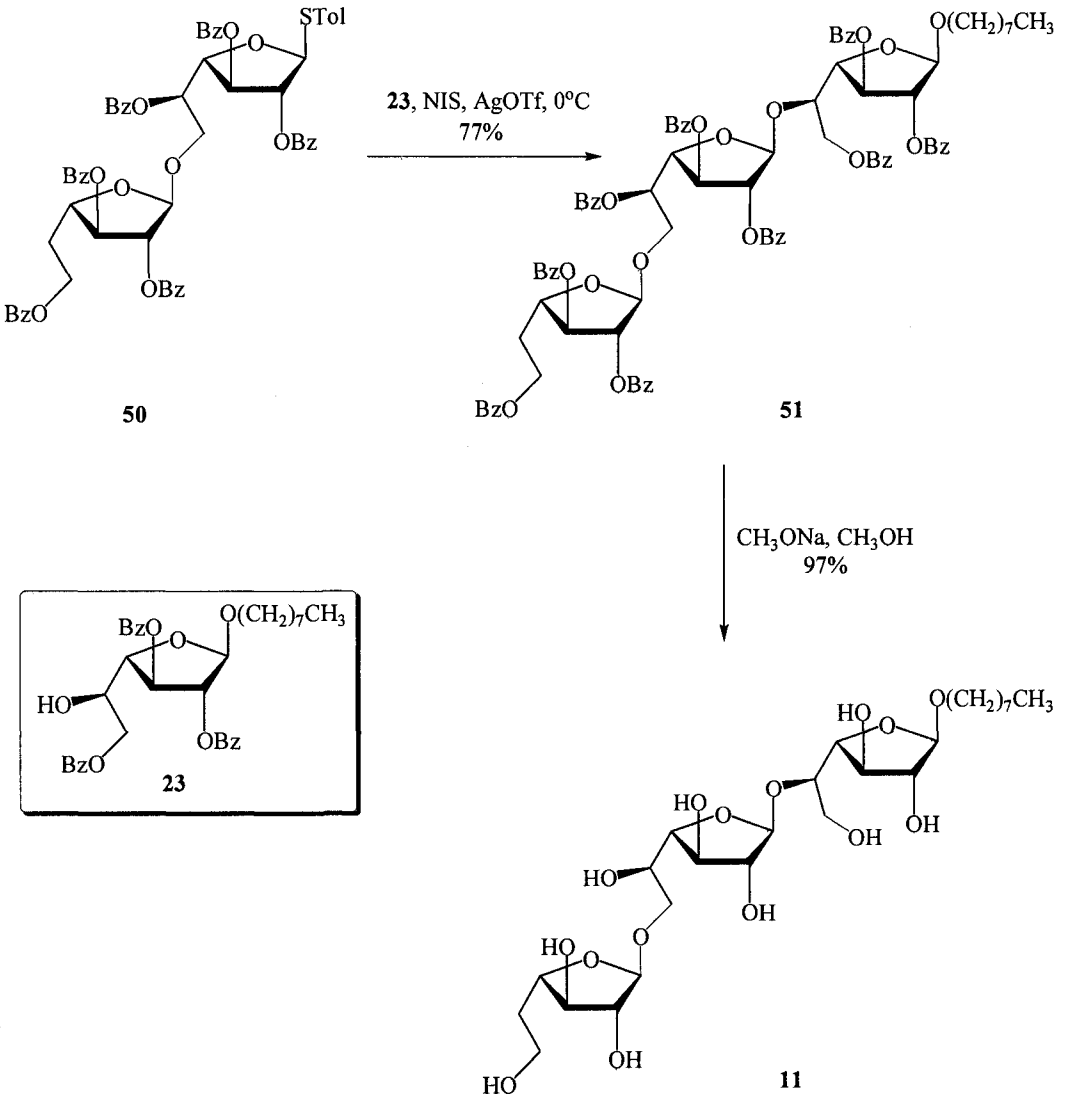


Figure 2.14 Synthesis of 5-deoxy-α-L-Arahex*f*-(1→6)-β-D-Gal*f*-(1→5)-β-D-Gal*f*-ocyl **11**.

2.2.3 Synthesis of 5",6"-Dideoxy-trisaccharide 12

The key in the synthesis of **12** was to prepare a 5,6-dideoxy galactofuranoside building block. Because the alkene **44** was prepared in **Section 2.3**, it was expected that it

could be reduced to give an appropriate precursor to the targets. This was the case and when compound **44** was subjected to catalytic hydrogenation,[98] the anticipated dideoxygenated intermediate was produced in quantitative yield (**Figure 2.15**). Without purification, the intermediate was treated with HBr in HOAc and then water to give 5,6-dideoxy reducing sugar **28** in 90% yield. Before forming the new glycosidic bond, **28** was converted into the trichloroacetimidate donor as was done before, and then TMSOTf was utilized to promote the coupling of the imidate intermediate from **28** with the acceptor, tolyl 2,3,5-tri-*O*-benzoyl-1-thio-β-D-galacto-furanoside (**24**), to produce the disaccharide **52** in 87% yield (**Figure 2.15**).

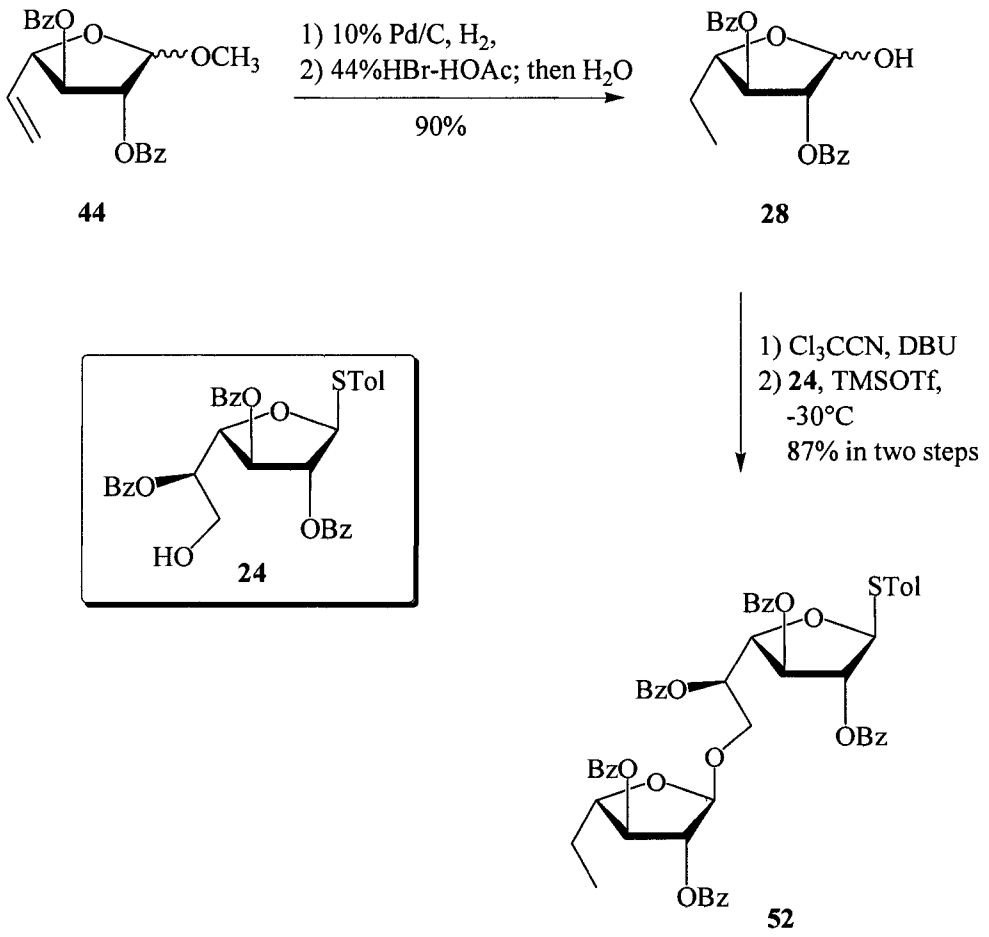


Figure 2.15 Synthesis of dideoxygenated disaccharide **52**.

Having synthesized **52**, it was coupled with octyl 2,3,6-tri-*O*-benzoyl-β-D-galacto-furanoside **23** using NIS and silver triflate promotion to produce trisaccharide **53** in an excellent 88% yield (**Figure 2.16**). After the glycosylation, the benzoyl groups of the trisaccharide were removed by sodium methoxide in methanol to afford the final product **12** in 97% yield **(Figure 2.16)**. To elucidate the stereochemistry of the 5",6"-dideoxy-trisaccharide **12**, I investigated the coupling constants of $^{3}J_{H\text{-}1,H\text{-}2}$ in the ^{1}H NMR spectrum which were all zero and chemical shift of all anomeric carbons in the ^{13}C NMR spectrum, which were 108.5 ppm, 108.3 ppm and 108.2 ppm, respectively. All of these data proved that the glycosidic bonds of **12** have the anticipated configuration shown in **Figure 2.16**.

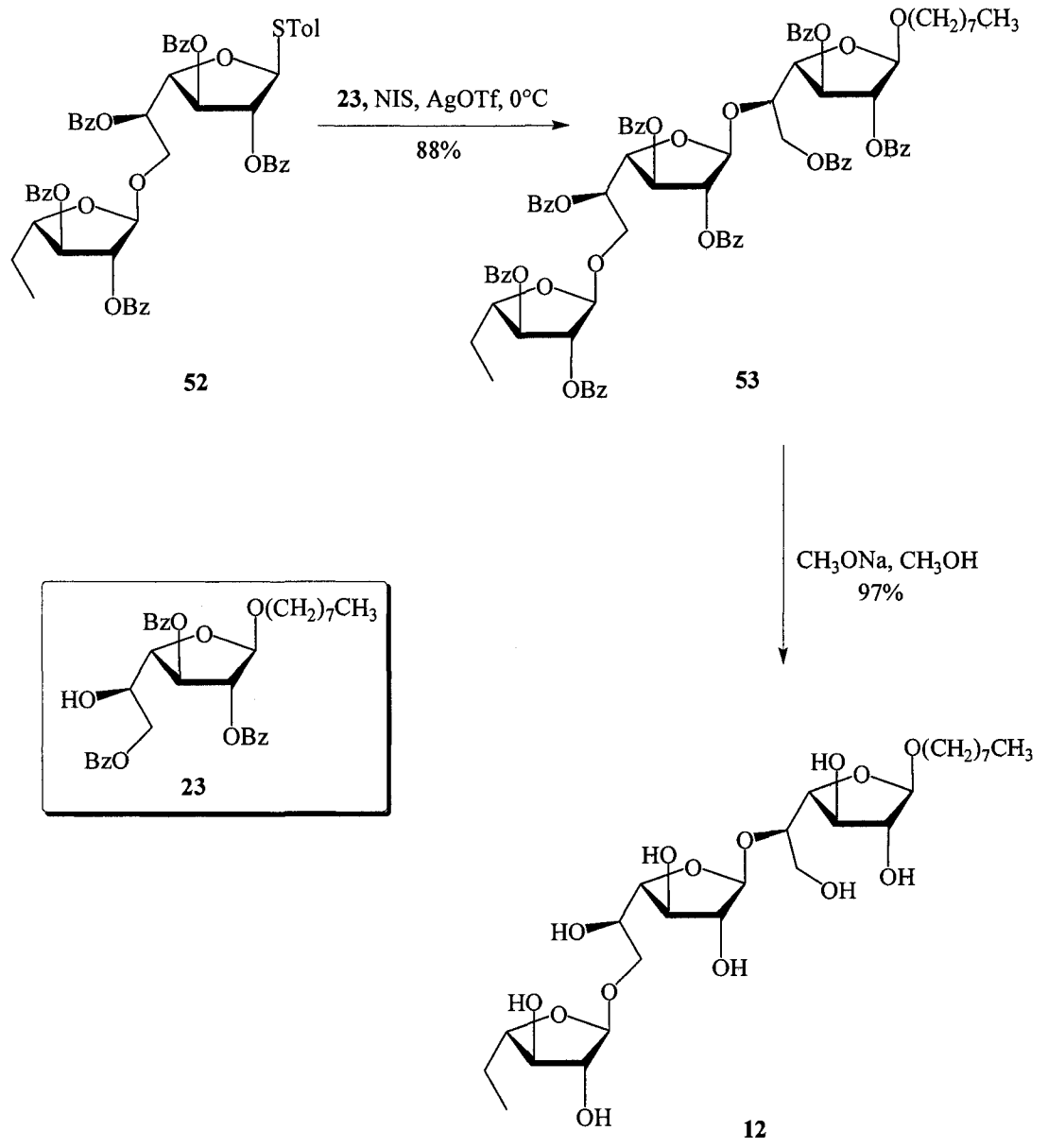


Figure 2.16 Synthesis of 5,6-dideoxy-α-L-Arahex*f*-(1→6)-β-D-Gal*f*-(1→5)-β-D-Gal*f*-octyl **12**.

2.2.4 Synthesis of 5,6-Dideoxy-trisaccharide 13

To complete the final trisaccharide target, **13**, the 5,6-dideoxy monosaccharide **28** was converted, as described above, into the trichloroacetimidate donor (**Figure 2.17**). After a quick passage through a silica column, the donor was mixed with acceptor **25** and

dried overnight in the presence of P_2O_5. Coupling of the two compounds promoted by TMSOTf generated disaccharide **54** in 81% yield. The resulting disaccharide was attached to acceptor **22** under the promotion of NIS and AgOTf to offer the trisaccharide **55** in 88% yield. Finally, the fourth trisaccharide, **13**, was obtained by debenzoylation of **55** in sodium methoxide in methanol in 96% yield **(Figure 2.17)**. Like the other trisaccharides, the stereochemistry of **13** was confirmed by NMR spectroscopy including ^{1}H NMR and ^{13}C NMR.

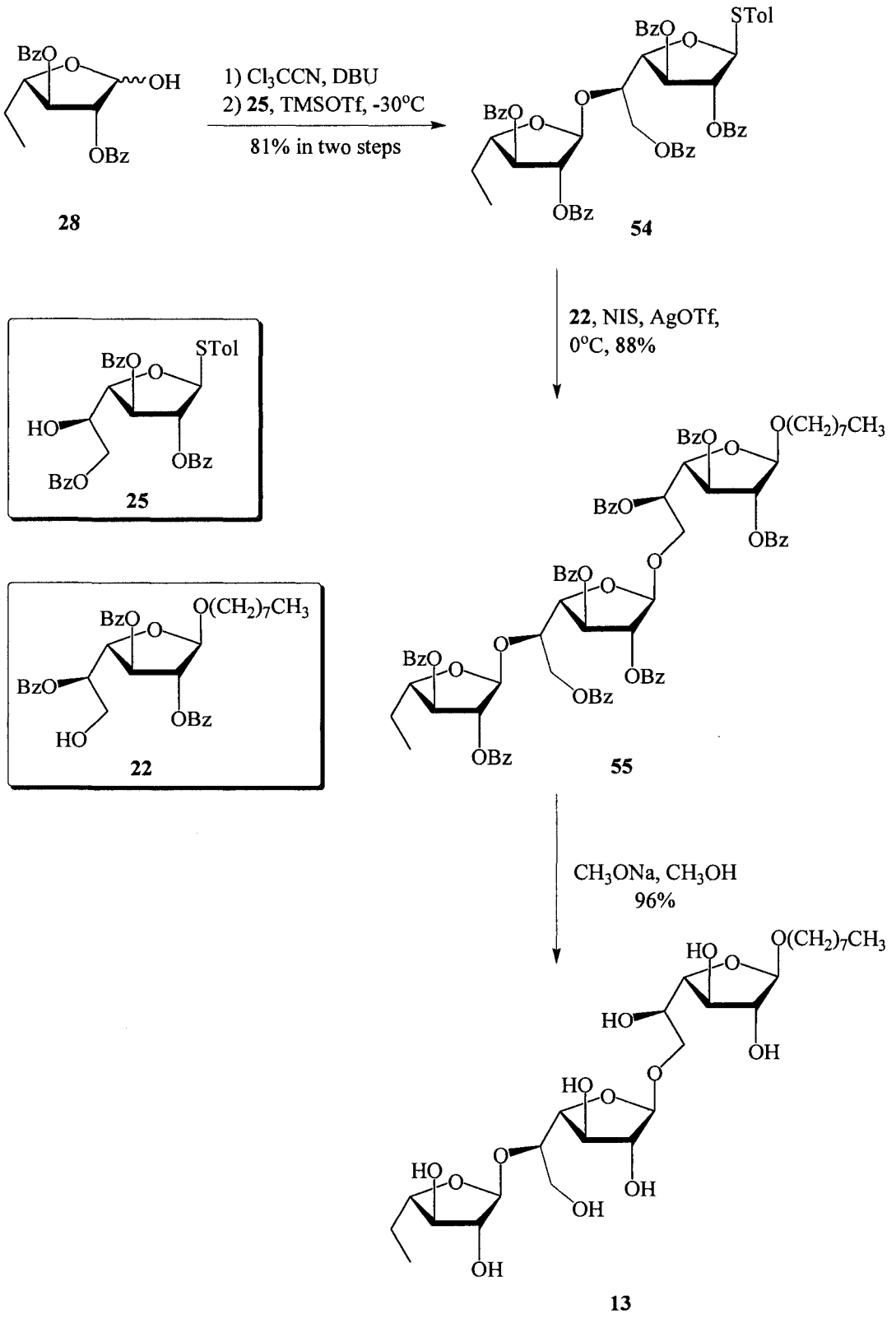


Figure 2.17 Synthesis of 5,6-dideoxy-α-L-Arahex*f*-(1→5)-β-D-Gal*f*-(1→6)-β-D-Gal*f*-octyl **13**.

2.3 Summary and Future Work

GlfT2 is one of galactosyltransferases which are responsible for the formation of galactan in mAG complex. Now people have confirmed that GlfT2 is a bifunctional enzyme which can direct the formation of two types of bonds, 1→5 and 1→6 glycosyl linkages. As my first project, I have synthesized four modified trisaccharides in which one or both of the hydroxyl groups on the acyclic diol of the terminal monosaccharide residue have been deoxygenated. These compounds, designed to be inhibitors of the GlfT2, were used to probe the substrate specificity of the enzyme. To synthesize the targets, I prepared, using standard synthetic methods, a series of monosaccharide building blocks that were assembled to give the final compounds; the products were all characterized by NMR spectroscopy and mass spectrometry. Jean pearcy was responsible to evaluate the bioactivity of the prepared oligosaccharides. When screened against GlfT2, the results showed that the 6"-deoxy trisaccharide **10** and both dideoxy analogs **12** and **13** inhibit the enzyme, but the 5"-deoxy-trisaccharide **11** is not, and instead surprisingly acts as a substrate. The result indicates that the C-6 OH group of the terminal residue maybe better interact with GlfT2 than the C-5 hydroxyl group in the acceptors.

Although these modified oligosaccharides have displayed some specificities of GlfT2 to us, the detailed interaction between the enzyme and substrates is still not clear. To further elucidate the issue, one of our extensions should be to identify whether the other hydroxyl groups of the oligosaccharides also influence the biological activity of the enzyme or not. For example, it may be possible to improve the inhibition ability of the compounds via methylation or fluorination of the respective hydroxyl groups, instead of deoxygenation. These issues will be studied in our further work.

Chapter 3: Synthesis of UDP-Gal*f* Donor Analogs

3.1 Project Aim

We also wanted to probe the substrate specificity of GlfT2 with respect to the donor, UDP-Gal*f*. As was true for the acceptors, analogs of the donor are anticipated to be potential inhibitors of the enzyme. To probe the steric and hydrogen-bonding requirements of GlfT2 further, we deemed it necessary to prepare a panel of analogs (**14–21**, **Figure 3.1**) in which each hydroxyl group has been deoxygenated or methylated. Through testing these compounds against the enzyme, we hope that the important interactions between each hydroxyl group and the active site can be identified.

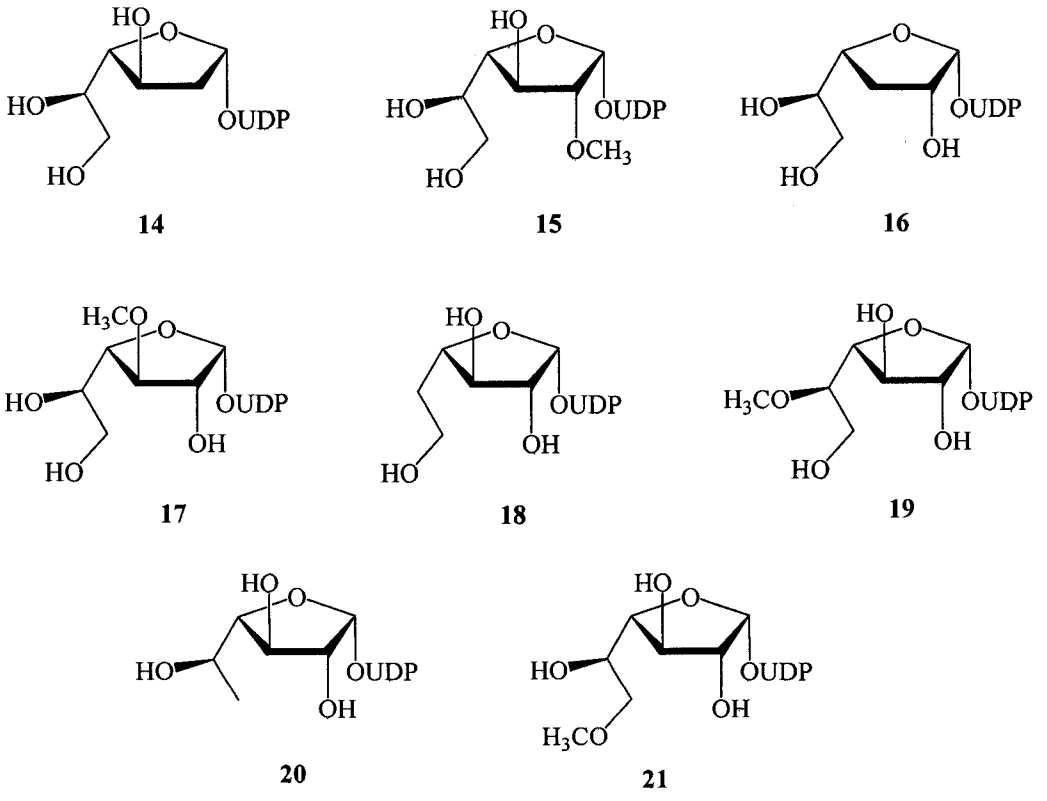


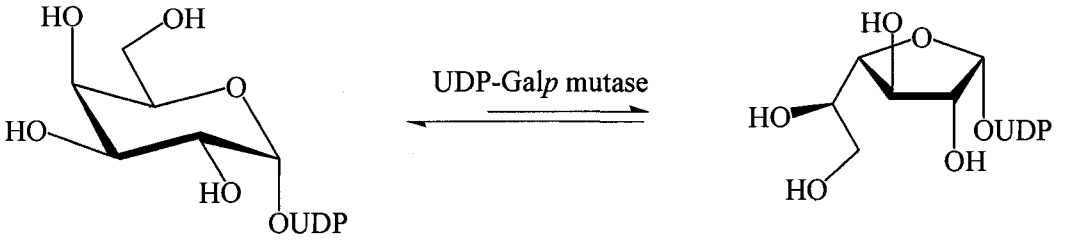
Figure 3.1 Structure of proposed UDP-Gal*f* donor analogs.

3.2 Overall Synthetic Approach

3.2.1 General Pathways to Prepare UDP-Gal*f* 9

In my project, all deoxygenated and methylated building blocks will be converted into the UDP-galactofuranose (UDP-Gal*f*) analogs via a general pathway. A brief introduction is available here to compare the current methodologies, which achieve the transformation from galactofuranoside to UDP-Gal*f*. To date, there are three procedures to prepare these UDP sugar analogs: enzymatic synthesis, chemical synthesis and chemoenzymatic synthesis.

Enzymatic synthesis employs UDP-Gal*p* mutase to catalyze the interconversion between UDP-Gal*p* **56** and UDP-Gal*f* **9** (**Figure 3.2**).[99,100] The procedure can offer labeled substrate **9** from the labeled UDP-Gal*p* which is very useful to determine the enzymes involved in the metabolism of Gal*f*, but its application is limited by the low yield of UDP-Gal*f*. In the equilibrium position of the reaction, UDP-Gal*p* is heavily favored compared to UDP-Gal*f* by a 93:7 ratio.



56 (93%) **9** (7%)

Figure 3.2 Enzymatic approach to UDP-Gal*f* **9**.

A chemical approach[101,102] for preparing the donor **9** has been reported (**Figure 3.3**),[103] which involves reaction of a galactofuranosy-1-phosphate **8** and activated

5'-*N*-methyl phosphorylimidazolide nucleoside **57**. Due to the poor yield (~35%), this method is not ideal for providing a large amount of product.

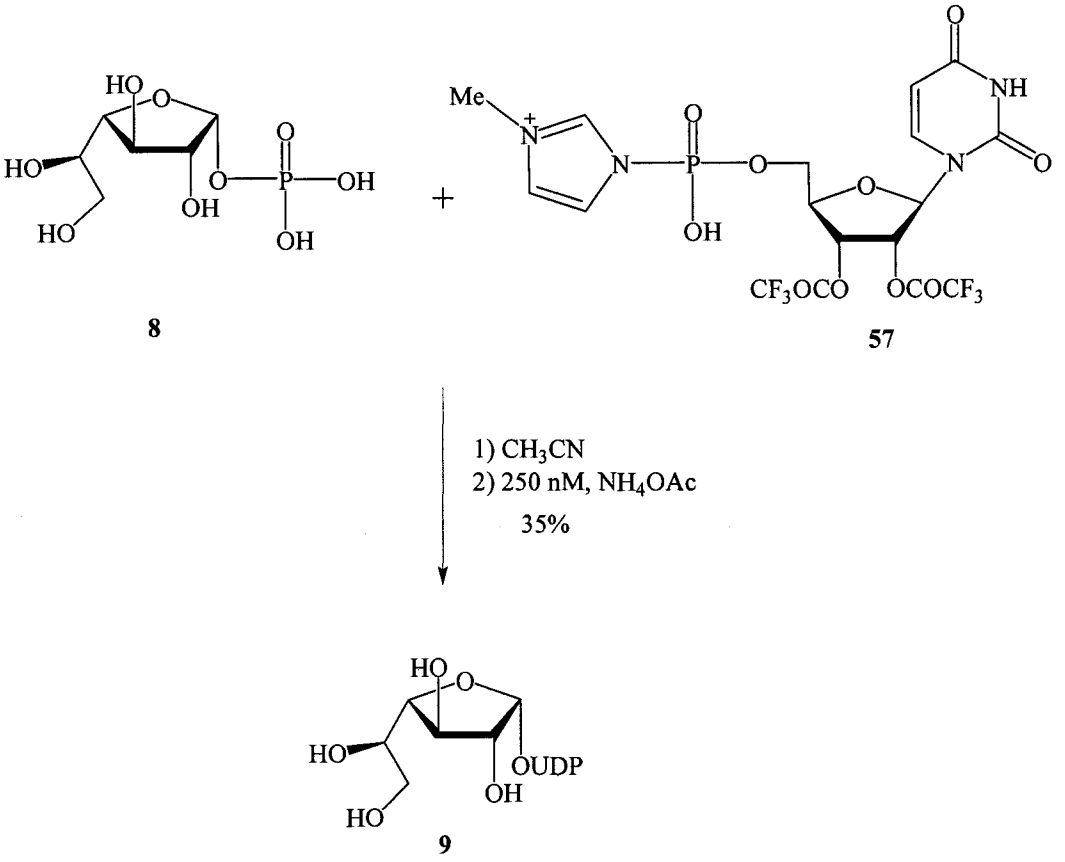


Figure 3.3 Chemical synthesis of UDP-Gal*f* **9**.

In this project, Ruixiang Blake Zheng and me used a chemoenzymatic approach to prepare the UDP-Gal*f* analogs which has been previously reported by Field and co-workers[66] (**Figure 3.4**). There are several enzymes involved in the process. Initially, the galactofuranosyl-1-phosphate **8** is converted to UDP-Gal*f* catalyzed by the enzyme, galactose-1-phosphate uridyltransferase, using UDP-Glc as the source of UMP. To move the equilibrium to right, the resulting by product, Glc-1-phosphate, is recycled by UTP under catalysis of the enzyme, UDP-Glc pyrophosphorylase to afford UDP-Glc. Finally,

the pyrophosphate is converted into phosphate by pyrophosphorylase. Compared with the above two pathways, the chemoenzymatic approach has higher yield (~70%) and can provide sufficient amount of substrates for our sequential research. Thus my initial goal was to synthesize a panel of modified Gal*f*-1-phosphate analogs.

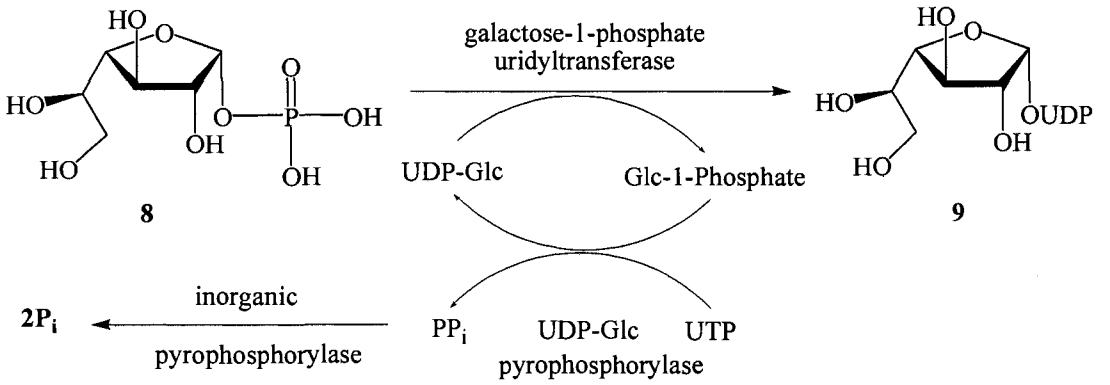


Figure 3.4 Chemoenzymatic approach to UDP-Gal*f* **9**.

3.2.2 Synthesis of 6-deoxy UDP-Gal*f* Analog 20

Methyl 2,3,5-tri-*O*-benzoyl-6-deoxy-α/β-D-galactofuranoside **38**, a key intermediate in the synthesis of the 6-deoxy-UDP-Galf analog **20**, was prepared in the course of synthesizing the trisaccharides, as described in Chapter 2. Here, a shorter synthesis route was developed to prepare **38** (**Figure 3.5**). After compound **58** was prepared from compound **31** in a very good yield as described in **Section 3.2.8**, the released C-6 hydroxyl group was substituted by iodide.[104] This reaction was carried out by stirring a solution of **58** with triphenylphosphine and iodine at room temperature. The product, **59,** was obtained in 96% yield. To prepare the 6-deoxy methyl glycoside building block, catalytic hydrogenation was used to reduce iodo-substituted glycoside **59**.[105] In the earlier synthesis (**Section 2.2**), although higher pressure hydrogen (45 PSI) had to be

employed for reduction of the bromo-substituted methyl glycoside **37**, compound **59**, which has a more reactive C–I bond, was reduced with 15 PSI H_2 to give compound **38** in 90% yield.

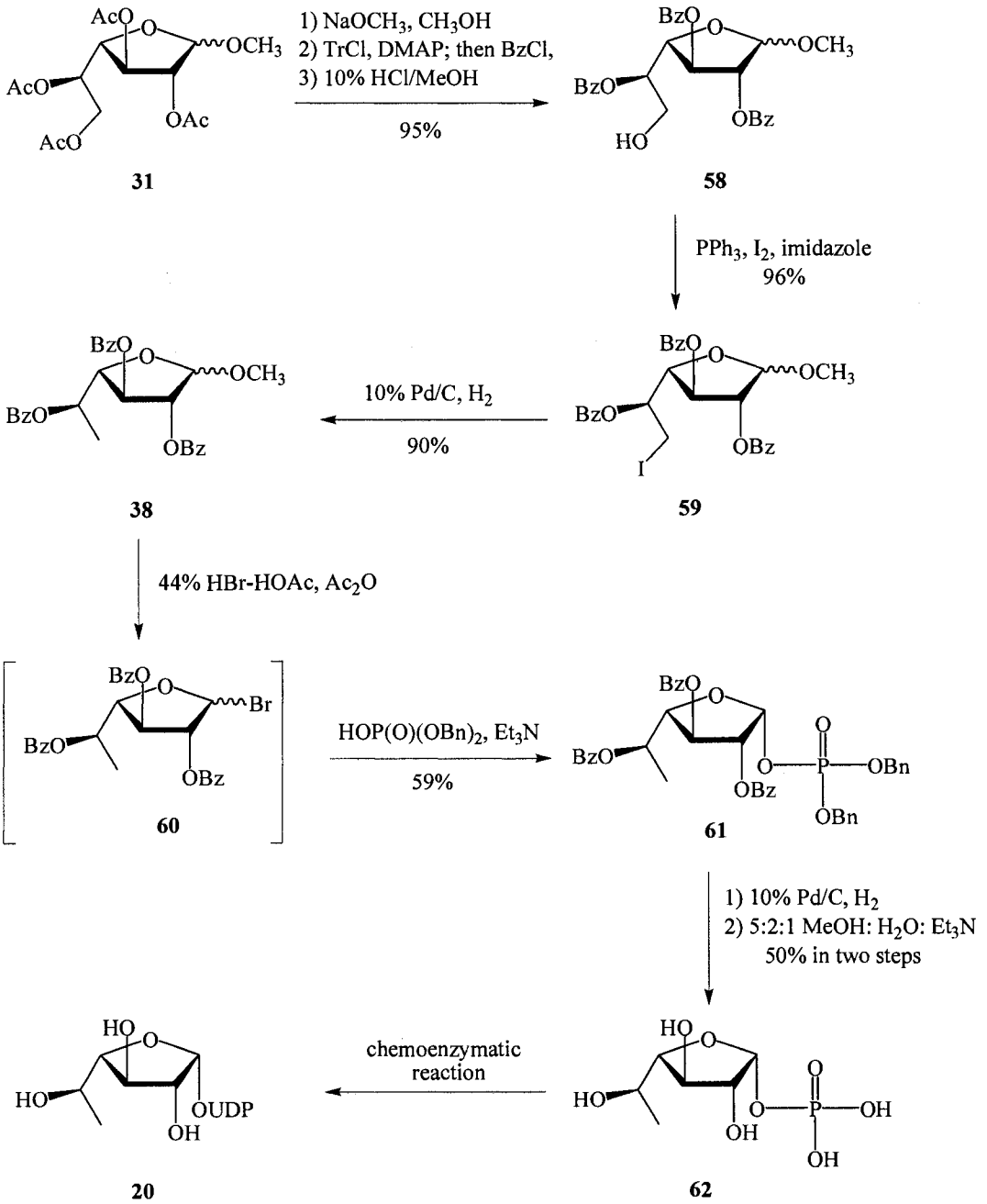


Figure 3.5 Synthesis of 6-deoxy-α-D-galatofuranosyl phosphate **20**.

With the 6-deoxy compound methyl glycoside **38** in hand, I next prepared the α glycosyl phosphate. This was achieved first by reaction of **38** with 44% HBr in HOAc to prepare the corresponding glycosyl bromide donor **60** as was done in the synthesis of the trisaccharides (see Chapter 2).[106] However unlike before, this donor was not hydrolyzed with water to generate the hemiacetal (e.g., **Figure 2.4**). In this case, it was reacted with dibenzyl phosphate. The α-galactofuranosyl phosphate **61** was isolated as a single isomer in 59% yield.[107] The reaction is not stereoselective because it produces both the α-isomer and β-isomer. After silica gel chromatography column, only the α-isomer **61** was obtained while the β isomer apparently decomposed during purification, which rationalizes the modest yield of the reaction. After the glycosylation reaction, the two benzyl groups were successfully removed by the catalytic hydrogenation. Following this, the remaining benzoyl groups were removed by 5:2:1 methanol–ammonium hydroxide–water to afford the 6-deoxy-α-D-galaco- furanoyl phosphate **62** in 50% overall yield.

Having synthesized **62**, I collaborated with another group member, Blake Zheng, to produce the 6-deoxy-UDP-Gal*f* target using the multi-enzyme reaction described in the introduction to this chapter. The reaction was monitored and then purified by reverse-phase HPLC. Because UDP-Gal*f* is unstable to high salt concentrations, a Sephadex G15 column was used to remove salts from the product. The whole process took several days but gave **20** in 70% overall yield, which was likely better than a purely chemical synthetic approach. To elucidate the stereochemistry, the coupling constant of ^{1}H NMR was identified. The anomeric proton is doublet of doublets, resulting from $^3J_{H\text{-}1,H\text{-}2}$ and $^3J_{H\text{-}1,\ P\text{-}\alpha}$, whose value are 5.9 and 4.2 Hz, respectively. These data support the stereochemical assignment of **20**.

3.2.3 Synthesis of 5-Deoxy UDP-Gal*f* Analog 18

As will be described in more detail in Section 3.2.8, I developed new conditions for the preparation of 2,3,6-tri-*O*-benzoyl-α/β-D-galactofuranoside **63** from methyl 2,3,5-tri-*O*-benzoyl α/β-galactofuranoside **58** by reaction with silver oxide in DMF (**Figure 3.6**). With **63** in hand, the hydroxyl group was substituted with iodine. Initially, when the reaction was carried out at room temperature, no product was obtained while the same condition gave a high yield for preparing primary iodide **59** from **58** (see **Figure 3.5**). However, after the reaction temperature was raised to 90 °C, the reaction was successfully achieved and the 5-iodo compound **64** was obtained in 96% yield.

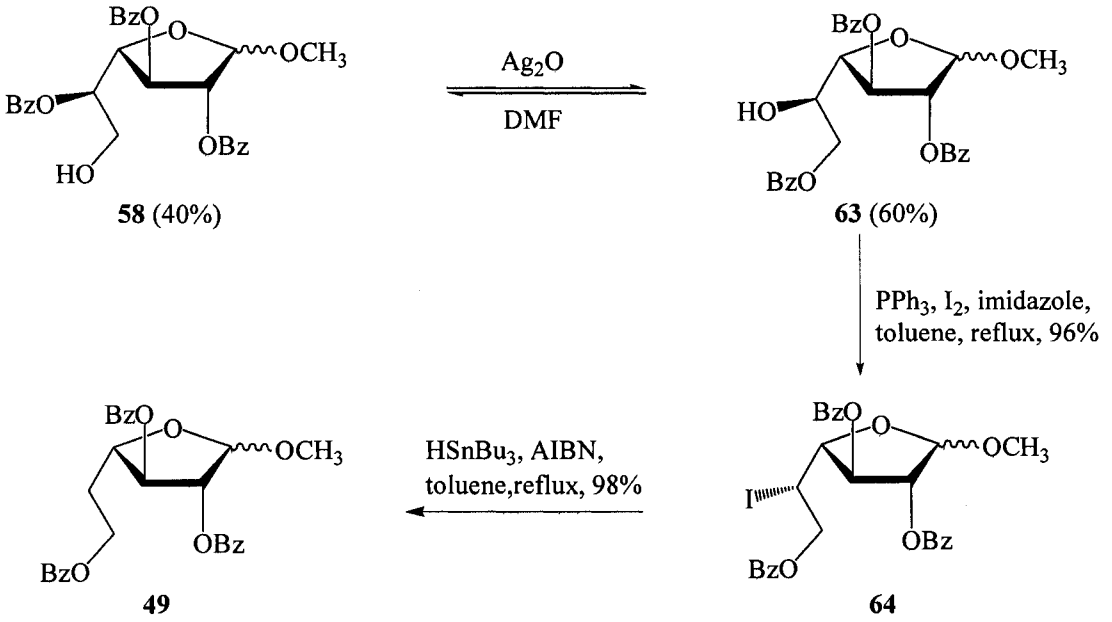


Figure 3.6 Synthesis of methyl 2,3,6-tri-*O*-benzoyl-5-deoxy-α/β-D-galactofuranoside **49**.

To reduce compound **64**, catalytic hydrogenation was first studied. Unfortunately, none of required product was obtained although the same conditions led to a high yield in

the reduction of the primary iodide **59** (see **Figure 3.5**). Even after enhancing the hydrogen pressure to 45 PSI (3 atm), no product was obtained. Due to the unsuccessful hydrogenation, a radical deoxygenation reaction was employed to remove iodine.[108] Compound **64** was heated at reflux with tin hydride and AIBN for two hours to afford **49** in 98% yield. As described in **Section 3.2.1**, compound **49** (**Figure 3.7**) was converted into a glycosyl bromide donor by reaction with 44% HBr in HOAc, and this product treated with dibenzyl phosphate and triethylamine to afford 5-deoxy-α-D-galacto-furanoside phosphate analog **65** in 48% overall yield. The product was debenzylated by hydrogenation with 10% Pd–C catalyst, and then debenzoylated to yield compound **66** in 53% yield.

Following this, chemoenzymatic synthesis (with the assistance of Blake Zheng) was applied to **66** to prepare the 5-deoxy-UDP-Gal*f* analog **18**. The crude compound was purified by reverse-phase HPLC and Sephadex G15 column to afford **18** in overall yield of 70%. As mentioned above for **20**, the stereochemistry of **18** was proved from the $^{3}J_{\text{H-1,H-2}}$. For the remainder of the targets, I was unable to complete their synthesis. I describe in the following sections the preparation of the methyl glycoside precursors for **14–17**, **19** and **21**.

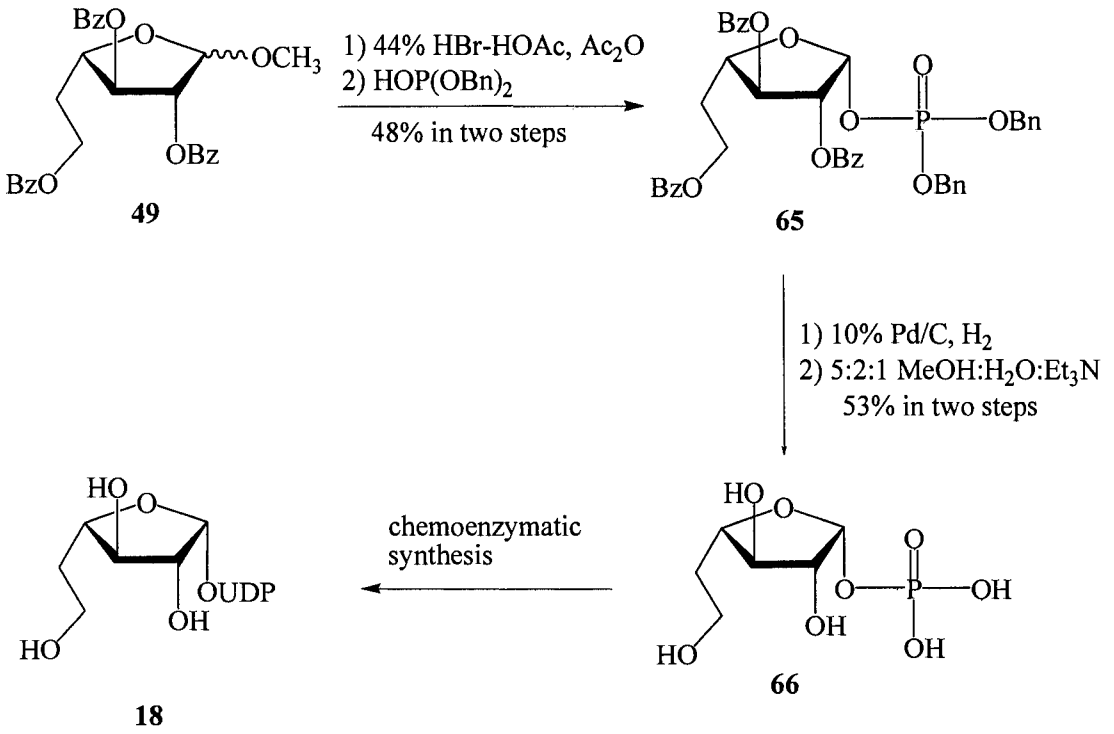


Figure 3.7 Synthesis of 5-Deoxy UDP-Gal*f* **18**.

3.2.4 Synthesis of 2-Deoxy-methyl Glycoside 74

The synthesis of the 2-deoxy methyl glycoside started from compound **31**, which was quantitatively deacetylated by treatment of sodium methoxide in methanol to give **67**. Following this, the two acyclic hydroxyl groups of **67** were then selectively protected with 2,2-dimethyoxypropane to afford a mixture of **68** and **69** in a 10:3 ratio, and the overall yield is 84% (**Figure 3.8**).[109] To identify their stereochemistry, I used the ^{1}H NMR and ^{13}C NMR spectra to distinguish the two isomers. The α-isomer **68** has a doublet (J = 4.5 Hz) for its anomeric proton and chemical shift of C-1 is 101.9 ppm. As the comparison, the anomeric proton of β-isomer **69** is a singlet and the chemical shift of its C-1 was much more downfield than the α-isomer (110.6 ppm). The mixture can be

separated by chromatography and only the β isomer **69** was carried out for the reaction procedure; the α isomer **68** was stored for the preparation of another building block.

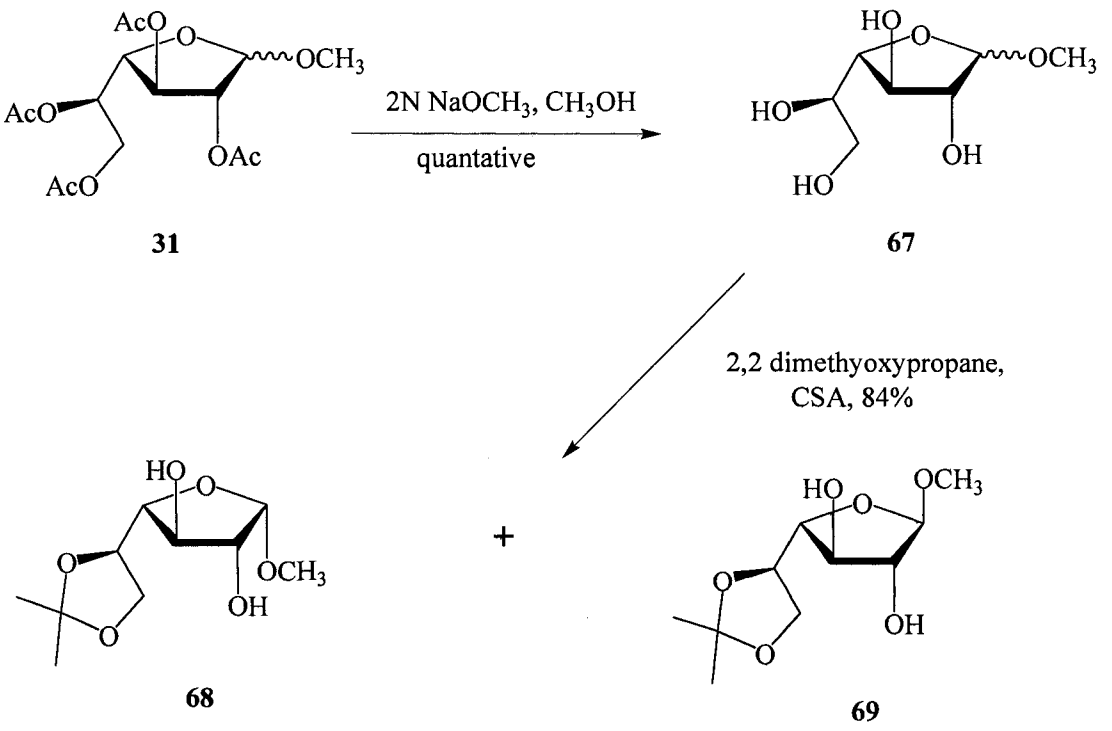


Figure 3.8 Synthesis of methyl 5,6-*O*-isopropylidene-α-D-galactofuranoside **68** and methyl 5,6-*O*-isopropylidene-β-D-galactofuranoside **69**.

To selectively protect either the C-2 or C-3 hydroxyl groups of **69**, one pathway is to convert this diol into an epoxide through a Mitsunobu reaction, followed by a selective cleavage by the appropriate nucleophiles (**Figure 3.9**). It is noteworthy that the anomeric methoxyl group of **69** would greatly influence the regioselectivity of the epoxide opening reaction due to the steric hindrance. Compound **69** was converted to epoxide **70** as expected in quantitative yield.[110] Epoxide **70** is a vital intermediate in this project. By selectively opening the epoxide ring with different reagents, such as methoxide anion and *p*-toluenethiolate anion, the compound was used to prepare several required building

blocks. Before moving to the next step, it was necessary to elucidate the stereochemistry of the epoxide **70** first. One piece of evidence is the coupling constant of $^{3}J_{H\text{-}1,\,H\text{-}2}$, whose value is zero as would be expected for the trans H1–H2 conformation.

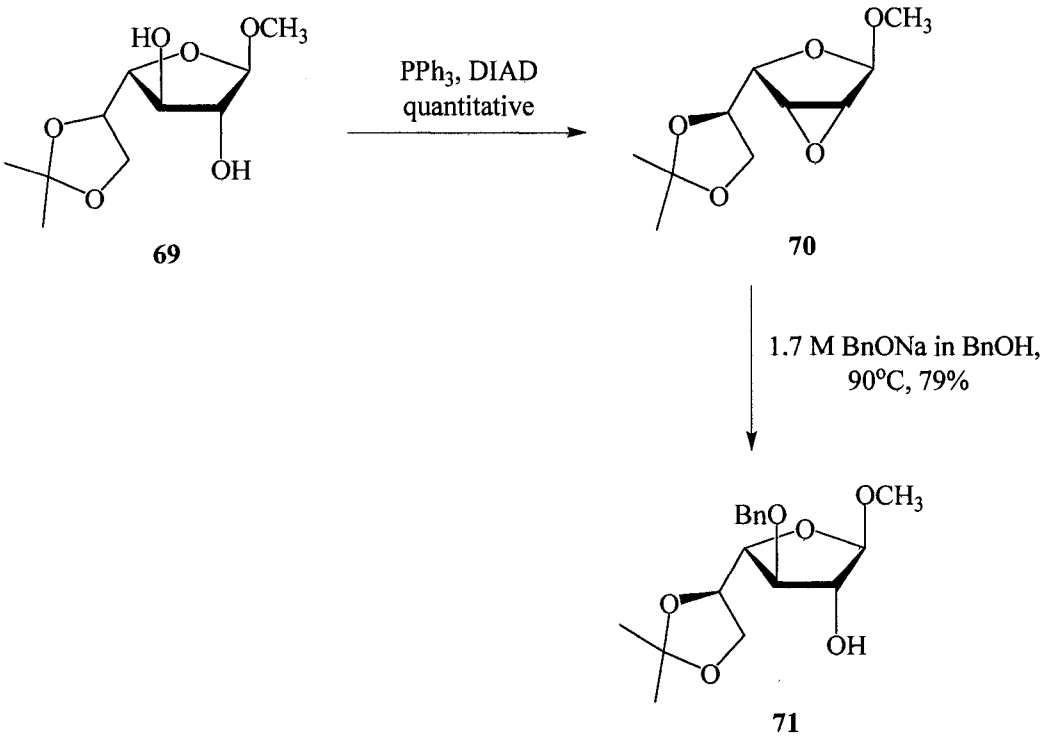


Figure 3.9 Synthesis of **71** from **69**.

For the synthesis of the 2-deoxy methyl glycoside, **70** was reacted with the sodium salt of benzyl alcohol.[111] When **70** was stirred with this alkoxide in benzyl alcohol at 90 °C, the epoxide ring was opened to afford **71** in 79% yield. The sequence is equivalent to selectively protecting the C-3 with a benzyl group, and the hydroxyl group in the product **71** can easily be removed or methylated.

First, the deoxygenated derivative was synthesized. A variety of methods are available for the deoxygenation of alcohols. Among them, the Barton–McCombie

deoxygenation[112] is a very popular method and this was the approach I used. This method requires the preparation of an appropriate precursor substance, which I envisioned could be prepared by commercially available reagents, such as *O*-phenyl chlorothionoformate or *O*-pentafluorophenyl chlorothionoformate.[113] Unfortunately, no product was obtained in these reactions, even when these reagent mixtures were stirred at higher temperature. A possible reason is that the isopropylidene ketal group of **71** prevents these large groups from reacting at the hydroxyl group of C-2. With this in mind, I decided to prepare a xanthante, which involved two much smaller reagents, CS_2 and MeI.[114] This reaction was very successful and gave xanthate **72** in 88% overall yield (**Figure 3.10**). With this precursor in hand, the Barton-McCombie reaction was applied to **72** to afford 2-deoxy-methyl glycoside **73** in a moderate yield of 59%. After the isopropylidene ketal was deprotected with HCl in methanol, the benzyl group was removed by catalytic hydrogenation to produce an unprotected 2-deoxy methyl galactofuranoside, which was immediately benzoylated to give **74** in 82% overall yield.

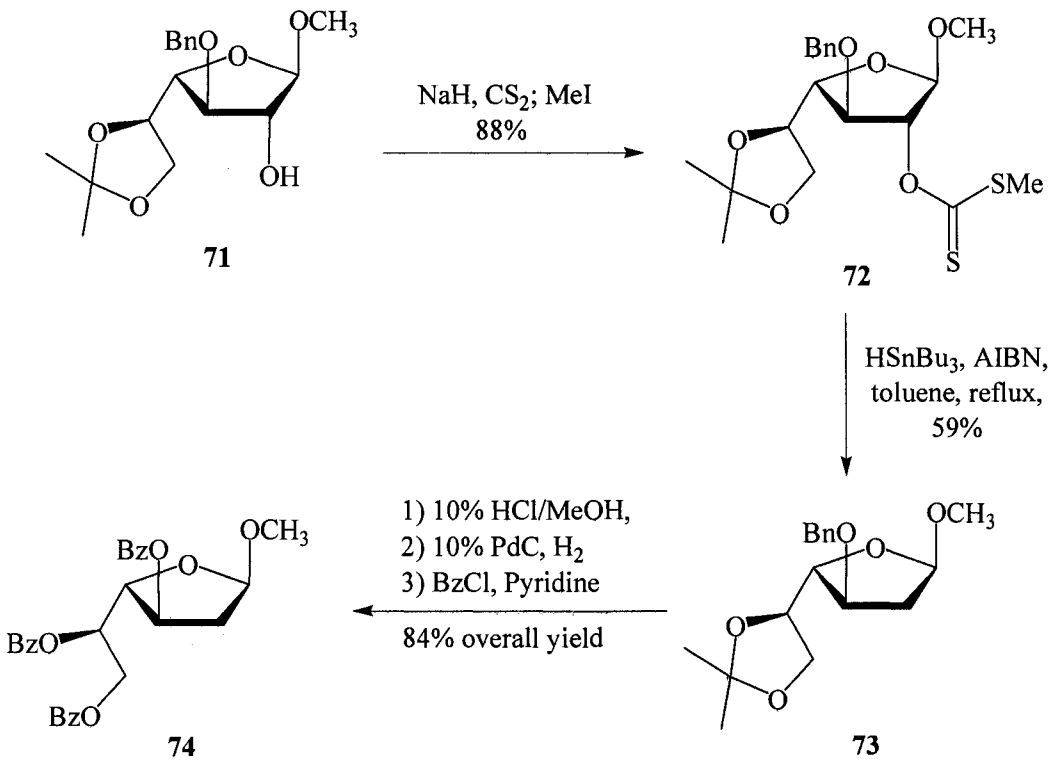
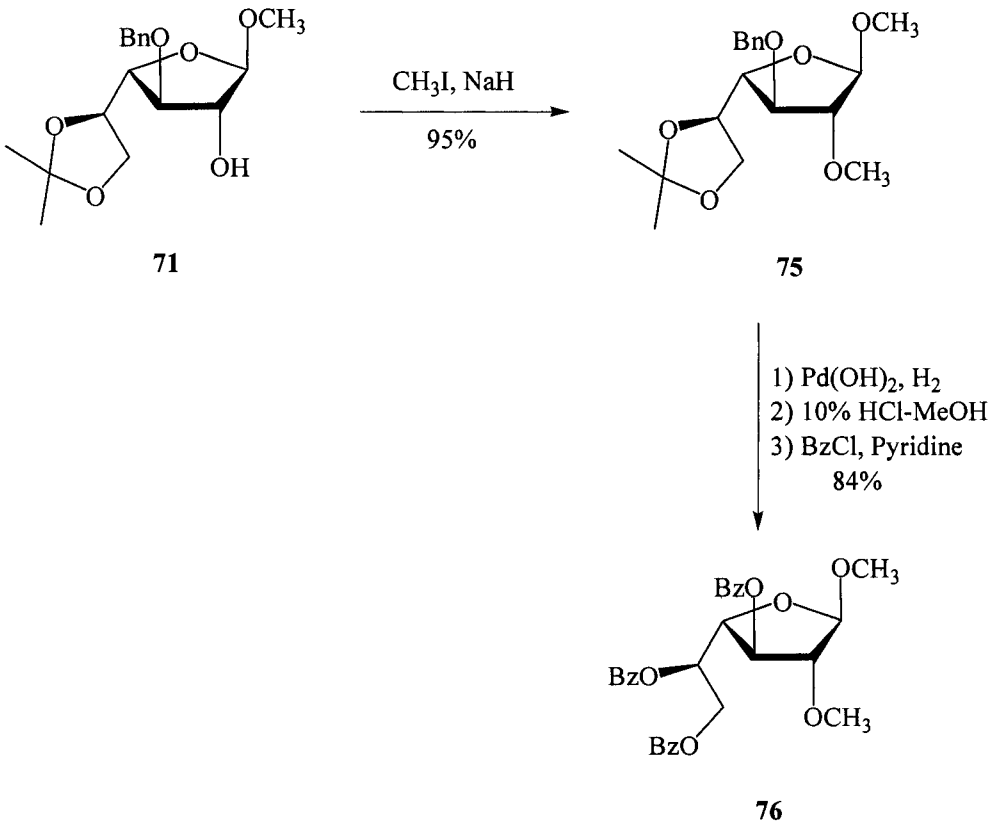


Figure 3.10 Synthesis of compound **74**.

3.2.5 Synthesis of 2-*O*-Methyl-methyl Glycoside 76

The 2-*O*-methyl analog was very easily synthesized from intermediate **71** as illustrated in **Figure 3.11**. First, The C-2 hydroxyl group of compound **71** was methylated by methyl iodide to afford intermediate **75** in 95% yield.[115] After debenzylation by the catalytic hydrogenation, the isopropylidene acetal of **75** was removed by reaction with 10% HCl in methanol. Finally, all three hydroxyl groups were protected by benzoyl chloride to provide **76** in 84% overall yield.



71 75 76

Figure 3.11 Synthesis of compound **76**.

3.2.6 Synthesis of 3-Deoxy-methyl Glycoside 79

The synthesis of 3-deoxy methyl glycoside (**79**) is shown in **Figure 3.12.** Epoxide **70** was treated with *p*-toluenethiol and sodium hydride at 90 °C for 90 minutes to produce **77** in 87%, which was expected as a precursor for the following radical deoxygenation reaction. Compound **77** was heated at reflux with AIBN and tributyltin hydride, after two hours, 3-deoxy glycoside **78** was achieved in 81% yield. To remove the isopropylidene acetal, 10% HCl in methanol was employed, and the resulting intermediate was benzoylated to produce **79** in 84% yield.

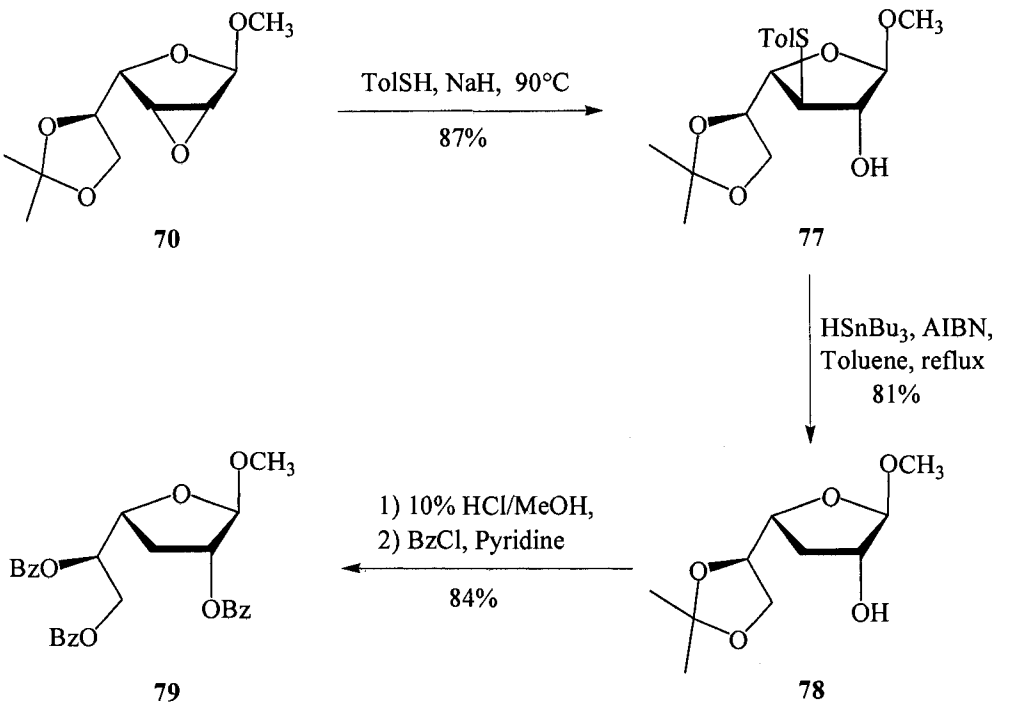


Figure 3.12 Synthesis of compound **79**.

3.2.7 Synthesis of 3-*O*-Methyl-methyl Glycoside 81

So far, I have described the use of sodium benzylate and *p*-toluenethiolate to cleave the epoxide **70** to afford the required products. As expected, this intermediate could also be converted into the building block **80** by heating at reflux in the presence of methoxide anion (**Figure 3.13**). Initially, the reaction was attempted at 50 °C with sodium methoxide in methanol. However, no product was obtained. Heating at higher temperature was not possible due to the low boiling poing of methanol; therefore, THF was used as the solvent. Although the reaction mixture was heated at reflux overnight with 10 equivalents of sodium methoxide, no reaction occurred either. Considering that this is a classic S_N2 reaction, a polar aprotic solvent should be favorable for it. Finally, the reaction was carried out in DMF with 10 equivalents sodium methoxide at 90 °C. After one hour, the

product **80** was successfully achieved in a modest yield of 63%. With the methyl group installed, the isopropylidene ketal of compound **80** was removed by 10% HCl in methanol to afford an intermediate that was subsequently benzoylated to produce **81** in 83% overall yield.

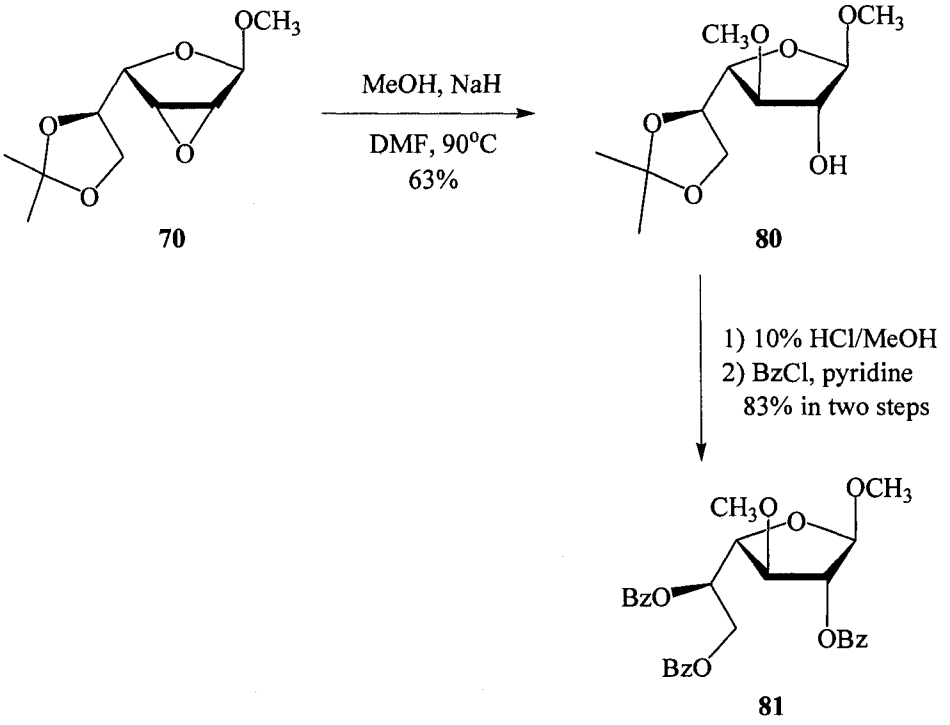


Figure 3.13 Synthesis of compound **81**.

3.2.8 Synthesis of 6-*O*-Methyl-methyl Glycoside 85

When we designed the pathway for the preparation of the 6-*O*-methylated UDP-Gal*f* analog, both this compound and the 6-deoxy analog were expected to be synthesized from the same intermediate, as was done for the building blocks 2-deoxy **74** and 2-methoxy **76**. To this end, we explored the route illustrated in **Figure 3.14**. The protected methyl glycoside **31** was deactylated and then differentially protected by first

tritylation of the primary hydroxyl group and benzoylation of the remaining hydroxyl groups, followed by a detritylation to give **58** in 95% yield, the product was the substrate for the methylation reaction. Because the benzoyl groups are unstable under basic conditions, and often migrate during alkylation reactions, we used weakly basic conditions to carry out this reaction, which has been reported. Our initial attempt involved stirring compound **58** for 12 hours in the presence of solid Ag_2O and methyl iodide. Analysis of the NMR spectra of the product indicated that it was not the desired compound **85**, but instead 2,3,6-tri-*O*-benzoyl-α/β-D-galactofuranoside mixture **63**, in which the benzoyl group had migrated from O-5 to O-6.

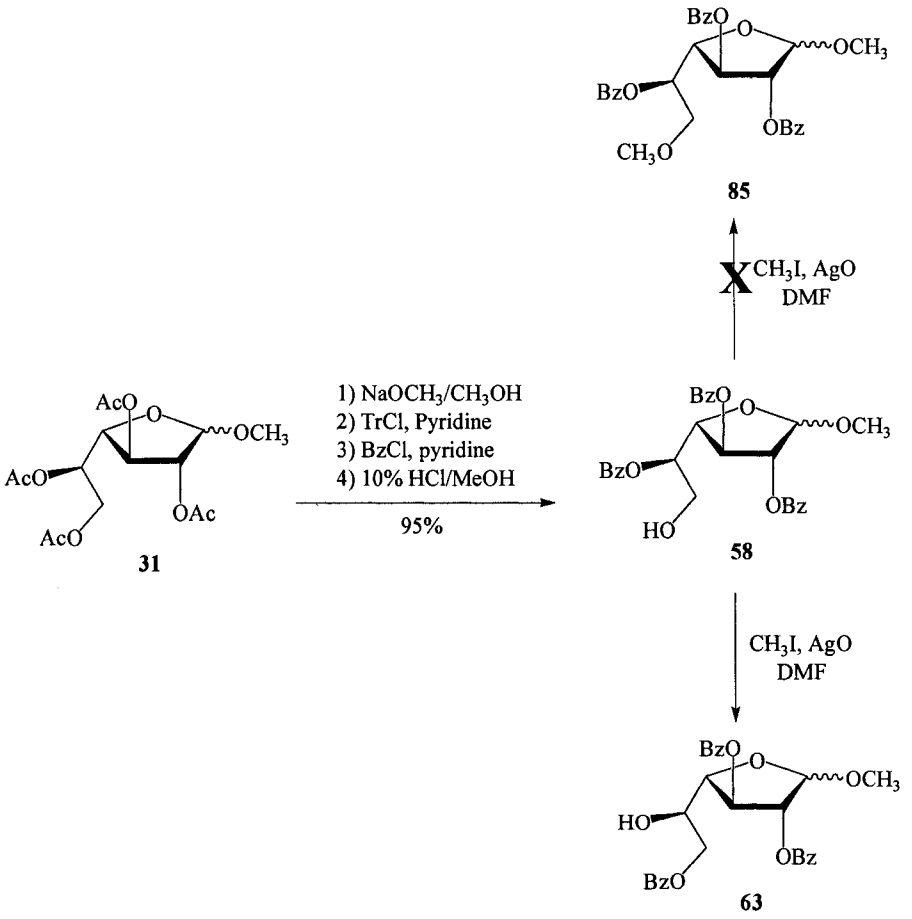


Figure 3.14 Initial pathway to the methyl 2,3,5-tri-*O*-benzoyl-6-*O*-methyl-α/β-D-galactofuranoside **85**.

Although this reaction did not give the expected product, I realized that this is an option to prepare an intermediate with the 5-OH group free through the migration of the benzoyl group from O-5 to O-6. Although the adduct with the benzoyl group on O-6 is more thermodynamically stable than the isomer with the group at O-5, only a small portion (30–40%) of thermodynamic stable product could be obtained under the acidic conditions often used for this migration[116] and the reaction time is frequently up to 2–4 days. I explored other conditions to do this reaction and found that it could be done in a shorter time (12 h), and in approximately 60% yield by carrying out the Ag_2O reaction in DMF, instead of THF or CH_2Cl_2.

While this migration reaction was of use in preparing **49** (see **Section 3.2.3**), another route was needed to provide the desired 6-*O*-methyl compound. The successful route is shown in **Figure 3.15**, and involves the replacement of benzoate esters with benzyl ethers. After removing the acetyl groups from the starting material **31**, the C-6 hydroxyl group was selectively protected by trityl chloride and then the other hydroxyl groups were benzylated to afford **82** in 46% overall yield. Following this, the trityl group was deprotected with 10% HCl in methanol to afford **83** in 87% yield. A methylation reaction of **83** involving sodium hydride and methyl iodide afforded **84** in excellent yield. Following this, the benzyl groups were changed to benzoyl groups, which provided the desired building block **85** in 85% overall yield.

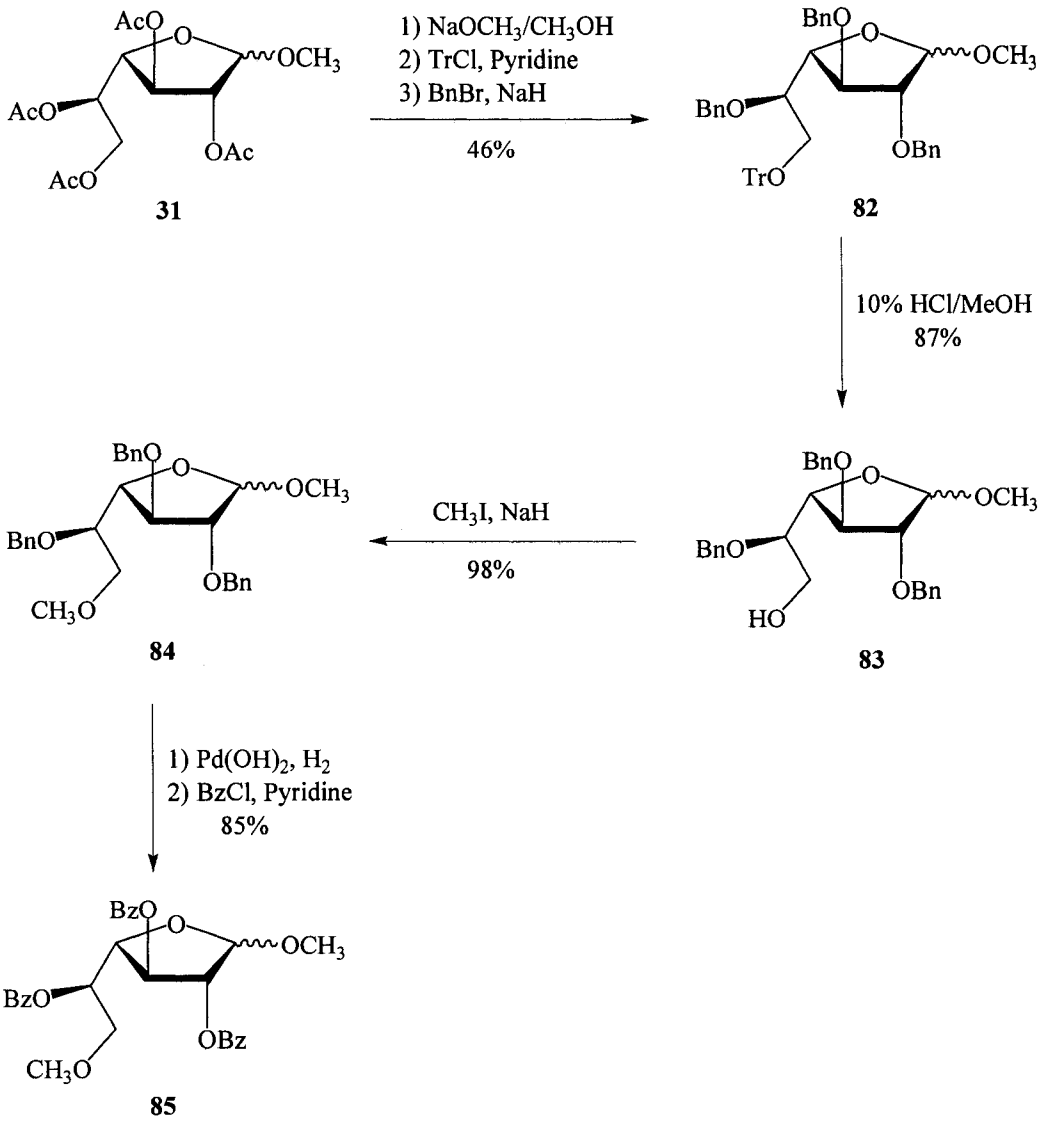


Figure 3.15 Synthesis of methyl 2,3,5-tri-*O*-benzoyl-6-*O*-methyl-α-D-galactofuranoside **85**.

3.2.9 Synthesis of 5-*O*-Methyl-methyl Glycoside 89

As mentioned in **Section 3.2.3**, when the C-5 and C-6 hydroxyl groups of methyl α/β-D-galactofuranoside (**Figure 3.8**) were protected with 2,2-dimethyoxypropane, two separable isomer products, **68** and **69**, were obtained. In the above discussion, the β-isomer **69** served as the key intermediate in the preparation of some building blocks. To

improve the efficiency of the synthetic route, the α isomer **68** was employed to prepare the last building block, methyl 2,3,6-tri-*O*-benzoyl-5-*O*-methyl-α/β-D-galacto- furanoside **89**.

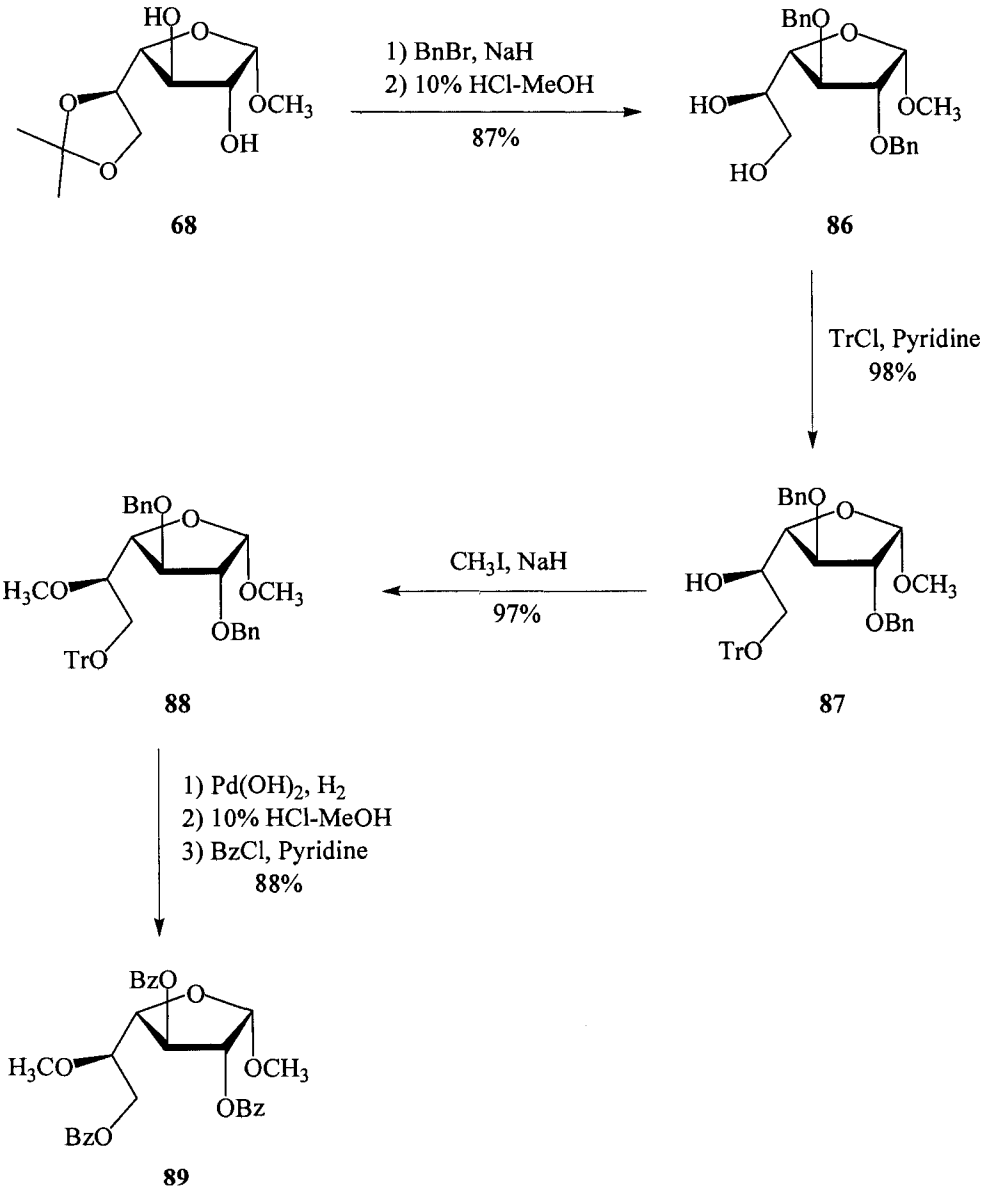


Figure 3.16 Synthesis of methyl 2,3,6-tri-*O*-benzoyl-5-*O*-methyl-α-D-galactofuranoside **89**.

First, the C-2 and C-3 hydroxyl groups of isomer **68** were protected by using benzyl bromide and sodium hydride (**Figure 3.16**), and then the isopropylidene ketal of intermediate from **68** was immediately deprotected in acidic solution to afford **86** in 87% overall yield. Following this, the C-6 hydroxyl group of **86** was selectively protected by reaction with trityl chloride and pyridine to give **87** in excellent yield. Next, methyl iodide and sodium hydride were employed to methylate the C-5 hydroxyl group, which produced **88** in 97% yield. Following this, the compound was treated with H_2 and $Pd(OH)_2$ to remove the benzyl groups, and then the trityl group was cleaved with 10% HCl in methanol, after benzoylation, building block **89** was synthesized in 88% yield.

3.3 Summary and Future Work

In this project, I worked toward the synthesis of UDP-Gal*f* analogs in which each hydroxyl group has been deoxygenated or methylated. This panel of compounds will allow us to probe the steric and hydrogen-bonding requirements of GlfT2. The route developed involved the chemical synthesis of a panel of modified galactofuranosyl phosphates, which were converted to the sugar nucleotides by an enzymatic method. Two of the eight sugar nucleotides, 5-deoxy-UDP-Gal*f* (**18**) and 6-deoxy-UDP-Gal*f* (**20**), have been synthesized by this approach. The preparation of these compounds requires skills in both traditional organic synthesis, and also enzymatic reactions, as well as purification by HPLC. To date, all precursors for sugar nucleotides have been prepared and the remaining UDP-Gal*f* analogs are in the process by another group member (Myles Poulin).

The bioactivity evaluation of these modified sugar nucleotides is the key step to construct an enzyme map of GlfT2. By far, the two UDP-Gal*f* analogs synthesized have

been tested as substrates and inhibitors of GlfT2. The 5-deoxy UDP-Gal*f* analog (**18**) displayed inhibition ability, while the 6-deoxy-UDP-Gal*f* (**20**) was a substrate but not an inhibitor. The result indicates the C-5 hydroxyl group of sugar nucleotides should be an important bonding site to GlfT2, which C-6 hydroxyl group is not. After the panel of modified UDP-Gal*f* are prepared and evaluated, we can obtain a relative comprehensive structure and activation relationship between the sugar nucleotide donor and acceptors which can help us to develop more efficient inhibitors for GlfT2.

To date, all biological tests including oligosaccharides were processed *in vitro*. Because *in vitro* test condition is not the same as the condition inside of organism, the results maybe vary from that arising *in vivo*. If *in vitro* tests can identify some compounds having significant inhibition ability to GlfT2, *in vivo* bioactivity test will be the second stage of our projects.

Chapter 4: Experimental Procedures

4.1 General Methods

All reagents used in my experiments were purchased from commercial sources without further purification, while solvents used in reactions were purified by PURESOLV-400 System from innovative Technology Inc.. Unless noted, all reactions were performed under a positive pressure of argon and were monitored by TLC on silica gel G-25 UV_{245} (0.25mm, Macherey-Nagel). Spots were detected under UV light and/or stained by charring with acidified ethanolic anisaldehyde. Solvents were evaporated under reduced pressure and below 50 °C (water bath). Column chromatography was carried out on silica gel 60 (40-60 mM). The ration between silica gel and crude product ranged from 100:1 to 20:1 (w/w). ^{1}H NMR spectra were processed on INOVA-NMR spectrometers at 500 or 600 MHz, and chemical shifts are referenced to either TMS (0.0, $CDCl_3$) or HOD (4.78, CD_3OD). ^{13}C NMR spectra were recorded at 100 or125 MHz whose chemical shift was reference to $CDCl_3$ (77.23 $CDCl_3$) or CD_3OD (48.9, CD_3OD). ^{31}P NMR was performed at 162 MHz. ^{1}H NMR data were reported as though they were first order, and the peaks were made by 2D-NMR spectra. ESI-MS spectra were recorded on samples suspended in CH_3Cl or CH_3OH and added NaCl. Optical rotations were measured on PERKIN-ELMER 241 polarimeter with sodium D line (589 nm).

4.1.1 Procedure for the Chemoenzymatic Synthesis of UDP-Gal*f* derivatives

To a solution of 6-deoxy Gal*f*-1-phosphate (10 mg, 18 μmol) in HEPES buffer (50 mM, pH 8.0) containing $MgCl_2$ (10 mM), KCl (5 mM) were added UTP (12 mg, 18 μmol), and glucose-1-phosphate uridyltransferase (8 μL,19 mg/mL). Following this, 0.25 units/μL inorganic pyrophosphatase (8 μL) and the immobilized galactose-1- phosphate uridyltransferase (0.4 mL) were added the above solution in a total volume of 1.0 mL. Finally, the reaction was initiated by addition of 50 mM UDP-Glc (2 μL). The solution was incubated with shaking at room temperature under protection of nitrogen. After 12 h, when HPLC analysis (See **Section 4.1.2)** of the spin-filtered (Amicon YM10) solution indicated that UTP was consumed, the reaction mixture was transferred to a BD column cartridge to remove immobilized GalU and GalPUT by cold Milli-Q water (5 × 1 mL). The filtrate was collected by passage through a Millipore Amicon Ultra-15 centrifuge tube with a molecular weight cut off of 10,000 Dalton. Following the centrifugation, the resulting filtrate was loaded on a Sephadex G-15 column with a flow rate 1 mL per minute to remove the remaining salts. After concentration, the crude compound was purified by HPLC (See **Section 4.1.2**) to give the 6-Deoxy-α-D-galactofuranosyl uridine diphosphate (32 mg, 70%) as foam. The purity and identity of UDP-Gal*f* was confirmed by ^{1}H NMR spectroscopy.

4.1.2 HPLC

HPLC instrument: Waters 600E HPLC having a photodiode array (PDA) detector with monitoring at 262 nm. The system was controlled by Empower chromatography software.

Condition for HPLC analysis: Phenomenex C18 (4.6 × 250 mm) column, protected with a C18 guard column cartridge. The gradient elution of two buffers: Buffer A (200 mM Et_3N–HOAc, pH6.6) and buffer B (200 mM, Et_3N–HOAc, pH6.6 containing 5% CH_3CN) was also used.

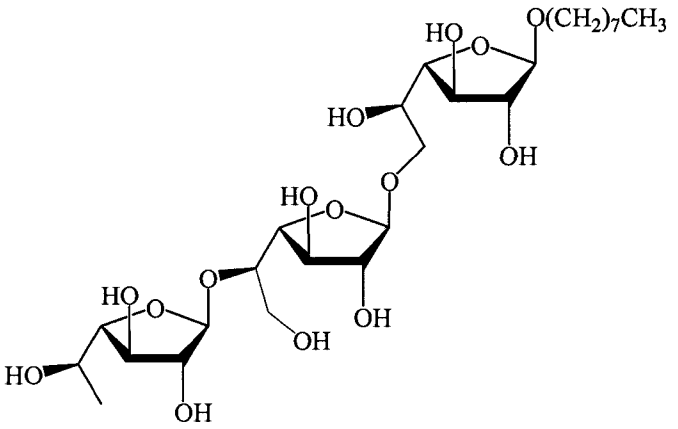
Table 4.1 Gradient conditions for monitoring enzymatic reaction

Time	Flow rate (mL/min)	%A	%B	Curve
0	0.8	96	4	N/A
10	0.8	96	4	6
25	0.8	0	100	6
35	0.8	0	100	6
36	0.8	96	4	6
45	0.8	96	4	6

Condition for HPLC Purification: Phenomenex C18 (21.2 × 250 mm) column, protected with a C18 guard column cartridge. The gradient elution of two buffers: Buffer C (5 mM Sodium phosphate, pH 6.8), Buffer D (MilliQ water) was used. The gradient conditions were: 0→100% buffer D for 12 min, 100% buffer D for 8 min followed by an equilibration phase buffer C for 5 min. The flow-rate was set at 6 mL/min.

4.2 Synthetic Procedures and Data Assignments

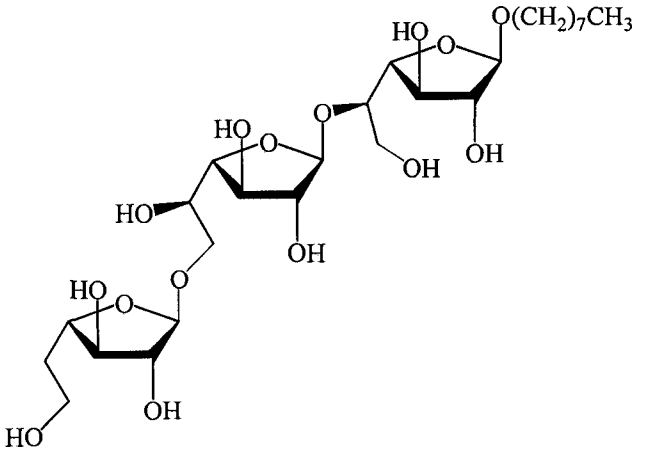
Octyl 6-Deoxy-β-D-galactofurnaosyl-(1→5)-β-D-galactofuranosyl-(1→6)-β-D-galactofuranoside (10)



To a solution of **40** (126 mg, 0.08 mmol) in 1:1 MeOH–CH_2Cl_2 (10 mL) was added 2 N $NaOCH_3$ (0.05 mL). The solution was stirred overnight, and then 10% HOAc in MeOH was used to neutralize the reaction mixture. The solution was concentrated to give a crude residue, which was purified by chromatography on Iatrobeads (7:1 CH_2Cl_2– MeOH) to give **10** (48 mg, 96%) as a white foam. R_f 0.47 (4:1 CH_2Cl_2–MeOH); $[\alpha]_D$ -11.2 (*c* 1.2, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 5.22 (s, 1 H, H-1"), 5.01 (s, 1 H, H-1'), 4.97 (s, 1 H, H-1), 4.16–4.12 (m, 2 H, H-2', H-3"), 4.12–4.08 (m, 2 H, H-3', H-2), 4.08–4.02 (m, 2 H, H-3, H-4), 3.99–3.88 (m, 6 H, H-4', H-5', H-5, H-2", H-4", H-5"), 3.85 (dd, 1 H, *J* = 10.8, 3.6 Hz, H-6), 3.79 (d, 1 H, *J* = 2.0 Hz, H-6'), 3.78 (d, 1 H, *J* = 3.5 Hz, H-6'), 3.75 (dt, 1 H, *J* = 10.0, 6.9 Hz, octyl C$\underline{H}_2$), 3.61 (dd, 1 H, *J* = 10.8, 6.7 Hz, H-6), 3.57 (dt, 1 H, *J* = 10.0, 6.6 Hz, octyl C$\underline{H}_2$), 1.63–1.56 (m, 2 H, octyl C$\underline{H}_2$), 1.38–1.24 (m, 13 H, octyl C$\underline{H}_2$, 2 × H-6"), 0.86 (dd, 3 H, *J* = 7.1, 6.4 Hz, octyl C$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 108.6 (C-1'), 107.9 (C-1"), 107.8 (C-1), 88.0 (C-2"), 83.3 (C-4'), 82.8 (C-2/C-2'),

82.3 (C-2'/C-2), 81.9 (C-3'/C-3), 81.9 (C-3/C-3'), 78.1 (C-4"), 77.5 (C-3"/C-4), 77.3 (C-4/C-3"), 76.6 (C-5'), 70.2 (C-5), 70.1 (C-6), 69.4 (octyl $\underline{C}H_2$), 68.2 (C-5"), 61.9 (C-6'), 31.9 (octyl $\underline{C}H_2$), 29.5 (octyl $\underline{C}H_2$), 29.2 (2 × octyl $\underline{C}H_2$), 26.0 (octyl $\underline{C}H_2$), 22.9 (octyl $\underline{C}H_2$), 19.2 (C-6"), 14.3 (octyl $\underline{C}H_3$). HRMS (ESI) *m/z* Calc. for (M+Na) $C_{26}H_{48}O_{15}Na$: 623.2887. Found: 623.2885.

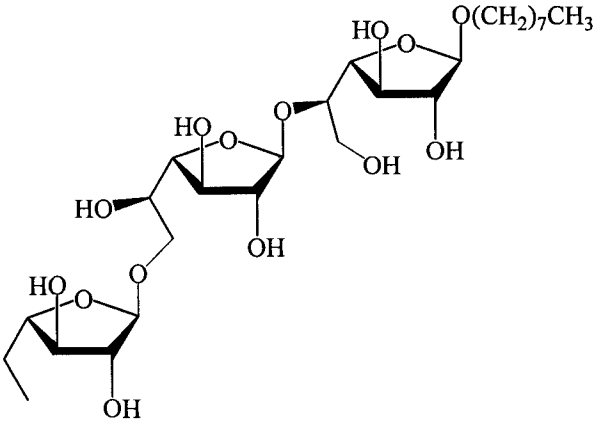
Octyl 5-Deoxy-α-L-*arabino*-hexofuranosyl-(1→6)-β-D-galactofuranosyl-(1→5)-β-D-galactofuranoside (11)



To a solution of **51** (260 mg, 0.17 mmol) in 1:1 MeOH–CH_2Cl_2 (20 mL) was added 2 N $NaOCH_3$ (0.05 mL). The reaction mixture was stirred for overnight followed by neutralization with 10% HOAc in MeOH. The solution was then concentrated to give a crude residue that was purified by chromatography on Iatrobeads (7:1 CH_2Cl_2–MeOH) to give **11** (98 mg, 97%) as a solid. R_f 0.47 (4:1 CH_2Cl_2–MeOH); $[\alpha]_D$ -113.9 (*c* 1.3, CH_3OH); ^1H NMR (600 MHz, $CDCl_3$, δ_H) 5.24 (s, 1 H, H-1'), 5.02 (s, 1 H, H-1"), 4.93 (s, 1 H, H-1), 4.16 (s, 1 H, H-2'), 4.12–4.04 (m, 5 H, H-2", H-4, H-3', H-3, H-4"), 4.03 (s, 1 H, H-2), 4.01–3.92 (m, 3 H, H-4', H-5', H-5), 3.89–3.85 (dd, 1 H, J = 10.9, 3.4 Hz, H-6'), 3.84 (dd, 1 H, J = 6.3, 3.5 Hz, H-3"), 3.82–3.66 (m, 5 H, 2 × H-6, 2 × H-6", octyl $C\underline{H}_2$),

3.61 (dd, 1 H, J = 10.9, 7.7 Hz, H-6'), 3.52 (dd, 1 H, J = 9.7, 6.8 Hz, octyl C$\underline{H}_2$), 2.02–1.84 (m, 2 H, H-5"), 1.63–1.57 (m, 2 H, octyl C$\underline{H}_2$), 1.36–1.25 (m, 10 H, 5 × octyl C$\underline{H}_2$), 0.88 (t, 3 H, J = 6.8 Hz, octyl C$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 108.6 (C-1"), 108.2 (C-1'), 108.0 (C-1), 83.9 (C-4"), 82.4 (C-2/C-2'), 82.2 (C-2'/C-2), 82.1 (C-3"/C-2"), 82.0 (C-2"/C-3"), 81.5 (C-4'/C-3"), 81.2 (C-3"/C-4'), 77.5 (C-4/C-3'), 77.4 (C-3'/C-4), 76.8 (C-5'), 70.5 (C-5), 70.1 (C-6'), 69.2 (octyl $\underline{C}H_2$), 62.5 (C-6), 59.3 (C-6"), 36.0 (C-5"), 32.3 (octyl $\underline{C}H_2$), 29.8 (octyl $\underline{C}H_2$), 29.7 (octyl $\underline{C}H_2$), 29.6 (octyl $\underline{C}H_2$), 26.4 (octyl $\underline{C}H_2$), 23.1 (octyl $\underline{C}H_2$), 14.5 (octyl $\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{26}H_{48}O_{15}Na$: 623.2886. Found: 623.2885.

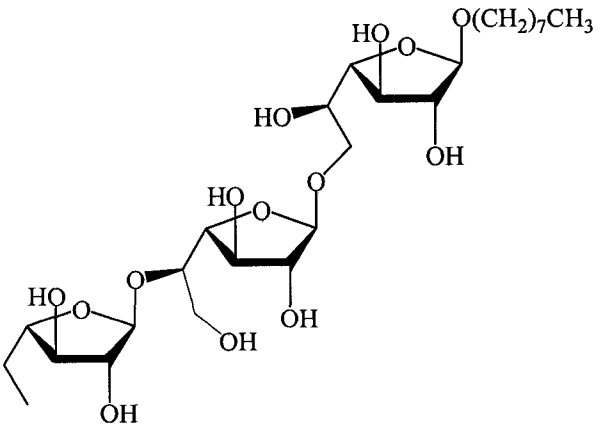
Octyl 5,6-Dideoxy-α/β-L-*arabino*-hexofuranosyl-(1→5)-β-D-galactofuranosyl-(1→6)-β-D-galactofuranoside (12)



To a solution of **53** (260 mg, 0.17 mmol) in 1:1 MeOH–CH_2Cl_2 (20 mL) was added 2 N $NaOCH_3$ (0.05 mL). The solution was stirred for overnight, and 10% HOAc in MeOH was used to neutralize the reaction mixture. The solution was concentrated to give a crude product that was purified by column chromatography on Iatrobeads (7:1 CH_2Cl_2–MeOH) to give **12** (98 mg, 97%) as a solid. R_f 0.47 (4:1 CH_2Cl_2–MeOH); $[\alpha]_D$ -101.0 (*c* 0.6,

CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$, δ_H) 5.24 (s, 1 H, H-1'), 5.01 (s, 1 H, H-1"), 4.92 (s, 1 H, H-1), 4.17 (s, 1 H, H-2'), 4.13–4.16 (m, 4 H, H-2", H-3', H-4, H-4'), 4.04 (s, 1 H, H-2), 4.00–3.94 (m, 3 H, H-3, H-5, H-5'), 3.92 (dd, 1 H, J = 12.6, 6.3 Hz, H-4"), 3.85 (dd, 1 H, J = 10.7, 3.6 Hz, H-6'), 3.82–3.76 (m, 3 H, H-3", 2 × H-6), 3.69 (dt, 1 H, J = 9.4, 6.9 Hz, octyl C$\underline{H}_2$), 3.61 (dd, 1 H, J = 10.7, 7.8 Hz, H-6'), 3.46 (dt, 2 H, J = 9.4, 6.9 Hz, octyl C$\underline{H}_2$), 1.76–1.64 (m, 2 H, 2 × H-5"), 1.64–1.58 (m, 2 H, octyl C$\underline{H}_2$), 1.38–1.26 (m, 10 H, 5 × octyl C$\underline{H}_2$), 1.00 (t, 3 H, J = 7.4 Hz, 2 × H-6"), 0.89 (t, 3 H, J = 6.9 Hz, octyl C$\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 108.5 (C-1"), 108.3 (C-1'), 108.2 (C-1), 85.6 (C-4), 83.9 (C-4/C-4'), 82.7 (C-4'/C-4), 82.3(9) (C-2/C-2'/C-2"), 82.3(4) (C-2'/C-2"/C-2), 82.1 (C-2"/C-2/C-2'), 80.8 (C-3"), 77.7 (C-3'), 77.6 (C-3/C-5"), 77.0 (C-5"/C-3), 70.2 (C-5'), 70.1 (C-6'), 69.0 (octyl $\underline{C}H_2$), 62.6 (C-6), 32.6 (octyl $\underline{C}H_2$), 30.1 (octyl $\underline{C}H_2$), 30.1 (octyl $\underline{C}H_2$), 30.0 (octyl $\underline{C}H_2$), 26.7 (C-5"/octyl $\underline{C}H_2$), 26.5 (octyl $\underline{C}H_2$/C-5"), 23.3 (octyl $\underline{C}H_2$), 14.6 (octyl $\underline{C}H_3$), 10.2 (C-6"); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{26}H_{48}O_{14}Na$: 607.2939. Found: 607.2936.

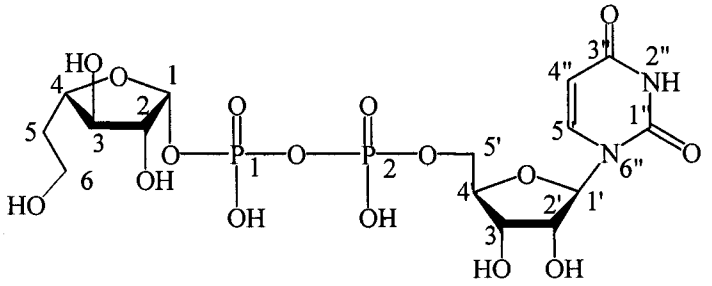
Octyl 5,6-Dideoxy-α/β-L-*arabino*-hexofuranosyl-(1→5)-β-D-galactofuranosyl- (1→6)-β-D-galactofuranoside (13)



To a solution of **55** (241 mg, 0.17 mmol) in 1:1 MeOH–CH_2Cl_2 (15 mL) was added 2 N $NaOCH_3$ (0.05 mL). After the reaction mixture was stirred overnight, 10% HOAc in MeOH solution was added to neutralize the reaction. The resulting solution was concentrated to give a crude residue, which was purified by chromatography on Iatrobeads (7:1 CH_2Cl_2–MeOH) to give **13** (95 mg, 96%) as a foam. R_f 0.47 (6:1 CH_2Cl_2–MeOH); $[\alpha]_D$ -79.2 (*c* 1.2, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 5.20 (s, 1 H, H-1'), 5.02 (s, 1 H, H-1"), 4.96 (s, 1 H, H-1), 4.15–4.05 (m, 5 H, H-2', H-2", H-3", H-4, H-4'), 4.04 (d, 1 H, J = 1.8 Hz, H-2), 4.02–3.96 (m, 2 H, H-4", H-5'), 3.95–3.90 (m, 2 H, H-3, H-5), 3.84 (dd, 1 H, J = 10.6, 3.4 Hz, H-6'), 3.82–3.75 (m, 3 H, H-3', 2 × H-6), 3.73 (dt, 1 H, J = 9.6, 6.9 Hz, octyl C$\underline{H}_2$), 3.63 (dd, 1 H, J = 10.6, 8.0 Hz, H-6'), 3.50 (dt, 1 H, J = 9.6, 6.5 Hz, octyl C$\underline{H}_2$), 1.78–1.58 (m, 4 H, 2 × H-5", octyl C$\underline{H}_2$), 1.40–1.25 (m, 10 H, 5 × octyl C$\underline{H}_2$), 1.11 (t, 3 H, J = 7.4 Hz, 3 × H-6), 0.90 (t, 3 H, J = 6.7 Hz, octyl C$\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 107.9 (C-1), 107.6 (C-1'), 107.2 (C-1"), 84.8 (C-2), 82.7 (C-3), 82.2 (C-2"/C-2'/C-3"/C-4"), 81.6 (C-2'/C-3"/C-4"/C-2"), 81.2 (C-3"/C-4"/C-2"/C-2'), 81.2 (C-4"/C-2"/C-2'/C-3"), 79.9 (C-3'), 76.9 (C-4/C-4'), 76.8 (C-4'/C-4), 76.0 (C-5),

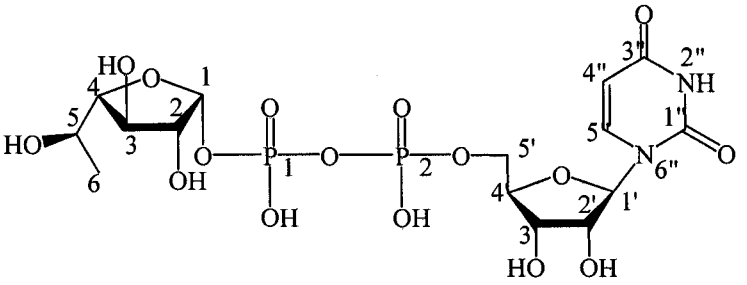
69.5 (C-6'), 69.4 (C-5'), 68.3 (octyl $\underline{C}H_2$), 60.8 (C-6), 31.6 ($\underline{C}H_2$ of Octyl), 29.1 (C-5"/2 × octyl $\underline{C}H_2$), 29.0 (2 × octyl $\underline{C}H_2$/C-5"), 28.9 (2 × octyl $\underline{C}H_2$/C-5"), 25.7 (octyl $\underline{C}H_2$), 25.6 (octyl $\underline{C}H_2$), 22.4 (octyl $\underline{C}H_2$), 13.7 (octyl $\underline{C}H_3$), 9.5 (C-6"); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{26}H_{48}O_{14}Na$: 607.2931. Found: 607.2936.

5-deoxy-α-L-*arabino*-hexofuranosyl uridine diphosphate (18)



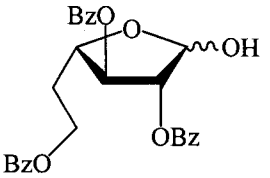
The general procedure (**Section 4.1.1**) was applied to 5-deoxy-5-deoxy-β-L-*arabino*-hexofuranosyl phosphate **66** (10 mg, 18 mmol) to give 6-deoxy UDP-Gal*f* **18** (34 mg, 71%), $[\alpha]_D$ 10.9 (*c* 0.13, H_2O); ^1H NMR (500 MHz, D_2O, δ_H) 7.98 (d, 1 H, *J* = 8.1 Hz, H-5"), 5.99–5.94 (m, 2 H, H-1', H-4"), 5.60 (dd, 1 H, *J* = 5.9, 4.2 Hz, H-1), 4.37 (m, 2 H, H-2', H-3'), 4.30–4.26 (m, 1 H, H-4'), 4.21 (ddd, 1 H, *J* = 11.8, 4.4, 2.6 Hz, H-5'), 4.18 (ddd, 1 H, *J* = 11.8, 5.6, 2.9 Hz, H-5'), 4.10 (ddd, 1 H, *J* = 7.8, 4.2, 2.0 Hz, H-2), 4.04 (dd, 1 H, *J* = 8.2, 7.8 Hz, H-3), 3.92 (ddd, 1 H, *J* = 8.2, 5.5, 5.5 Hz, H-4), 3.78–3.69 (m, 2 H, 2 × H-6), 1.98–1.93 (m, 2 H, 2 × H-5); ^{13}C (125 MHz, $CDCl_3$, δ_C) 167.1 (C-3"), 152.7 (C-1"), 142.5 (C-5"), 103.5 (C-4"), 98.8 (d, 1 C, *J* = 5.8 Hz, C-1), 89.2 (C-1'), 84.1 (d, 1 C, *J* = 9.4 Hz, C-4'), 80.4 (C-4), 78.5 (C-3),77.5 (d, 1 C, *J* = 7.6 Hz, C-2), 74.6 (C-2'/C-3'), 70.5 (C-3'/C-2'), 65.7 (d, 1 C, *J* = 5.75 Hz, H-5'), 59.2 (C-6), 37.6 (C-5); ^{31}P (162 MHz, D_2O, δ_C) -7.57 (d, 1 P, *J* = 20.7 Hz, P-1), -9.18 (d, 1 P, *J* = 20.7 Hz, P-2); HRMS (ESI) *m/z* Calc. for (M-H) $C_{15}H_{23}N_2O_{16}P_2$: 549.0518. Found: 549.0517.

6-Deoxy-α-D-galactofuranosyl uridine diphosphate (20)



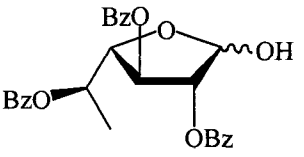
The general procedure (see **Section 4.1.1**) was applied to 6-deoxy Galf-1-phosphate **62** (10 mg, 18 mmol) to give 6-deoxy UDP-Gal*f* **20** (32 mg, 70%), ^{1}H NMR (500 MHz, CD_2Cl_2, δ_H) 7.95 (d, 1 H, J = 8.1 Hz, H-5"), 5.98–5.95 (m, 2 H, H-1', H-4"), 5.63 (dd, 1 H, J = 5.8, 4.3 Hz, H-1), 4.38–4.34 (m, 2 H, H-2', H-3'), 4.27 (dd, 1 H, J = 2.8, 2.5 Hz, H-4'), 4.23 (ddd, 1 H, J = 11.8, 4.3, 2.5 Hz, H-5'), 4.18 (ddd, 1 H, J = 11.8, 5.6, 2.8 Hz, H-5'), 4.11 (ddd, 1 H, J = 8.2, 4.3, 2.5 Hz, H-2), 4.06 (dd, 1 H, J = 8.2, 7.0 Hz, H-3), 3.88 (1 H, dd, J = 7.0, 6.5 Hz, H-5), 3.61 (t, 1 H, J = 7.0 Hz, H-4), 1.19 (d, 3 H, J = 6.5 Hz, 3 × H-6); ^{13}C (125 MHz, CD_2Cl_2, δ_C) 168.7 (C-3"), 154.3 (C-1"), 144.1 (C-5"), 105.2 (C-4"), 100.1 (d, 1C, J = 6.1 Hz, C-1), 90.9 (C-1'), 88.6 (C-4), 85.7 (d, 1C, J = 9.1 Hz, C-4'), 79.7 (d, 1C, J = 7.9 Hz, C-2), 77.0 (C-3), 76.3 (C-3'), 72.1(C-2'/C-3'), 71.7 (C-3'/C-2'), 67.4 (C-5'); ^{31}P NMR (162 MHz, CD_2Cl_2, δ_P) -10.2 (d, 1 P, J = 20.7 Hz, P-1), -10.6 (d, 1 P, J = 20.7 Hz, P-2); HRMS (ESI) *m/z* Calc. for (M–H) $C_{15}H_{23}N_2O_{16}P_2$: 549.0517. Found: 549.0517.

2,3,6-Tri-*O*-benzoyl-5-deoxy-α/β-L-*arabino*-hexofuranose (26)



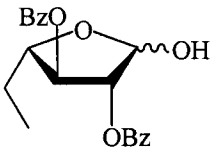
To a solution of **49** (52 mg, 0.11 mmol) in CH_2Cl_2 (3 mL) was added 44% HBr in HOAc (0.5 mL) at 0 °C. The solution was stirred for 1.5 h, and then diluted with CH_2Cl_2 (10 mL). The resulting solution was washed with ice-water (10 mL × 2), cold sat. aq. $NaHCO_3$ solution (10 mL × 2), brine (10 mL) and dried ($MgSO_4$). The dried organic layer was concentrated to afford a syrup, which was purified by column chromatography (3:1 hexane–EtOAc) to give **26** (47 mg, 93%, α:β = 0.3:1) as a foam. R_f 0.58 (2:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–7.95 (m, 7.8 H, Ar), 7.61–7.50 (m, 3.9 H, Ar), 7.47–7.36 (m, 7.8 H, Ar), 5.83 (d, 0.3 H, J = 4.4 Hz, H-1α), 5.75 (dd, 0.3 H, J = 4.8, 4.8 Hz, H-3α), 5.63 (s, 1 H, H-1β), 5.53–5.50 (m, 1.3 H, H-2α, H-2β), 5.42 (dd, J = 4.7, 1.6 Hz, H-3β), 4.65 (ddd, 1 H, J = 8.8, 4.7, 4.5 Hz, H-4β), 4.62–4.50 (m, 2.6 H, 2 × H-6α, 2 × H-6β), 4.30 (ddd, 0.3 H, J = 9.3, 4.8, 4.3 Hz, H-4α), 2.52–2.26 (m, 2.6 H, 2 × H-5α, 2 × H-5β); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.5 (α C=O), 166.5 (β C=O), 165.8 (α C=O), 165.8 (β C=O), 165.7 (α C=O), 165.4 (C=O), 133.5 (Ar), 133.5 (Ar), 132.9 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.5 (Ar), 129.1 (Ar), 129.0 (Ar), 128.5 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 100.75 (C-1β), 95.4 (C-1α), 82.6 (C-2β), 80.5 (C-3β/C-4β), 80.3 (C-4β/C-3β), 79.5 (C-3α), 78.1 (C-2α/C-4α), 77.7 (C-2α/C-4α), 61.6 (C-6α), 61.5 (C-6β), 34.3 (C-5α), 32.6 (C-5β); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{27}H_{24}O_8Na$: 499.1369. Found: 499.1363.

2,3,5-Tri-*O*-benzoyl-6-deoxy-α/β-D-galactofuranose (27)



To a solution of **38** (2.5 g, 5.1 mmol) in CH_2Cl_2 (30 mL) was added 44% HBr in HOAc (8 mL) at 0 °C. After 1.5 h, the solution was concentrated to give a syrup that was dissolved in EtOAc (60 mL). The solution was washed with ice-water (30 mL × 2), cold sat. aq. $NaHCO_3$ solution (30 mL), brine (30 mL) and dried ($MgSO_4$). The dried organic layer was concentrated to afford a crude residue, which was purified by column chromatography (3:1 hexane–EtOAc) to give **27** (2.27 g, 94%, α:β = 0.3:1) as a white foam. R_f 0.56 (2:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.11–7.99 (m, 7.8 H, Ar), 7.61–7.24 (m, 11.7 H, Ar), 5.98 (t, 0.3 H, *J* = 5.1 Hz, H-3α), 5.80 (t, 0.3 H, *J* = 4.6 Hz, H-1α), 5.69 (d, 1 H, *J* = 3.3 Hz, H-1β), 5.66–5.58 (m, 2.3 H, H-5α, H-3β, H-5β), 5.51 (dd, 0.3 H, *J* = 5.1, 4.6 Hz, H-2α), 5.49 (s, 1 H, H-2β), 4.63 (t, 1 H, *J* = 4.8 Hz, H-4β), 4.23 (dd, 0.3 H, *J* = 6.7, 5.1 Hz, H-4α), 3.76 (d, 0.3 H, *J* = 4.6 Hz, α O<u>H</u>), 3.36 (d, 1 H, 3.3 Hz, β O<u>H</u>), 1.52 (d, 3 H, *J* = 6.5 Hz, 3 × H-6β), 1.50 (d, 0.9 H, *J* = 6.4 Hz, 3 × H-6α); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.5 (<u>C</u>=O), 165.9 (<u>C</u>=O), 165.7(7) (<u>C</u>=O), 165.7(1) (<u>C</u>=O), 165.7(0) (<u>C</u>=O), 165.5 (<u>C</u>=O), 133.5(8) (Ar), 133.5(3) (Ar), 133.5(0) (2 × Ar), 133.1 (Ar), 133.0(Ar), 130.1 (2× Ar), 130.0 (2 × Ar), 129.9(3) (2 × Ar), 129.9(0) (2 × Ar), 129.8(6) (2 × Ar), 129.8(0) (2 × Ar), 129.1 (Ar), 129.0(4) (Ar), 129.0(2) (Ar), 129.0(0) (Ar), 128.5 (6 × Ar), 128.4(9) (2 × Ar), 128.4(8) (2 × Ar), 128.4 (2 × Ar), 128.3 (2 × Ar), 100.9 (C-1β), 95.9 (C-1α), 84.2 (C-4β), 83.1 (C-2β), 82.7 (C-4α), 78.0 (C-2α), 77.6 (C-3β), 76.0 (C-3α), 71.4 (C-5α), 69.8 (C-5β), 16.3 (C-6β), 16.2 (C-6α); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{27}H_{24}O_8Na$: 499.1363. Found: 499.1367.

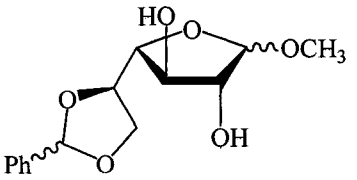
2,3-Di-*O*-benzoyl-5,6-dideoxy-α/β-L-*arabino*-hexofuranose (28)



To a solution of **44** (28 mg, 0.08 mmol) in MeOH (10 mL) was added 10% Pd–C (10 mg). The reaction mixture was stirred for 1 h under H_2 (1 atm) followed by removal of the catalyst by filtration. The filtrate was concentrated to afford a colorless syrup. TLC showed that a pink spot was produced at R_f 0.35 (19:1 hexane–EtOAc). The resulting syrup was dissolved in CH_2Cl_2 (10 mL) at 0 °C. To this solution was added 44% HBr in HOAc (1 mL) and Ac_2O (0.2 mL). After 1 h, CH_2Cl_2 (10 mL) was used to dilute the solution, which was washed with ice-water (5 mL × 3), satd aq. $NaHCO_3$ solution (5 × 2 mL), brine (5 mL), and dried (Na_2SO_4). Concentration of the organic extract gave a crude residue that was purified by column chromatography (4:1 hexane–EtOAc) to afford **28** (24 mg, 90%, α:β = 0.2:1) as a foam. R_f 0.27 (4:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–8.01 (m, 4.8 H, Ar), 7.62–7.57 (m, 2.4 H, Ar), 7.55–7.51 (m, 4.8 H, Ar), 5.75 (dd, 0.2 H, J = 6.1, 4.5 Hz, H-1α), 5.68 (t, 0.2 H, J = 5.19 Hz, H-3α), 5.58 (d, 1 H, J = 4.0 Hz, H-1β), 5.48–5.45 (m, 1.2 H, H-2α, H-2β), 5.32 (dd, 1 H, J = 5.0, 1.3 Hz, H-3β), 4.41 (dt, 1 H, J = 7.3, 5.0 Hz, H-4β), 4.02 (dt, 0.2 H, J = 7.9, 5.2 Hz, H-4α), 3.18 (d, 0.2 H, J = 6.1 Hz, α-O<u>H</u>), 3.12 (d, 1 H, J = 4.0 Hz, β-O<u>H</u>), 1.98–1.80 (m, 2.4 H, H-5α, H-5β), 1.11–1.05 (m, 3.6 H, H-6α, H-6β); ^{13}C (125 MHz, $CDCl_3$, δ_C) 165.7 (2 × <u>C</u>=O), 165.5 (2 × <u>C</u>=O), 133.5 (2 × Ar), 133.4 (2 × Ar), 129.9 (2 × Ar), 129.8(8) (4 × Ar), 129.8(1) (2 × Ar), 129.3 (2 × Ar), 129.2 (Ar), 129.0 (Ar), 128.5(1) (4 × Ar), 128.5(0) (4 × Ar), 100.6 (C-1β), 95.2 (C-1α), 84.2 (C-4β), 82.8 (C-2β), 81.9 (C-4α), 80.1 (C-3β), 78.7 (C-3α), 77.7

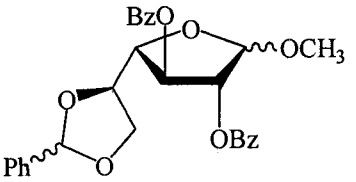
(C-2α), 28.0 (C-5α), 26.4 (C-5β), 9.9 (C-6α), 9.8 (C-6β); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{20}H_{20}O_6Na$: 379.1153. Found: 379.1152.

Methyl 5,6-Di-*O*-benzylidene-α/β-D-galactofuranoside (32)



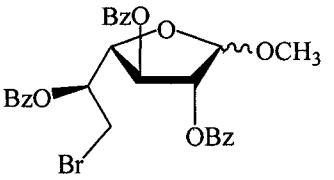
To a solution of **31** (190 mg, 0.52 mmol) in 1:1 CH_2Cl_2–MeOH (8 mL) was added 2 N $NaOCH_3$ in CH_3OH (0.2 mL). The solution was stirred for 2 h, and then neutralized by the addition of HOAc. Evaporation of the solution afforded a white residue, which was dissolved in dry DMF (5 mL). To this solution was added benzaldehyde dimethyl acetal (0.12 mL, 0.80 mmol) and CSA (60 mg, 0.26 mmol). The mixture was stirred for 6 h on a rotary evaporator at 40 °C. The reaction mixture was then neutralized with Et_3N (1 mL). Removal of the DMF under reduced pressure gave a crude syrup, which was purified by column chromatography (1:3 hexane–EtOAc) to give **32** (93 mg, 63%) as an oil. R_f0.33 (1:3 hexane–EtOAc, α_1:α_2:β_1:β_2 = 0.3:0.3:1:1); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 5.92 (s, 1H, β_1 benzylidene H), 5.79 (s, 1H, β_2 benzylidene H), 4.95 (s, 1H, β_1 H-1), 4.89 (s, 1H, β_2 H-1), 4.46–4.41 (m, 2H, β_1 H-5, β_2 H-5), 4.32–4.22 (m, 2H, β_1 H-5, β_2 H-6), 4.14–4.06 (m, 6H, β_1 H-3, β_1 H-4, β_1 H-6, β_2 H-3, β_2 H-4, β_2 H-6), 4.02–3.90 (m, 4H, β_1 H-2, β_1 2-OH, β_2 H-2, β_2 2-OH), 3.40 (s, 3H, β_1-OCH_3/β_2-OCH_3), 3.39 (s, 3H, β_2-OCH_3/β_1-OCH_3), 3.33–3.32 (m, 2H, β_1-OH, β_2-OH), ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 109.4 (β_1 C-1), 109.3 (β_2 C-1), 104.9 (β_1 benzylidene C), 104.7 (β_2 benzylidene C), HRMS (ESI) *m/z* Calc. for (M+Na) $C_{14}H_{18}O_6Na$: 305.0990. Found: 305.0995.

Methyl 5,6-*O*-Benzylidene-2,3-di-*O*-bezoyl-α/β-D-galactofuranoside (33)



To a solution of **32** (4.84 g, 17.2 mmol) in dry pyridine (80 mL) was added benzoyl chloride (5.76 mL, 41.2 mmol) dropwise at 0 °C. The solution was stirred for 12 h, and methanol (2mL) was added to quench the excess benzoyl chloride. Removal of pyridine gave a yellow syrup, which was dissolved in EtOAc (100 mL). The organic layer was washed with 1 M HCl (50 mL), sat. aq. $NaHCO_3$ (50 mL), brine (60 mL) and dried ($MgSO_4$). After concentration of the reaction mixture, the resulting syrup was purified by column chromatography (9:1 hexane–EtOAc) to give **5** (7.83 g, 98%) as an oil. R_f = 0.27 (9:1 hexane–EtOAc); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{28}H_{26}O_8Na$: 513.1521. Found: 513.1520. **33** contained four isomers, and its NMR spectrum was too complicated. The structure of **33** was identified by HRMS data.

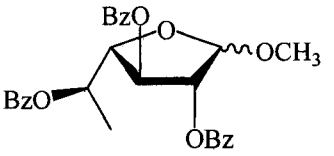
Methyl 2,3,5-Tri-*O*-benzoyl-6-bromo-6-deoxy-α/β-D-galactofuranoside (37)



To a solution of **33** (7.83 g, 17.3 mmol) in CCl_4 (200 mL) was added *N*-bromosuccimide (3.39 g, 19.0 mmol) and barium carbonate (1.88 g, 9.52 mmol) at room temperature. The mixture was heated at reflux for 3 h, and then the reaction mixture was cooled and filtered. The filtrate was concentrated and the resulting syrup was dissolved in ether (250 mL). The solution was washed with H_2O (75 mL × 3), brine (50 mL), and then dried

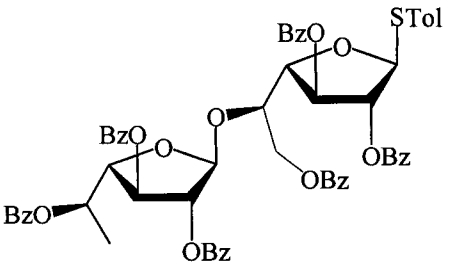
(Na_2SO_4). Evaporation of the organic solution afforded a pale yellow syrup, which was purified by chromatography (9:1 hexane–EtOAc) to yield compound **37** (6.65 g, 71.1%, α:β = 0.3:1) as a white foam. R_f 0.48 (4:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.20–8.18 (m, 0.6 H, Ar), 8.12–8.04 (m, 5.2 H, Ar), 7.96–7.92 (m, 2 H, Ar) 7.61–7.52 (m, 3.9 H, Ar), 7.48–7.41 (m, 3.9 H, Ar), 7.38–7.28 (m, 3.9 H, Ar), 6.11 (dd, 1 H, *J* = 7.6, 6.3 Hz, H-3α), 5.82 (ddd, 1H, *J* = 7.0, 3.1, 3.1 Hz, H-5β), 5.58 (ddd, 1 H, *J* = 6.5, 5.0, 5.0 Hz, H-5α), 5.53 (dd, 1 H, *J* = 5.6, 1.3 Hz, H-3β), 5.49 (d, 1 H, *J* = 1.3 Hz, H-2β), 5.49 (dd, 1 H, *J* = 7.6, 4.6 Hz, H-2α), 5.39 (d, 1 H, *J* = 4.6 Hz, H-1α), 5.23 (s, 1 H, H-1β), 4.9 (dd, 1 H, *J* = 5.6, 3.1 Hz, H-4β), 4.71 (dd, 1 H, *J* = 6.3, 4.9 Hz, H-4α), 3.84–3.73 (m, 4 H, 2 × H-6α, 2 × H-6β), 3.52 (s, 3 H, β-OC$\underline{H}_3$), 3.45 (s, 3 H, α-OC$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 165.9 ($\underline{C}$=O), 165.8 ($\underline{C}$=O), 165.7 ($\underline{C}$=O), 165.6 ($\underline{C}$=O), 165.5 ($\underline{C}$=O), 165.4 ($\underline{C}$=O), 133.5(6) (Ar), 133.5(3) (Ar), 133.4(6) (Ar), 133.4(3) (Ar), 133.3(7) (Ar), 133.(5) (Ar), 130.0 (4 × Ar) 129.9(9) (4 × Ar), 129.9(3) (2 × Ar), 129.8 (2 × Ar), 129.5 (Ar), 129.3 (Ar), 129.0(4) (Ar), 129.0(0) (Ar), 128.9(7) (Ar), 128.9(1) (Ar), 128.4 (12 × Ar), 106.5 (C-1β), 101.0(C-1α), 82.5 (C-2β), 81.0 (C-4β), 78.6 (C-4α), 77.7 (C-3β), 77.5 (C-2α), 74.6 (C-3α), 72.8 (C-5α), 71.8 (C-5β), 55.7 (α OCH_3), 54.9 (β O$\underline{C}H_3$), 29.2 (C-6α), 29.0 (C-6β); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{28}H_{25}BrO_8Na$: 591.0625. Found: 591.0626.

Methyl 2,3,5-Tri-*O*-benzoyl-6-deoxy-α/β-D-galactofuranoside (38)



To a solution of **37** (4.70 g, 8.27 mmol) in EtOAc (45 mL) was added 10% Pd–C (60 mg) and Et_3N (5 mL) at room temperature. The solution was allowed to stir under H_2 (45 PSI) for 12 h, and then the Pd–C was removed by filtration. Evaporation of the solution afforded a pale yellow syrup, which was purified by chromatography (6:1 hexane–EtOAc) to yield **38** (3.08 g, 76%, α:β = 0.3:1) as a white foam. R_f 0.58 (4:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.12–8.01 (m, 7.8 H, Ar), 7.60–7.48 (m, 3.9 H, Ar), 7.46–7.36 (m, 5.2 H, Ar), 7.32–7.26 (m, 2.6 H, Ar), 6.16 (dd, 0.3 H, J = 7.5, 6.4 Hz, H-3α), 5.67–5.62 (dq, 1 H, J = 6.5, 4.1 Hz, H-5β), 5.62–5.60 (m, 1 H, H-3β), 5.50–5.43 (m, 1.6 H, H-2α, H-5α, H-2β), 5.35 (d, 0.3 H, J = 4.6 Hz, H-1α), 5.20 (s, 1 H, H-1β), 4.48 (dd, 1 H, J = 5.7, 4.1 Hz, H-4β), 4.34 (t, 0.3 H, J = 6.4 Hz, H-4α), 3.51 (s, 3 H, β $OC\underline{H}_3$), 3.44 (s, 0.9 H, α $OC\underline{H}_3$), 1.56 (d, 3 H, J = 6.5 Hz, 3 × H-6β), 1.48 (d, 0.9 H, J = 6.5 Hz, 3 × H-6α); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.0 ($\underline{C}$=O), 165.9 ($\underline{C}$=O), 165.8 ($\underline{C}$=O), 165.6 ($\underline{C}$=O), 165.5 ($\underline{C}$=O), 165.5 ($\underline{C}$=O), 133.4(6) (Ar), 133.4(3) (Ar), 133.4(2) (Ar), 133.4(0) (Ar), 132.9(4) (Ar), 132.9(0) (Ar), 130.3 (Ar), 130.1 (2 × Ar), 130.0 (Ar), 129.9 (4 × Ar), 129.8(7) (4 × Ar), 129.8(4) (Ar), 129.7(5) (2 × Ar), 129.7(4) (2 × Ar), 129.2 (3 × Ar), 128.4(7) (4 × Ar), 128.4(4) (2 × Ar), 128.4(2) (4 × Ar), 106.7 (C-1β), 100.9 (C-1α), 83.4 (C-4β), 82.6 (C-2β), 81.3 (C-4α), 77.8 (C-2α), 77.4 (C-3β), 74.7 (C-3α), 71.3 (C-5α), 69.6 (C-5β), 55.5 (OCH_3α), 54.9 (C-6β), 16.2 (C-6β), 15.8 (C-6α); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{28}H_{26}O_8Na$: 513.1519. Found: 513.1519.

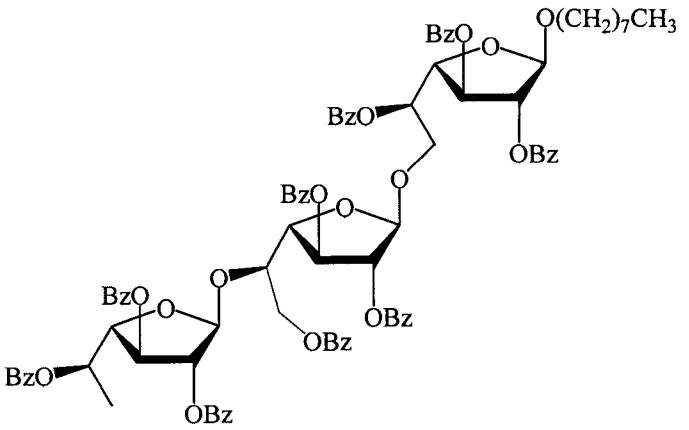
_p_-Tolyl 2,3,5-Tri-_O_-benzoyl-6-deoxy-β-D-galactofuranosyl-(1→5)-2,3,6-tri-_O_-benzoyl-β-D-galactofuranoside (39)



To a solution of **27** (500 mg, 1.05 mmol) in CH_2Cl_2 (12 mL) was added Cl_3CCN (1.26 mL, 12.6 mmol) and DBU (44 μL, 0.29 mmol) at 0 °C. The solution was stirred for 3 h at room temperature at which point TLC showed that a new compound was produced, R_f 0.41 (6:1 hexane–EtOAc). The mixture was concentrated, and the resulting residue was purified by quick passage through a column of silica gel (7:1 hexane–EtOAc) to afford 2,3,5-tri-*O*-benzoyl-6-deoxy-α/β-galactofuranosyl trichloro- imidate. The intermediate was mixed with the acceptor **25** (473 mg, 1.00 mmol) and dried for 12 h in the presence of P_2O_5. To this mixture was added CH_2Cl_2 (12 mL) and 4 Å molecular sieves (600 mg), and the solution was cooled to -30 °C. TMSOTf (38 μL, 0.21 mmol) was added, and the reaction was kept for 40 min before Et_3N (1 mL) was used to neutralize the mixture. The solids were removed by filtration, and the filtrate was concentrated to give a crude syrup that was purified by chromatography (40:1 toluene–EtOAc) to give **39** (705 mg, 72%) as a white foam. R_f 0.39 (25:1 toluene–EtOAc); $[\alpha]_D$ -62.1 (*c* 0.7, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.09–7.89 (m, 11 H, Ar), 7.59–7.02 (m, 23 H, Ar), 5.91 (dd, 1 H, J = 4.5, 2.0 Hz, H-3), 5.74 (d, 1 H, J = 2.4 Hz, H-1), 5.72 (s, 1 H, H-1'), 5.71 (dd, 1 H, J = 2.4, 2.0 Hz, H-2), 5.60 (d, 1 H, J = 1.0 Hz, H-2'), 5.55 (dd, 1 H, J = 5.6, 1.0 Hz, H-3'), 5.47 (dt, 1 H, J = 6.5, 4.4 Hz, H-5'), 4.82 (dd, 1 H, J = 4.5, 3.8 Hz, H-4), 4.77–4.64 (m, 4 H, H-4', H-5, 2 × H-6), 2.29 (s, 3 H, STol $C\underline{H}_3$), 1.35 (d, 3 H, J = 6.5 Hz, 3 × H-6'); ^{13}C

NMR (125 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 165.6 (C=O), 165.5 (2 × C=O), 165.3 (C=O), 165.2 (C=O), 138.1 (2 × Ar), 133.6 (Ar), 133.5 (Ar), 133.3(5) (Ar), 133.3(2) (Ar), 133.0 (Ar), 132.8 (Ar), 132.7 (2 × Ar), 130.1 (Ar), 129.9(8) (2 × Ar), 129.9(4) (2 × Ar), 129.8(9) (2 × Ar), 129.8(5) (2 × Ar), 129.8(0) (2 × Ar), 129.6(9) (2 × Ar), 129.6(7) (2 × Ar), 129.5 (Ar), 129.0(5) (Ar), 129.0(2) (Ar), 128.9(8) (Ar), 128.9(3) (Ar), 128.8 (Ar), 128.5(3) (Ar), 128.5(0) (2 × Ar), 128.4 (2 × Ar), 128.3(3) (2 × Ar), 128.3(0) (2 × Ar), 128.2 (2 × Ar), 105.8 (C-1'), 91.2 (C-1), 84.2 (C-4'), 82.7 (C-4/C-2'), 82.4 (C-2'/C-4), 81.5 (C-2), 77.5 (C-3/C-3'), 77.4 (C-3'/C-3), 74.2 (C-5), 69.7 (C-5'), 64.5 (C-6), 21.1 (STol CH_3), 16.1 (C-6'); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{61}H_{52}O_{15}NaS$: 1079.2875. Found: 1079.2919.

Octyl 2,3,5-Tri-*O*-benzoyl-6-deoxy-β-D-galactofurnaosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (40)

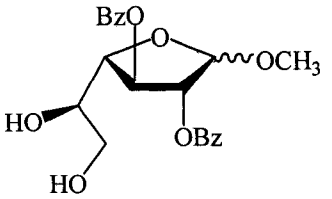


To a mixture of **39** (93 mg, 0.09 mmol) and the acceptor **22** (53 mg, 0.09 mmol) was added dry CH_2Cl_2 (4 mL) and 4Å molecular sieves (300 mg). The mixture was stirred for 30 min at 0 °C, then *N*-iodosuccimide (23 mg, 0.11 mmol) and silver triflate (2.5 mg, 0.02 mmol) were added. After 4 h, the solution became dark red, and was neutralized by the addition of Et_3N (0.5 mL). The mixture was then diluted with CH_2Cl_2 (6 mL) and filtered

through Celite. The filtrate was washed with a sat. aq. $Na_2S_2O_3$ solution (15 mL × 2), brine (15 mL), and dried (Na_2SO_4). The dried organic layer was concentrated to give a crude residue, which was purified by chromatography (40:1 toluene–EtOAc) to give **40** (126 mg, 93%) as a white foam. R_f 0.33 (25:1 toluene–EtOAc); $[\alpha]_D$ -18.9 (*c* 0.8, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.07–7.94 (m, 17 H, Ar), 7.56–7.16 (m, 28 H, Ar), 5.92 (ddd, 1 H, *J* = 6.8, 5.6, 3.9 Hz, H-5), 5.83 (d, 1 H, *J* = 5.0 Hz, H-3'), 5.76 (s, 1 H, H-1"), 5.65 (d, 1 H, *J* = 1.3 Hz, H-2"), 5.60 (d, 1 H, *J* = 5.0, H-3), 5.54 (dd, 1 H, *J* = 5.6 Hz, 1.3 Hz, H-3"), 5.51–5.46 (m, 2 H, H-2', H-5"), 5.44 (s, 1 H, H-2), 5.36 (s, 1 H, H-1'), 5.27 (s, 1 H, H-1), 4.78–4.64 (m, 6 H, H-4, H-4', H-4", H-5', 2 × H-6'), 4.20 (dd, 1 H, *J* = 10.6, 5.6 Hz, H-6), 4.05 (dd, 1 H, *J* = 10.6, 6.8 Hz, H-6), 3.73 (dt, 1 H, *J* = 9.5, 6.7 Hz, octyl OC$\underline{H}_2$), 3.51 (dt, 1 H, *J* = 9.5, 6.5 Hz, octyl OC$\underline{H}_2$), 1.66–1.54 (m, 2 H, 2 × octyl C$\underline{H}_2$), 1.40 (d, 3 H, *J* = 6.5 Hz, 3 × H-6"), 1.38–1.28 (m, 10 H, 10 × octyl C$\underline{H}_2$), 0.88–0.82 (t, 3 H, *J* = 6.9 Hz, octyl C$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.1 ($\underline{C}$=O), 165.8 ($\underline{C}$=O), 165.7 ($\underline{C}$=O), 165.6 ($\underline{C}$=O), 165.5 ($\underline{C}$=O), 165.4 ($\underline{C}$=O), 165.4 ($\underline{C}$=O), 165.1 ($\underline{C}$=O), 165.1 ($\underline{C}$=O), 133.3 (Ar), 133.2(8) (2 × Ar), 133.2(7) (2 × Ar), 133.2(1) (Ar), 133.0 (Ar), 132.9 (Ar), 132.7 (Ar), 130.2 (Ar), 129.9 (4 × Ar), 129.8(7) (2 × Ar), 129.8(6) (2 × Ar), 129.8(0) (2 × Ar), 129.7(7) (2 × Ar), 129.7(3) (2 × Ar), 129.6(9) (2 × Ar), 129.6(5) (2 × Ar), 129.2 (Ar), 129.1 (Ar), 129.0(8) (Ar), 129.0(2) (Ar), 128.9(6) (Ar), 128.9(0) (Ar), 128.4 (2 × Ar), 128.3(7) (8 × Ar), 128.3(3) (2 × Ar), 128.3(0) (2 × Ar), 128.2(5) (2 × Ar), 128.2(1) (2 × Ar), 128.1 (2 × Ar), 106.1 (C-1"), 105.6 (C-1'), 105.6 (C-1), 84.1 (C-4/C-4'/C-4"), 82.9 (C-4'/C-4"/C-4), 82.5 (C-2/C-2'/C-2"), 82.1 (C-2'/C-2"/C-2), 81.7 (C-2"/C-2/C-2'), 81.3 (C-4"/C-4/C-4'), 77.5 (C-3/C-3'/C-3"), 77.4 (C-3'/C-3"/C-3), 77.3 (C-3"/C-3/C-3'), 73.5 (C-5'), 71.3 (C-5), 69.7 (C-5"), 67.8 (octyl $\underline{C}H_2$), 66.0 (C-6),

59.1 (C-6'), 31.8 (octyl $\underline{C}H_2$), 29.4 (octyl $\underline{C}H_2$), 29.4 (octyl $\underline{C}H_2$), 29.2 (octyl $\underline{C}H_2$), 26.1 (octyl $\underline{C}H_2$), 22.6 (octyl $\underline{C}H_2$), 16.1 (C-6"), 14.0 (octyl $\underline{C}H_3$); HRMS (MALDI) *m/z* Calc. for (M+Na) $C_{89}H_{84}O_{24}Na$: 1559.53. Found: 1559.5245.

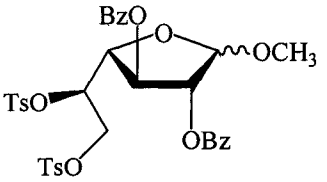
Methyl 2,3-Di-*O*-benzoyl-α/β-D-galactofuranoside (41)



70% HOAc in H_2O (40 mL) was added to **33** (1.54 g, 3.14 mmol). After stirring for 3 h at room temperature, the solution was concentrated to afford a crude resiude, which was dissolved in EtOAc (50 mL). The solution was washed with sat. aq. $NaHCO_3$ solution (30 mL × 2), brine (30 mL), and the organic extracts dried (Na_2SO_4). After concentration of the organic layer, the resulting syrup was purified by chromatography (1:3 hexane–EtOAc) to give **41** (275 mg, 97%, α:β = 0.3:1) as a solid. R_f 0.51 (25:1 CH_2Cl_2–MeOH); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–8.00 (m, 5.2 H, Ar), 7.60–7.55 (m, 2.6 H, Ar), 7.38–7.40 (m, 5.2 H, Ar), 6.03 (dd, 0.3 H, *J* = 6.3, 5.0 Hz, H-3α), 5.60 (dd, 1 H, *J* = 4.9, 1.5 Hz, H-3β), 5.50 (d, 1 H, *J* = 1.5 Hz, H-2β), 5.44 (dd, 0.3 H, *J* = 6.3, 4.9 Hz, H-2α), 5.36 (d, 0.3 H, *J* = 4.9 Hz, H-1α), 5.15 (s, 1 H, H-1β), 4.36–4.31 (m, 1.3 H, H-4α, H-4β), 4.16–4.02 (m, 1.3 H, H-5α, H-5β), 3.82–3.70 (m, 1.6 H, 2 × H-6α, H-6β), 3.45 (s, 3.9 H, α $OC\underline{H}_3$, β $OC\underline{H}_3$), 2.65 (b, 2.6 H, 2 × α O$\underline{H}$, 2 × β O$\underline{H}$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.1 ($\underline{C}$=O), 166.0 ($\underline{C}$=O), 165.9 ($\underline{C}$=O), 165.4 ($\underline{C}$=O), 133.5(8) (2 × Ar), 133.5(3) (2 × Ar), 129.9(8) (2 × Ar), 129.9(5) (2 × Ar), 129.8(8) (2 × Ar), 129.8(5) (2 × Ar), 129.0(8) (Ar), 129.0(6) (Ar), 129.0(2) (Ar), 129.0(0) (Ar), 128.5(4) (4 × Ar), 128.5(0) (4 × Ar), 106.9 (C-1β), 101.7 (C-1α), 83.8 (C-4β), 82.3 (C-4α), 81.5 (C-2β), 77.9 (C-3β), 77.8

(C-2α), 76.2 (C-3α), 71.4 (C-5α), 70.8 (C-5β), 64.2 (C-6β), 63.9 (C-6α), 56.7 (α O$\underline{C}H_3$), 55.0 (β O$\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{21}H_{22}O_8Na$: 425.1210. Found: 425.1207.

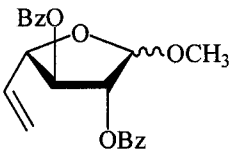
Methyl 2,3-Di-*O*-benzoyl-5,6-di-*O*-*p*-toluenesulfonate-α/β-D-galactofuranoside (42)



To a solution of **41** (1.0 g, 2.5 mmol) in $CHCl_3$ (15 mL) was added DMAP (1.82 g, 15.0 mmol), Et_3N (5 mL) and *p*-toluenesulfonyl chloride (2.90 g, 0.015 mmol) at 0 °C. After 10 min, the solution was heated at reflux for 3 h, and cooled to room temperature at which point MeOH was added to quench the excess *p*-toluenesulfonyl chloride. The solution was concentrated to give a crude residue, which was dissolved in EtOAc (50 mL). The solution was then washed with 1 M HCl (40 mL), sat. aq. $NaHCO_3$ solution (40 mL), brine (50 mL) and dried ($MgSO_4$). The resulting organic layer was concentrated to afford a syrup that was purified by chromatography (3:1 hexane–EtOAc) to give **42** (1.53 g, 87%, α:β = 0.4:1) as a foam. R_f 0.29 (3:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–7.79 (m, 5.6 H, Ar), 7.84–7.81 (m, 0.8 H, Ar), 7.76–7.71 (m, 4 H, Ar), 7.69–7.67 (m, 0.8 H, Ar), 7.64–7.56 (m, 2.4 H, Ar), 7.50–7.42 (m, 5.6 H, Ar), 7.34–7.25 (m, 4 H, Ar), 7.19–7.15 (m, 2 H, Ar), 5.75 (dd, 1 H, *J* = 7.4, 6.5 Hz, H-3α), 5.40 (s, 1 H, H-2β), 5.31 (dd, 0.4 H, *J* = 7.4, 4.4 Hz, H-2α), 5.26 (ddd, 1 H, *J* = 7.0, 6.2, 2.6 Hz, H-5β), 5.21 (d, 0.4 H, *J* = 6.6, 2.6 Hz, H-1α), 5.11–5.07 (m, 1.4 H, H-5α, H-3β), 5.04 (s, 1 H, H-1β), 4.40 (dd, 1 H, *J* = 4.7, 2.6 Hz, H-4β), 4.38–4.25 (m, 3.2 H, H-4α, 2 × H-6α, 2 × H-6β), 3.38 (s, 3 H, β OC$\underline{H}_3$), 3.26 (s, 3 H, α OC$\underline{H}_3$), 2.44 (s, 3 H, β Ts C$\underline{H}_3$), 2.42 (s, 3 H,

α Ts $C\underline{H}_3$), 2.40 (s, 3 H, α Ts $C\underline{H}_3$), 2.33 (s, 3 H, β Ts $C\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 165.7 ($\underline{C}$=O), 165.6 ($\underline{C}$=O), 165.3 ($\underline{C}$=O), 165.3 ($\underline{C}$=O), 145.3 (Ar), 145.2 (Ar), 145.1 (Ar), 145.0 (Ar), 133.5 (2 × Ar), 133.4 (2 × Ar), 133.2 (Ar), 132.6 (Ar), 132.2 (2 × Ar), 130.0(6) (2 × Ar), 130.0(0) (2 × Ar), 129.9(6) (8 × Ar), 129.9(3) (2 × Ar), 129.8 (2 × Ar), 129.7 (2 × Ar), 129.0 (2 × Ar), 128.9 (2 × Ar), 128.5 (2 × Ar), 128.4 (4 × Ar), 128.1 (2 × Ar), 128.1 (2 × Ar), 128.0 (2 × Ar), 128.0 (2 × Ar), 106.6 (C-1β), 100.9 (C-1α), 81.1 (C-2β), 80.8 (C-4β), 77.5 (C-3β), 77.3 (C-2α), 77.0 (C-5α), 75.8 (C-5β), 74.4 (C-3α), 66.6 (C-6α), 66.6 (C-6β), 55.6 (α $O\underline{C}H_3$), 54.9 (β $O\underline{C}H_3$), 21.6 (2 × β Ts $\underline{C}H_3$), 21.6 (2 × α Ts $\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{35}H_{34}O_{12}S_2Na$: 733.1386. Found: 733.1384.

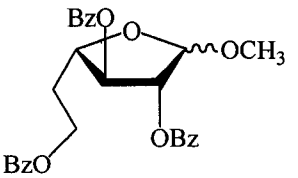
Methyl 2,3-Di-*O*-benzoyl-5,6-dideoxy-α/β-L-*arabino*-hex-5-enfuranoside (44)



To a solution of **42** (1.53 g, 2.15 mmol) in butanone (25 mL) was added NaI (2.8 g, 18.7 mmol), and then the solution was heated to 80 °C for 4 h. The other portion of NaI (2.8 g, 18.7 mmol) was added, and the solution was stirred for 12 h at 80 °C. After cooling, concentration of the solution gave a crude residue, which was dissolved in EtOAc (30 mL). This solution was washed with sat. aq. $Na_2S_2O_3$ solution (30 mL × 2), brine (30 mL), and dried ($MgSO_4$). The organic layer was evaporated to give a syrup that was purified by chromatography (19:1 hexane–EtOAc) to give **44** (0.74 g, 93%, α:β = 0.2:1) as a foam. R_f 0.26 (19:1 hexane–EtOAc); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–8.05 (m, 4.8 H, Ar), 7.61–7.56 (m, 2.4 H, Ar), 7.48–7.44 (m, 4.8 H, Ar), 6.14 (m, 1.2 H, H-5α, H-5β), 5.85 (dd, 0.2 H, J = 7.0, 5.5 Hz, H-3α), 5.52 (dd, 1 H, J = 17.2, 1.2 Hz, H-6β), 5.49 (d, 1 H, J = 2.0

Hz, H-2β), 5.44 (dd, 0.2 H, J = 7.0, 4.5 Hz, H-2α), 5.41 (dd, 0.2 H, J = 17.1, 1.0 Hz, H-6α), 5.37–5.33 (m, 2.2 H, H-1α, H-3β, H-6'β), 5.30 (dd, 0.2 H, J = 10.3, 1.0 Hz, H-6'α), 5.16 (s, 1 H, H-1β), 4.72 (dd, 1 H, J = 6.5, 5.3 Hz, H-4β), 4.54 (dd, 0.2 H, J = 7.0, 5.5 Hz, H-4α), 3.50 (s, 3 H, β $OC\underline{H}_3$), 3.44 (s, 0.6 H, α-$OC\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.0 ($\underline{C}$=O), 165.7 ($\underline{C}$=O), 165.6 ($\underline{C}$=O), 165.3 ($\underline{C}$=O), 137.0 (C-5α), 134.7 (C-5β), 133.4(7) (Ar), 133.4(2) (Ar), 133.4(0) (Ar), 133.3(7) (Ar), 129.9(9) (2 × Ar), 129.9(3) (2 × Ar), 129.8(8) (2 × Ar), 129.8(4) (2 × Ar), 129.3(6) (Ar), 129.3(1) (Ar), 129.2(3) (Ar), 129.2(1) (Ar), 128.4(8) (4 × Ar), 128.4(5) (2 × Ar), 128.4(3) (2 × Ar), 118.1 (C-6β), 117.9 (C-6α), 106.9 (C-1β), 101.3 (C-1α), 83.0 (C-4β), 82.0 (C-2β), 81.7 (C-4α), 80.7 (C-3β), 79.1 (C-3α), 77.6 (C-2α), 55.6 (α-$O\underline{C}H_3$), 55.0 (β-$O\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{21}H_{20}O_6Na$: 391.1149. Found: 391.1152.

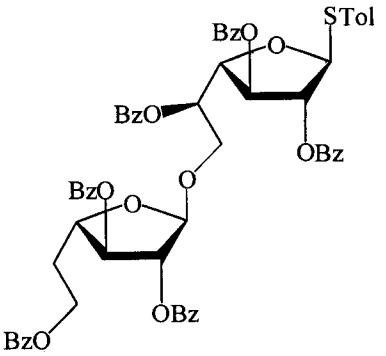
Methyl 2,3,6-Tri-*O*-benzoyl-5-deoxy-α/β-L-*arabino*-hexofuranoside (49)



To a solution of **44** (1.85 g, 5.02 mmol) in Et_2O (25 mL) was added silver trifluoroacetate (1.22 g, 5.52 mmol) and a solution of I_2 (1.40 g) in Et_2O (25 mL) dropwise. The solution was vigorously stirred with exclusion of light. After 10 min, the solid was removed by filtration, and the filtrate was washed with satd aq. $Na_2S_2O_3$ solution (2 × 50 mL). The organic layer was concentrated to give a syrup, which was dissolved in ethanol (50 mL) containing Et_3N (5 mL), and W-4 Raney Nickel was added. The solution was stirred under H_2 (1 atm) for 12 h. Then, waste solid catalyst was removed by filtration, and the filtrate was concentrated to afford a crude residue which was sequentially dissolved in

pyridine (40 mL). To the solution was added benzoyl chloride (0.60 mL, 4.88 mmol) dropwise at 0 °C, and then the reaction mixture was stirred for 12 h at room temperature. Evaporation of the solution gave a crude compound that was dissolved in EtOAc (50 mL). This solution was washed with 1 M HCl (40 mL), sat. aq. $NaHCO_3$ solution (40 mL), brine (40 mL) and dried ($MgSO_4$). Removal of solvent afforded a pale yellow syrup, which was purified by chromatography (9:1 hexane–EtOAc) to give **49** (1.95 g, 98%, α:β = 0.3:1) as a foam. R_f 0.61 (4:1 hexane–EtOAc); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–8.00 (m, 4.7 H, Ar), 7.60–7.51 (m, 3.9 H, Ar), 7.48–7.38 (m, 7.8 H, Ar), 5.81 (dd, 0.3 H, J = 6.6, 4.8 Hz, H-3α), 5.49 (d, 1 H, J = 1.8 Hz, H-2β), 5.47 (dd, 1 H, J = 6.6, 4.5 Hz, H-2α), 5.41 (dd, 1 H, J = 5.3, 1.8 Hz, H-3β), 5.33 (d, 0.3 H, J = 4.5 Hz, H-1α), 5.10 (s, 1 H, H-1β), 4.63–4.52 (m, 2.6 H, 2 × H-6α, 2 × H-6β), 4.46 (ddd, 1 H, J = 8.7, 5.3, 4.5 Hz, H-4β), 4.33 (ddd, 0.3 H, J = 10.3, 4.8, 4.3 Hz, H-4α), 3.46 (s, 3 H, β $OC\underline{H}_3$), 3.44 (s, 3 H, α $OC\underline{H}_3$), 2.56–2.27 (m, 2.6 H, 2 × H-5α, 2 × H-5β); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.4 (<u>C</u>=O), 166.4 (<u>C</u>=O), 166.0 (<u>C</u>=O), 165.9 (<u>C</u>=O), 165.8 (<u>C</u>=O), 165.4 (<u>C</u>=O), 133.4(8) (Ar), 133.4(5) (Ar), 133.4(2) (Ar), 133.4 (Ar), 132.8(7) (Ar), 132.8(5) (Ar), 130.2 (Ar), 130.1 (Ar), 129.9(6) (2 × Ar), 129.9(3) (2 × Ar), 129.8 (4 × Ar), 129.5 (4 × Ar), 129.3 (Ar), 129.2(5) (Ar), 129.2(2) (Ar), 129.2(0) (Ar), 128.5 (2 × Ar), 128.4(7) (2 × Ar), 128.4(4) (4 × Ar), 128.3(2) (2 × Ar), 128.3(0) (2 × Ar), 106.6 (C-1β), 101.5 (C-1α), 82.2 (C-2β), 80.6 (C-3β), 79.7 (C-3α), 79.5 (C-4β), 78.3 (C-4α), 77.8 (C-2α), 61.7 (C-6α), 61.6 (C-6β), 55.6 (α $O\underline{C}H_3$), 54.8 (β $O\underline{C}H_3$), 34.9 (C-5α), 32.4 (C-5β); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{28}H_{26}O_8Na$: 513.1516. Found: 513.1520.

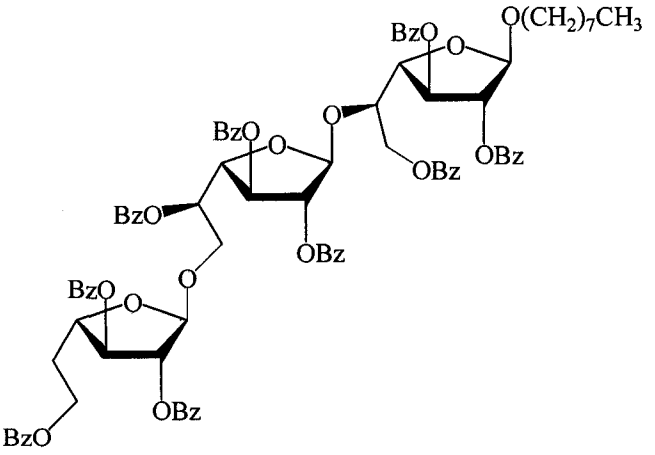
***p*-Tolyl 2,3,6-Tri-*O*-benzoyl-5-deoxy-α-L-*arabino*-hexofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (50)**



To a solution of **26** (460 mg, 0.97 mmol) in CH_2Cl_2 (10 mL) was added Cl_3CCN (0.48 mL, 4.83 mmol) and DBU (58 μL, 0.38 mmol) at 0 °C. The solution was warmed to room temperature and stirred for 3 h. TLC showed a spot at R_f 0.41, which presumably corresponds to the expected tricholoracetimidate (6:1 hexane–EtOAc), The solution mixture was concentrated, and then purified by a quick purification using column chromatography (7:1 hexane–EtOAc). After concentration of the relevant column fractions, the resulting compound was mixed with the acceptor **24** (458 mg, 0.97 mmol). This mixture was dried for 12 h in the presence of P_2O_5. To the mixture was added dry CH_2Cl_2 (10 mL) and 4 Å molecular sieves (600 mg). After the mixture was cooled to -30 °C, TMSOTf (36 μL, 0.20 mmol) was added, and after 40 min Et_3N (1 mL) was used to neutralize the solution. After filtration, the filtrate was evaporated to give a crude syrup that was purified by chromatography (40:1 toluene– EtOAc) to give **50** (767 mg, 75%) as a foam. R_f 0.39 (25:1 toluene–EtOAc); $[\alpha]_D$ -41.2 (*c* 0.9, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.09–7.88 (m, 12 H, Ar), 7.58–7.24 (m, 20 H, Ar), 7.07–7.03 (m, 2 H, Ar), 5.90 (ddd, 1 H, J = 6.1, 5.9, 5.0 Hz, H-5), 5.74 (d, 1 H, J = 1.8 Hz, H-1), 5.67 (dd, 1 H, J = 5.0, 1.8 Hz, H-3), 5.62 (t, 1 H, J = 1.8 Hz, H-2), 5.42 (s, 1 H, H-2'), 5.35 (d, 1 H, J = 4.8 Hz, H-3'), 5.27 (s, 1 H, H-1'), 4.97 (t, 1 H, J = 5.0 Hz, H-4), 4.56–4.41 (m, 3 H, H-4',

2 × H-6'), 4.18 (dd, 1 H, J = 10.5, 5.9 Hz, H-6), 3.97 (dd, 1 H, J = 10.5, 6.1 Hz, H-6), 2.40–2.20 (m, 5 H, 2 × H-5', STol $C\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.3 ($\underline{C}$=O), 165.7 ($\underline{C}$=O), 165.7 ($\underline{C}$=O), 165.4 ($\underline{C}$=O), 165.3 ($\underline{C}$=O), 165.0 ($\underline{C}$=O), 137.9 (Ar), 133.4(6) (Ar), 133.4(1) (Ar), 133.4(0) (Ar), 133.3 (Ar), 133.1 (Ar), 132.8 (Ar), 132.6 (2 × Ar), 130.1 (Ar), 130.0 (2 × Ar), 129.9(3) (2 × Ar), 129.9(0) (2 × Ar), 129.8(4) (2 × Ar), 129.8(1) (2 × Ar), 129.7 (2 × Ar), 129.6 (2 × Ar), 129.5 (2 × Ar), 129.1 (Ar), 129.0 (Ar), 129.0 (Ar), 128.8 (Ar), 128.4(5) (2 × Ar), 128.4(3) (2 × Ar), 128.4(2) (2 × Ar), 128.4(0) (2 × Ar), 128.3 (2 × Ar), 128.2 (2 × Ar), 105.8 (C-1'), 91.3 (C-1), 82.4 (C-2), 81.9 (C-2'), 81.3 (C-4), 80.5 (C-3'/C-4'), 80.4 (C-4'/C-3'), 77.7 (C-3), 71.2 (C-5), 65.6 (C-6), 61.6 (C-6'), 32.3 (C-5'), 21.0 (STol $\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{61}H_{52}O_{15}Na$: 1079.2927. Found: 1079.1927.

Octyl 2,3,6-Tri-*O*-benzoyl-5-deoxy-α-L-*arabino*-hexofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (51)

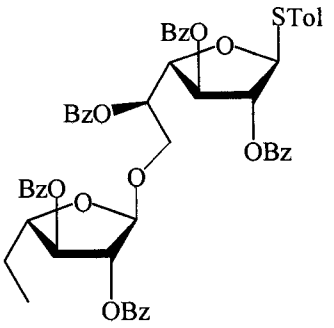


To a mixture of **50** (232 mg, 0.22 mmol) and the acceptor **23** (120 mg, 0.20 mmol) was added dry CH_2Cl_2 (10 mL) and 4 Å molecular sieves (600 mg). The reaction mixture was stirred for 20 min at 0 °C followed by the addition of *N*-iodosuccimide (108 mg, 0.48

mmol) and silver triflate (10.3 mg, 0.04 mmol). After stirring for 2 h at 0 °C, the solution became dark red, and was then neutralized by Et_3N (0.5 mL). This solution was diluted with CH_2Cl_2 (30 mL) and filtered through Celite. The resulting filtrate was washed with satd. aq. $Na_2S_2O_3$ solution (30 mL × 2), brine (30 mL), and the organic solution was dried ($NaSO_4$). Concentration of the organic extract yielded a crude residue, which was purified by chromatography (15:1 toluene–EtOAc) to afford **51** (260 mg, 77%) as a foam. R_f 0.23 (25:1 toluene–EtOAc); $[\alpha]_D$ -1.9 (*c* 2.3, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.02–7.93 (m, 12 H, Ar), 7.91–7.88 (m, 2 H, Ar), 7.85–7.78 (m, 4 H, Ar), 7.60–7.54 (m, 1 H, Ar), 7.51–7.26 (m, 20 H, Ar), 7.22–7.17 (m, 6 H, Ar), 5.92 (ddd, 1 H, *J* = 7.8, 3.9, 3.7 Hz, H-5'), 5.82 (dd, 1 H, *J* = 5.0, 0.9 Hz, H-3), 5.77 (s, 1 H, H-1'), 5.65 (s, 1 H, H-2'), 5.62 (dd, 1 H, *J* = 4.9, 1.4 Hz, H-3'), 5.50 (d, 1 H, *J* = 0.9 Hz, H-2), 5.35 (d, 1 H, *J* = 1.4 Hz, H-2"), 5.32 (dd, 1 H, *J* = 4.9, 1.4 Hz, H-3"), 5.22 (s, 1 H, H-1), 5.15 (s, 1 H, H-1"), 4.92 (dd, 1 H, *J* = 4.9, 3.7 Hz, H-4'), 4.78–4.66 (m, 3 H, H-5, 2 × H-6), 4.53–4.38 (m, 4 H, H-4, H-4'', 2 × H-6"), 4.12 (dd, 1 H, *J* = 11.4, 3.9 Hz, H-6'), 3.98 (dd, 1 H, *J* = 11.4, 7.8 Hz, H-6'), 3.68 (dt, 1 H, *J* = 9.5, 6.8 Hz, octyl C$\underline{H}_2$), 3.57 (dt, 1 H, *J* = 9.5, 6.8 Hz, octyl C$\underline{H}_2$), 2.34–2.18 (m, 2 H, 2 × H-5"), 1.63−1.52 (m, 2 H, octyl C$\underline{H}_2$), 1.39–1.18 (m, 10 H, 5 × octyl C$\underline{H}_2$), 0.86 (t, 3 H, *J* = 7.0 Hz, octyl C$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.3 (<u>C</u>=O), 166.1 (<u>C</u>=O), 165.8 (<u>C</u>=O), 165.7 (<u>C</u>=O), 165.6 (<u>C</u>=O), 165.4 (<u>C</u>=O), 165.4 (<u>C</u>=O), 165.1 (<u>C</u>=O), 164.9 (<u>C</u>=O), 133.3(5) (Ar), 133.3(3) (Ar), 133.3(1) (Ar), 133.1(8) (Ar), 133.1(5) (Ar), 133.1(0) (Ar), 132.9 (Ar), 132.8 (Ar), 132.7 (Ar), 130.2 (Ar), 129.9(2) (4 × Ar), 129.9(0) (2 × Ar), 129.7(7) (8 × Ar), 129.7(0) (2 × Ar), 129.6 (2 × Ar), 129.1(7) (2 × Ar), 129.1(6) (2 × Ar), 129.1(1) (Ar), 129.0 (Ar), 128.9 (Ar), 128.9 (Ar), 128.5 (2 × Ar), 128.4 (2 × Ar), 128.3 (4 × Ar), 128.2(9) (2 × Ar), 128.2(7) (2 × Ar),

128.2(3) (4 × Ar), 128.1 (2 × Ar), 106.3 (C-1), 105.5 (C-1'/C-1"), 105.3 (C-1"/C-1'), 82.3 (C-4), 82.2 (C-4'), 82.0 (C-2/C-2'/C-2"), 81.9 (C-2'/C-2"/C-2), 81.7 (C-2"/C-2/C-2'), 80.3 (C-4"), 80.1 (C-3"), 77.8 (C-3'), 77.1 (C-3), 73.1 (C-5), 71.7 (C-5'), 67.6 (octyl $\underline{C}H_2$/C-6'), 67.3 (C-6'/octyl $\underline{C}H_2$), 64.7 (C-6), 61.5 (C-6"), 32.3 (C-5"), 31.8 (octyl $\underline{C}H_2$), 29.5 (octyl $\underline{C}H_2$), 29.4 (octyl $\underline{C}H_2$), 29.2 (octyl $\underline{C}H_2$), 26.1 (octyl $\underline{C}H_2$), 22.6 (octyl $\underline{C}H_2$), 14.1 (octyl $\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{89}H_{84}O_{24}Na$: 1559.5252. Found: 1559.5250.

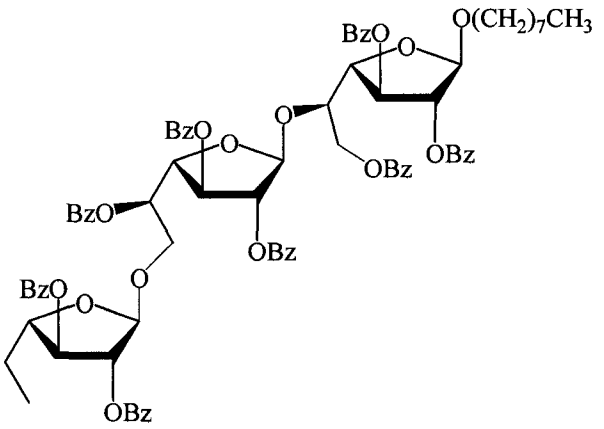
_p_-Tolyl 2,3-Di-*O*-benzoyl-5,6-dideoxy-α/β-L-*arabino*-hexofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (52)



To a solution of **28** (110 mg, 0.31 mmol) in CH_2Cl_2 (4 mL) was added Cl_3CCN (0.46 mL, 4.65 mmol) and DBU (15 μL, 0.10 mmol) at 0 °C. The reaction solution was warmed to room temperature and kept for 45 min. TLC showed that a spot was produced at R_f 0.54 (6:1 hexane–EtOAc). The solution was concentrated and purified by chromatography (8:1 hexane–EtOAc). The collected fraction were concentrated to give a yellow syrup, which was mixed with the acceptor (180 mg, 0.31 mmol). After the mixture was dried for 12 h in the presence of P_2O_5, dry CH_2Cl_2 (5 mL) and 4 Å molecular sieves (120 mg) were added. Then, the mixture was cooled to -30 °C, and TMSOTf (10 μL, 0.06 mmol) was added. After 40 min, Et_3N (0.5 mL) was used to neutralize the acid. Molecular sieves

were removed by filtration, and the filtrate was concentrated to afford a crude residue that was purified by column chromatography (6:1 hexane–EtOAc) to give **52** (252 mg, 87%) as a foam. R_f 0.29 (6:1 hexane–EtOAc); $[\alpha]_D$ -54.0 (*c* 0.9, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–8.04 (m, 6 H, Ar), 8.02–7.97 (m, 2 H, Ar), 7.94–7.89 (m, 2 H, Ar), 7.61–7.26 (m, 17 H, Ar), 7.10–7.08 (m, 2 H, Ar), 5.92 (ddd, 1 H, *J* = 6.0, 5.9, 4.6 Hz, H-5), 5.79 (d, 1 H, *J* = 1.8 Hz, H-1), 5.71 (dd, 1 H, *J* = 4.6, 1.8 Hz, H-3), 5.66 (t, 1 H, *J* = 1.8 Hz, H-2), 5.41 (s, 1 H, H-2'), 5.28–5.25 (m, 2 H, H-1', H-3'), 5.02 (dd, 1 H, *J* = 4.6, 4.5 Hz, H-4), 4.06 (dd, 1 H, *J* = 6.7, 5.2 Hz, H-4'), 4.04 (dd, 1 H, *J* =10.5, 5.9 Hz, H-6), 4.00 (dd, 1 H, *J* = 10.5, 6.0 Hz, H-6), 2.30 (s, 3 H, STol C$\underline{H}_3$), 1.83–1.62 (m, 2 H, 2 × H-5'), 1.00 (t, 3 H, *J* = 7.4 Hz, 3 × H-6'); ^{13}C (125 MHz, $CDCl_3$, δ_C) 165.7 (<u>C</u>=O), 165.7 (<u>C</u>=O), 165.5 (<u>C</u>=O), 165.3 (<u>C</u>=O), 165.1 (<u>C</u>=O), 138.0 (Ar), 133.5 (Ar), 133.4 (2 × Ar), 133.3 (Ar), 133.1 (Ar), 132.7 (2 × Ar), 130.0 (2 × Ar), 129.9 (2 × Ar), 129.8 (2 × Ar), 129.8 (2 × Ar), 129.8 (2 × Ar), 129.7 (2 × Ar), 129.7(2) (Ar), 129.7(0) (Ar), 129.4 (Ar), 129.3 (Ar), 129.0 (Ar), 128.9 (Ar), 128.4(7) (2 × Ar), 128.4(6) (2 × Ar), 128.4(3) (4 × Ar), 128.4(0) (2 × Ar), 105.7 (C-1), 91.3 (C-1'), 83.9 (C-4), 82.4 (C-2/C-2'), 82.2 (C-2'/C-2), 81.1 (C-4'), 79.9 (C-3), 77.7 (C-3'), 71.4 (C-5'), 65.5 (C-6'), 26.1 (C-5), 21.1 (STol <u>C</u>H_3), 9.5 (C-6); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{54}H_{48}O_{13}SNa$: 959.2705. Found: 959.2707.

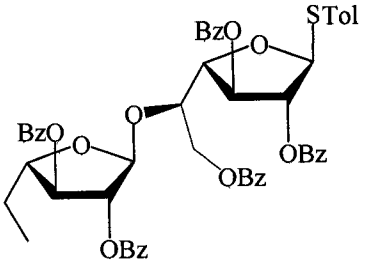
Octyl 2,3-Di-*O*-benzoyl-5,6-dideoxy-α/β-L-*arabino*-hexofuranosyl-(1→5)-2,3,6-tri-*O*- benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galacto-furanoside (53)



To a mixture of **52** (202 mg, 0.22 mmol) and the acceptor **23** (130 mg, 0.22 mmol) was added dry CH_2Cl_2 (10 mL) and 4Å molecular sieves (150 mg). After the reaction mixture was stirred for 30 min at 0 °C, *N*-iodosuccimide (62 mg, 0.28 mmol) and silver triflate (11 mg, 0.04 mmol) were added and solution was stirred for 4 h. The solution was neutralized with Et_3N (0.5 mL), and diluted with CH_2Cl_2 (10 mL). Molecular sieves were removed by filtration, and the filtrate was washed with satd aq. $Na_2S_2O_3$ solution (15 mL × 2), brine (15 mL), and organic layer was dried (Na_2SO_4). The organic layer was concentrated to give a crude residue, which was purified by column chromatography (40:1 toluene–EtOAc) to afford **53** (270 mg, 88%) as a foam. R_f 0.25 (40:1 toluene–EtOAc); $[\alpha]_D$ -2.5 (*c* 0.9, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.05–7.92 (m, 14 H, Ar), 7.87–7.78 (m, 4 H, Ar), 7.60–7.55 (m, 2 H, Ar), 7.54–7.18 (m, 20 H, Ar), 5.95 (ddd, 1 H, *J* = 8.1, 3.9, 3.3 Hz, H-5'), 5.84 (d, 1 H, *J* = 5.1 Hz, H-3), 5.78 (s, 1 H, H-1'), 5.67 (s, 1 H, H-2'), 5.64 (d, 1 H, *J* = 5.1 Hz, H-3'), 5.52 (s, 1 H, H-2), 5.32 (s, 1 H, H-2"), 5.23 (s, 1 H, H-1), 5.20 (d, 1 H, *J* = 5.4 Hz, H-3"), 5.10 (s, 1 H, H-1"), 4.97 (dd, 1 H, *J* = 5.1, 3.4 Hz,

H-4'), 4.80–4.67 (m, 3 H, H-5, 2 × H-6), 4.52 (dd, 1 H, J = 5.1, 3.8 Hz, H-4), 4.22 (dd, 1 H, J = 11.5, 5.4 Hz, H-4"), 4.14 (dd, 1 H, J = 11.1, 3.4 Hz, H-6'), 3.99 (dd, 1 H, J = 11.1, 8.1 Hz, H-6'), 3.74 (dt, 1 H, J = 9.2, 6.8 Hz, octyl C$\underline{H}_2$), 3.48 (dt, 1 H, J = 9.2, 6.3 Hz, octyl C$\underline{H}_2$), 1.84–1.70 (m, 2 H, 2 × H-5"), 1.62–1.54 (m, 2 H, octyl C$\underline{H}_2$), 1.40–1.20 (m, 10 H, 5 × octyl C$\underline{H}_2$), 0.98 (t, 3 H, J = 7.3 Hz, 3 × H-6"), 0.87 (dd, 3 H, J = 7.1, 6.3 Hz, octyl C$\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 166.1 ($\underline{C}$=O), 165.9 ($\underline{C}$=O), 165.7 ($\underline{C}$=O), 165.6 ($\underline{C}$=O), 165.5 ($\underline{C}$=O),), 165.4 ($\underline{C}$=O), 165.2 ($\underline{C}$=O), 164.0 ($\underline{C}$=O), 133.3(6) (Ar), 133.3(2) (Ar), 133.3(0) (Ar), 133.2 (2 × Ar), 133.1 (Ar), 133.0 (Ar), 132.9 (Ar), 129.9 (6 × Ar), 129.7 (8 × Ar), 129.7 (2 × Ar), 129.3(6) (Ar), 129.3(2) (Ar), 129.1 (2 × Ar), 129.0(3) (Ar), 129.0(0) (Ar), 128.9 (2 × Ar), 128.5 (2 × Ar), 128.3(6) (6 × Ar), 128.3(0) (2 × Ar), 128.2(7) (2 × Ar), 128.2(4) (2 × Ar), 128.1 (2 × Ar), 106.2 (C-1"), 105.5 (C-1'/C-1), 105.3 (C-1/C-1'), 83.6 (C-4"), 82.4 (C-4'/C-4), 82.3 (C-4/C-4'), 82.1 (C-2"), 82.0 (C-2'), 81.7 (C-2), 79.8 (C-3"), 77.8 (C-3'), 77.2 (C-3), 73.1 (C-5), 71.8 (C-5'), 67.5 (octyl $\underline{C}H_2$), 67.0 (C-6'), 64.6 (C-6), 31.8 (octyl $\underline{C}H_2$), 29.5 (octyl $\underline{C}H_2$), 29.4 (octyl $\underline{C}H_2$), 29.2 (octyl $\underline{C}H_2$), 26.1 (C-5"/octyl $\underline{C}H_2$), 26.0 (octyl $\underline{C}H_2$/C-5"), 22.6 (octyl $\underline{C}H_2$), 14.1 (octyl $\underline{C}H_3$), 9.5 (C-6"); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{82}H_{80}O_{22}Na$: 1439.5041. Found 1439.5033.

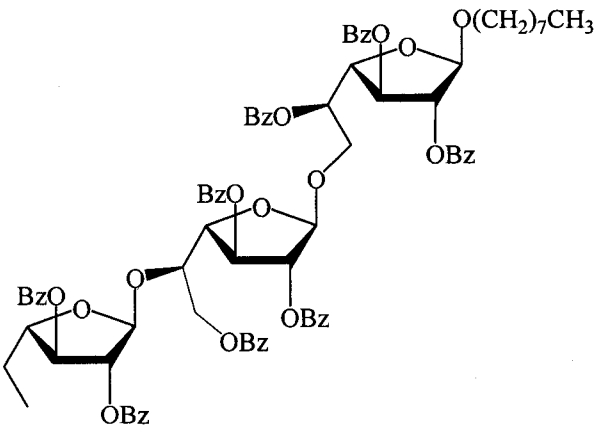
***p*-Tolyl 2,3-Di-*O*-benzoyl-5,6-dideoxy-α/β-L-*arabino*-hexofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (54)**



To a solution of **28** (96 mg, 0.27 mmol) in CH_2Cl_2 (3 mL) was added Cl_3CCN (0.41 mL, 4.4 mmol) and DBU (15 μL, 0.10 mmol) at 0 °C. The reaction mixture was warmed to room temperature and kept for 1 h. TLC showed a spot at R_f 0.54 (6:1 hexane–EtOAc). The solution was concentrated and purified by quick passage through a column of silica gel (8:1 hexane–EtOAc). The resulting trichloroacetimidate was mixed with the acceptor **25** (140 mg, 0.27 mmol). After the mixture was dried for 12 h in the presence of P_2O_5, dry CH_2Cl_2 (5 mL) and 4 Å molecular sieves (150 mg) were added. Then, the mixture was cooled to -30 °C followed by the addition of TMSOTf (8 μL, 0.05 mmol). The reaction proceeded for 40 min, and Et_3N (0.5 mL) was used to neutralize the acid. After removal of molecular sieves by filtration, the solution was concentrated to give a crude product, which was purified by column chromatography (6:1 hexane–EtOAc) to give **54** (203 mg, 81%) as a foam. R_f 0.29 (6:1 hexane–EtOAc). $[\alpha]_D$ -21.2 (*c* 1.2, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–7.90 (m, 10 H, Ar), 7.62–7.56 (m, 2 H, Ar), 7.52–7.42 (m, 9 H, Ar), 7.38–7.24 (m, 6 H, Ar), 7.10–7.08 (m, 2 H, Ar), 5.90 (d, 1 H, J = 3.8 Hz, H-3), 5.73 (s, 1 H, H-1), 5.69 (s, 1 H, H-2), 5.64–5.61 (m, 2 H, H-1', H-2') 5.32 (d, 1 H, J = 5.6 Hz, H-3'), 4.82 (dd, 1 H, J = 3.9, 3.8 Hz, H-4), 4.76–4.64 (m, 3 H, H-5, 2 × H-6), 4.47 (ddd, 1 H, J = 5.6, 5.6, 5.6 Hz, H-4'), 2.31 (s, 3 H, STol C$\underline{H_3}$), 1.88–1.74 (m, 2 H, 2

× H-5'), 0.97 (dd, 2 H, J = 7.6, 6.6 Hz, 3 × H-6'); ^{13}C (125 MHz, $CDCl_3$, δ_C) 166.2 (C=O), 165.6 (C=O), 165.5 (C=O), 165.4 (C=O), 165.1 (C=O), 138.1 (Ar), 133.5 (Ar), 133.4(2) (Ar), 133.4(0) (Ar), 133.2 (Ar), 133.0 (2 × Ar), 132.8 (2 × Ar), 130.0 (2 × Ar), 129.8(4) (5 × Ar), 129.8(0) (2 × Ar), 129.7 (Ar), 129.6 (2 × Ar), 129.4 (Ar), 129.2 (2 × Ar), 129.1 (Ar), 128.9 (Ar), 128.4(8) (5 × Ar), 128.4(3) (2 × Ar), 128.3(1) (2 × Ar), 128.3(0) (2 × Ar), 105.9 (C-1), 91.1 (C-1'), 83.9 (C-4),82.5 (C-2), 81.9 (C-2'/C-4'), 81.6 (C-4'/C-2'), 79.9 (C-3), 77.5 (C-3'), 73.9 (C-5'), 64.7 (C-6'), 26.1 (C-5), 21.1 (STol CH_3), 9.5 (C-6); HRMS (ESI) *m/z* Calc. for (M+Na) $C54H48O_{13}SNa$: 959.2704. Found: 959.2708.

Octyl 2,3-Di-*O*-benzoyl-5,6-dideoxy-α/β-L-*arabino*-hexofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (55)

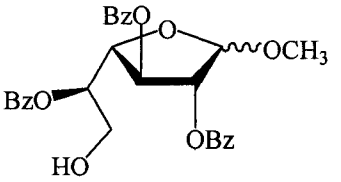


To a mixture of **54** (202 mg, 0.22 mmol) and the acceptor **22** (130 mg, 0.22 mmol) was added dry CH_2Cl_2 (20 mL) and 4Å molecular sieves (150 mg) at 0 °C. After the reaction mixture was stirred for 20 min, *N*-iodosuccimide (62 mg, 0.28 mmol) and silver triflate (11 mg, 0.04 mmol) were added. The solution was stirred for 4 h, and the color became

dark red. The solution was neutralized by Et_3N (1.0 mL), and then diluted with CH_2Cl_2 (10 mL). Molecular sieves were removed by filtration, and the resulting filtrate was washed with satd. aq. $Na_2S_2O_3$ solution (25 mL × 2), brine (25 mL), and dried (Na_2SO_4). The dried organic layer was concentrated to give a crude residue, which was purified by chromatography (40:1 toluene–EtOAc) to give **55** (270 mg, 88%) as a foam. R_f 0.24 (40:1 toluene–EtOAc); $[\alpha]_D$ -7.7 (*c* 2.1, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.08–7.86 (m, 16 H, Ar), 7.60–7.26 (m, 24 H, Ar), 5.90 (ddd, 1 H, *J* = 6.8, 5.7, 3.7 Hz, H-5), 5.80 (d, 1 H, *J* = 5.4 Hz, H-3'), 5.61 (d, 1 H, *J* = 2.2 Hz, H-2"), 5.60 (s, 1 H, H-1"), 5.59 (d, 1 H, *J* = 5.1 Hz, H-3), 5.45–4.43 (m, 2 H, H-2, H-2'), 5.32 (s, 1 H, H-1'), 5.29 (dd, 1 H, *J* = 5.7, 2.2 Hz, H-3"), 5.27 (s, 1 H, H-1), 4.76–4.59 (m, 5 H, H-4, H-4', H-5', 2 × H-6'), 4.41 (dd, 1 H, *J* = 6.6, 5.7 Hz, H-4"), 4.19 (dd, 1 H, *J* = 10.6, 5.7 Hz, H-6), 4.12 (dd, 1 H, *J* = 10.6, 6.8 Hz, H-6), 3.76 (dt, 1 H, *J* = 9.6, 6.7 Hz, octyl C$\underline{H}_2$), 3.50 (dt, 1 H, *J* = 9.6, 6.5 Hz, octyl C$\underline{H}_2$), 1.82–1.70 (m, 2 H, 2 × H-5"), 1.64–1.54 (m, 2 H, octyl C$\underline{H}_2$), 1.40–1.18 (m, 10 H, octyl C$\underline{H}_2$), 0.97 (t, 3 H, *J* = 7.4 Hz, 3 × H-6"), 0.86 (t, 3 H, *J* = 6.9 Hz, octyl C$\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 166.1 (<u>C</u>=O), 165.8 (<u>C</u>=O), 165.6 (<u>C</u>=O), 165.6 (<u>C</u>=O), 165.5 (<u>C</u>=O), 165.4 (<u>C</u>=O), 165.2 (<u>C</u>=O), 165.0 (<u>C</u>=O), 133.3 (Ar), 133.2(8) (Ar), 133.2(6) (Ar), 133.2(3) (2 × Ar), 133.2(0) (Ar), 133.0 (Ar), 132.9 (Ar), 129.9(5) (4 × Ar), 129.9(0) (2 × Ar), 129.8(3) (2 × Ar), 129.8(1) (6 × Ar), 129.7(9) (Ar), 129.6 (2 × Ar), 129.3 (2 × Ar), 129.2(7) (Ar), 129.2(3) (Ar), 128.1(2) (Ar), 129.1(0) (Ar), 129.0 (Ar), 128.3(8) (8 × Ar), 128.3(4) (2 × Ar), 128.3(1) (2 × Ar), 128.2(5) (2 × Ar), 128.2(2) (2 × Ar), 106.2 (C-1'), 105.9 (C-1"), 105.7(C-1), 83.6 (C-4), 82.4 (C-2"), 82.3 (C-2/C-2'), 82.1 (C-2'/C-2), 81.9 (C-4/C-4'), 81.3 (C-4/C-4'), 79.7 (C-3"), 77.5 (C-3/C-3'), 77.2 (C-3'/C-3), 73.6 (C-5'), 71.3 (C-5), 67.8 (octyl <u>C</u>H_2), 66.0 (C-6), 65.2 (C-6'), 31.8 (octyl

$\underline{C}H_2$), 29.5 (octyl $\underline{C}H_2$), 29.4 (octyl $\underline{C}H_2$), 29.2 (octyl $\underline{C}H_2$), 26.1 (C-5"/ octyl $\underline{C}H_2$), 26.0 (octyl $\underline{C}H_2$/C-5"), 22.6 (octyl $\underline{C}H_2$), 14.0 (octyl $\underline{C}H_3$), 9.4 (C-6"); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{82}H_{80}O_{22}Na$: 1439.5033. Found: 1439.5033.

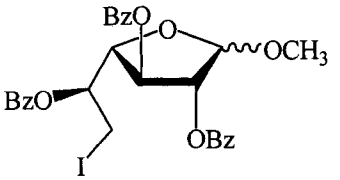
Methyl 2,3,5-Tri-*O*-benzoyl-α/β-D-galactofuranoside (58)



To a solution of **67** (900 mg, 4.63 mmol) in dry pyridine (25 mL) was added trityl chloride (1.55 g, 5.56 mmol) and DMAP (113 mg, 0.93 mmol). After the solution was stirred at 40 °C for 12 h and then cooled to 0 °C, benzoyl chloride (2.1 mL, 16 mmol) was added. The reaction mixture was stirred for 6 h while warming to room temperature and MeOH (5 mL) was added to quench excess benzoyl chloride. After pyridine was evaporated, the resulting crude compound was dissolved in EtOAc (50 mL). The solution was washed with 1 M HCl solution (30 mL), satd aq. $NaHCO_3$ solution (30 mL), brine (30 mL), and the organic layer was dried ($MgSO_4$). The dried organic layer was concentrated to afford a syrup, which was dissolved in 1:1 CH_2Cl_2–CH_3OH (50 mL). To the solution was added 10% HCl in MeOH (1 mL). The solution was then stirred at room temperature for 1 h, and Et_3N (1 mL) was added to neutralize the solution. After concentration, the resulting residue was dissolved in EtOAc, and the solution was washed with water (30 mL), brine (30 mL) and the organic layer was dried ($MgSO_4$). Concentration of the organic layer afforded a syrup that was purified by column chromatography (3:1 hexane–EtOAc) to give **58** (2.0 g, 95%, α:β = 0.3:1) as an amorphous solid. R_f 0.37 (2:1 hexane–EtOAc); ^1H NMR (500 MHz, $CDCl_3$, δ_H)

8.19–7.98 (m, 7.8 H, Ar), 7.61–7.26 (m, 11.7 H, Ar), 6.17 (dd, 0.3 H, J = 7.2, 6.0 Hz, H-3α), 5.68–5.59 (ddd, 1 H, J= 4.8, 4.8, 3.9 Hz, H-5β), 5.61 (d, 1 H, J = 5.2 Hz, H-3β), 5.49–5.45 (m, 1.6 H, H-2α, H-5α, H-2β), 5.38 (d, 0.3 H, J = 4.7 Hz, H-1α), 5.22 (s, 1 H, H-1β), 4.66 (dd, 1 H, J = 5.2, 3.9 Hz, H-4β), 4.58 (dd, 0.3 H, J = 6.0, 5.2 Hz, H-4α), 4.10–4.06 (m, 2.6 H, 2 × H-6α, 2 × H-6β), 3.51 (β $OC\underline{H}_3$), 3.42 (α OCH_3); ^{13}C (125 MHz, $CDCl_3$, δ_C) 166.5 ($\underline{C}$=O), 165.3 ($\underline{C}$=O), 165.9 ($\underline{C}$=O), 165.8(7) ($\underline{C}$=O), 165.8(3) ($\underline{C}$=O), 165.5 ($\underline{C}$=O), 133.5 (2 × Ar), 133.4 (2 × Ar), 133.2 (2 × Ar), 130.0 (2 × Ar), 129.9(7) (2 × Ar), 129.9(4) (4 × Ar), 129.9(0) (4 × Ar), 129.8 (Ar), 129.6 (Ar), 129.1 (Ar), 129.0(1) (Ar), 129.0(0) (Ar), 128.9 (Ar), 128.5 (4 × Ar), 128.4 (4 × Ar), 128.3 (2 × Ar), 106.8 (C-1β), 101.2 (C-1α), 82.1 (C-2β), 81.9 (C-4β), 79.2 (C-4α), 77.7 (C-3β), 77.5 (C-2α), 75.0 (C-3α), 74.9 (C-5α), 73.5 (C-5β), 62.6 (C-6β), 62.2 (C-6α), 55.8 (α $O\underline{C}H_3$), 55.0 (β $O\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{28}H_{26}O_9Na$: 529.1469. Found: 529.1469.

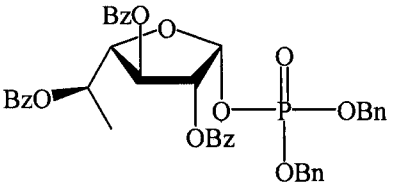
Methyl 2,3,5-Tri-*O*-benzoyl-6-deoxy-iodo-α/β-D-galactofuranoside (59)



To a solution of **58** (53 mg, 0.11 mmol) in dry CH_2Cl_2 (4 mL) was added PPh_3 (52 mg, 0.2 mmol), imidazole (14 mg, 0.2 mmol) and I_2 (51 mg, 0.2 mmol) at 0 °C. The solution was stirred for 1 h at room temperature, at which point TLC (2:1 hexane–EtOAc) indicated that no more starting material remained. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and the solution was washed with satd aq. $Na_2S_2O_3$ solution (10 mL × 2), brine (10 mL), and the organic layer dried ($MgSO_4$). Concentration afforded a pale yellow syrup, which was purified by column chromatography (9:1 hexane–EtOAc) to

give **51** (63 mg, 96%, α:β = 0.3:1) as an amorphous solid. R_f0.31 (9:1 hexane–EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 8.19–7.93 (m, 7.8 H, Ar), 7.60–7.28 (m, 11.7 H, Ar), 6.09 (dd, 1 H, J = 7.3, 6.3 Hz, H-3α), 5.70 (ddd, 1 H, J = 7.0, 7.0, 3.1 Hz, H-5β), 5.50 (d, 1 H, J = 5.6 Hz, H-3β), 5.48–5.47 (m, 1.3 H, H-2α, H-2β), 5.38–5.36 (m, 0.6 H, H-1α, H-5α), 5.22 (s, 1 H, H-1β), 4.82 (dd, 1 H, J = 5.6, 3.1 Hz, H-4β), 4.69 (dd, 0.3 H, J = 6.3, 5.1 Hz, H-4α), 3.62–3.57 (m, 2.6 H, 2 × H-6α, 2 × H-6β), 3.53 (s, 3 H, β $OC\underline{H}_3$), 3.44 (s, 3 H, α $OC\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 165.9 ($\underline{C}$=O), 165.8 ($\underline{C}$=O), 165.7 ($\underline{C}$=O), 165.6 ($\underline{C}$=O), 165.5(1) ($\underline{C}$=O), 165.5(0) ($\underline{C}$=O), 133.5(4) (Ar), 133.5(0) (Ar), 133.4(4) (Ar), 133.4(2) (Ar), 133.3(7) (Ar), 133.3(2) (Ar), 130.0(2) (4 × Ar), 130.0(0) (4 × Ar), 129.9 (2 ×Ar), 129.8 (2 ×Ar), 129.6 (Ar), 129.4 (Ar), 129.0 (2 ×Ar), 128.9(9) (Ar), 128.9(4) (Ar), 128.4(6) (4 × Ar), 128.4(4) (4 × Ar), 128.4(1) (4 × Ar), 106.5 (C-1β), 101.1 (C-1α), 82.6 (C-2β), 81.7 (C-4β), 79.6 (C-4α), 77.7 (C-3β), 77.6 (C-2α), 74.7 (C-3α), 72.9 (C-5α), 72.1 (C-5β), 55.7 (α $O\underline{C}H_3$), 54.9 (β $O\underline{C}H_3$), 1.8 (C-6α), 1.1 (C-6β); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{28}H_{25}O_8INa$: 639.0484. Found: 639.0486.

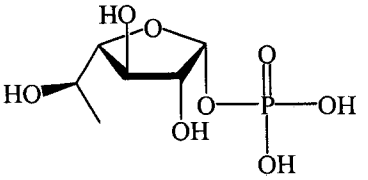
6-Deoxy-2,3,5-Tri-*O*-benzoyl-α-D-galactofuranosyl-phosphate (61)



To a solution of **38** (230 mg, 0.46 mmol) in dry CH_2Cl_2 (5 mL) was added 44% HBr in HOAc (1 mL) at 0 °C. After 1 h, TLC (15:1 toluene–EtOAc) indicated that there were two intermediates (presumably an α/β mixture of glycosyl bromides) whose R_f values were 0.49 and 0.37. The solvent was removed by co-evaporation with toluene to afford a pale yellow syrup, which was dissolved in dry toluene (5 mL). To this solution was added

dibenzyl phosphate (143 mg, 0.51 mmol) and Et_3N (0.5 mL) at 0 °C. After 3 h, the solution was concentrated to give a crude compound that was purified by chromatography (2:1 hexane–EtOAc) to afford **61** (203 mg, 59%, β only) as a amorphous solid. R_f 0.2 (2:1 hexane–EtOAc); $[\alpha]_D$ +55.2 (*c* 0.3, $CHCl_3$); ^1H NMR (500 MHz, CD_2Cl_2, δ_H) 8.11–8.01 (m, 6 H, Ar), 7.62–7.54 (m, 2 H, Ar), 7.51–7.44 (m, 3 H, Ar), 7.41–7.36 (m, 2 H, Ar), 7.31–7.22 (m, 8 H, Ar), 7.20–7.16 (m, 2 H, Ar), 7.11–6.90 (m, 2 H, Ar), 6.30 (dd, 1 H, J = 5.8, 4.5 Hz, H-1), 6.12 (dd, 1 H, J = 7.8, 6.7 Hz, H-3), 6.70 (ddd, 1 H, J = 7.8, 4.5, 2.0 Hz, H-2), 5.45 (dq, 1 H, J = 6.5, 4.8 Hz, H-5), 5.03–4.80 (m, 4 H, 2 × PhC$\underline{H}_2$), 4.30 (dd, 1 H, J = 6.7, 4.8 Hz, H-4), 1.45 (d, 3 H, J = 6.5 Hz, 3 × H-6); ^{13}C (125 MHz, CD_2Cl_2, δ_C) 166.0 ($\underline{C}$=O), 165. 9 ($\underline{C}$=O), 165.8 ($\underline{C}$=O), 136.13 (d, 1 C, J = 7.7 Hz, Ar), 136.03 (d, 1 C, J = 7.7 Hz, Ar), 134.0 (2 × Ar), 133.4 (Ar), 130.5 (Ar), 130.3 (2 × Ar), 130.2 (2 × Ar), 130.1 (2 × Ar), 129.4 (Ar), 129.2 (Ar), 129.0(6) (2 × Ar), 128.9 (2 × Ar), 128.7(9) (2 × Ar), 128.7(7) (2 × Ar), 128.7(3) (2 × Ar), 128.7(1) (Ar), 128.7(0) (Ar), 128.1 (2 × Ar), 128.0 (2 × Ar), 97.9 (d, 1 C, J = 5.0 Hz, C-1), 82.8 (C-4), 77.1 (d, 1 C, J = 6.6 Hz, C-2), 73.6 (C-3), 70.6 (C-5), 69.5 (d, 1 C, J = 3.8 Hz, Ph$\underline{C}H_2$), 69.5 (d, 1 C, J = 3.8 Hz, Ph$\underline{C}H_2$), 15.9 (C-6); ^{31}P (162 MHz, CD_2Cl_2, δ_P) 0.45 (P); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{41}H_{37}O_{11}PNa$: 759.1965. Found: 759.1965.

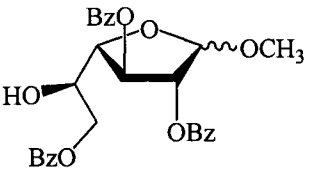
6-Deoxy-2, 3, 5-tri-O-benzoyl-α-D-galactofuranosyl-phosphate (62)



To a solution of **61** (85 mg, 0.11 mmol) in EtOAc (2 mL) was added Et_3N (0.4 mL) and 10% Pd–C (12 mg). The reaction mixture was stirred for 12 h under H_2 (1 atm). The

catalyst was removed by filtration, and the filtrate was concentrated to afford a syrup. This intermediate was dissolved in MeOH–H_2O–Et_3N (5:2:1, 5 mL) and stirred for 4 days. Concentration of the solution gave a crude compound, which was purified by the reverse phase chromatography (10:2:1 EtOH– NH_4OH –H_2O) to give **62** (31 mg, 50%) as a solid. R_f 0.26 (10:2:1 EtOH–NH_4OH–H_2O); ^1H NMR (500 MHz, D_2O, δ_H) 5.52 (dd, 1 H, J = 5.4, 4.3 Hz, H-1), 4.13 (ddd, 1 H, J = 8.2, 4.3, 2.3 Hz, H-2), 4.07 (dd, 1 H, J = 8.2, 6.9 Hz, H-3), 3.87 (dq, 1 H, J = 6.9, 6.5 Hz, H-5), 3.62 (dd, 1 H, J = 6.9, 6.9 Hz, H-4), 1.20 (d, 1 H, J = 6.5 Hz, 3 × H-6); ^{13}C (125 MHz, CD_2Cl_2, δ_C) 97.8 (d, 1 C, J = 5.8 Hz, C-1), 86.7 (C-4), 77.9 (d, 1 C, J = 7.8 Hz, C-2), 75.2 (C-3), 69.9 (C-5), 53.2 (Et_3N $C\underline{H}_2$), 18.4 (C-6), 7.5 (Et_3N $C\underline{H}_3$); ^{31}P NMR (162 MHz, CD_2Cl_2, δ_P) 0.07 (P); HRMS (ESI) *m/z* Calc. for (M–H) $C_6H_{12}O_8P$: 243.0276. Found: 243.0263.

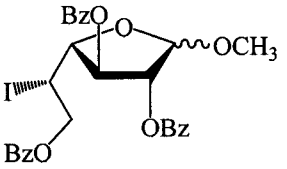
Methyl 2,3,6-Tri-*O*-Benzoyl-α/β-D-galactofuranoside (63)



To a solution of **58** (1.74 g, 3.50 mmol) in DMF (40 mL) was added solid Ag_2O (6 g). The reaction mixture was stirred at room temperature for 12 h and then filtered. The filtrate was concentrated to give a syrup, which was purified by column chromatography (3:1 hexane–EtOAc) to give **63** (1.0 g, 60%, α: β = 0.2:1) as a foam. R_f 0.45 (2:1 hexane–EtOAc); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.11–8.01 (m, 7.2 H, Ar), 7.62–7.38 (m, 10.8 H, Ar), 6.10 (dd, 0.2 H, J = 6.1, 4.7 Hz, H-3α), 5.66 (dd, 1 H, J = 5.1, 1.4 Hz, H-3β), 5.53 (d, 1 H, J = 1.4 Hz, H-2β), 5.49 (dd, 0.2 H, J = 6.1, 5.0 Hz, H-2α), 5.39 (d, 0.2 H, J = 5.0 Hz, H-1α), 5.17 (s, 1 H, H-1β), 4.64–4.42 (m, 3.8 H, H-4α, H-5α, 2 × H-6α, H-5β, 2

× H-6β), 4.39 (dd, 1 H, J = 5.1, 2.2 Hz, H-4β), 3.51 (s, 0.6 H, OC<u>H</u>$_3$), 3.45 (s, 3 H, OC<u>H</u>$_3$), 3.40 (d, 0.2 H, J = 5.6 Hz, α O<u>H</u>), 2.74 (d, 1 H, J = 8.6 Hz, β O<u>H</u>); ^{13}C (125 MHz, $CDCl_3$, δ_C) 166.6 (<u>C</u>=O), 166.3 (<u>C</u>=O) 166.2 (<u>C</u>=O), 166.1 (<u>C</u>=O), 165.5 (2 × <u>C</u>=O), 133.7(1) (2 × Ar), 133.7(0) (2 × Ar), 133.2(4) (Ar), 133.2(0) (Ar), 130.1 (4 × Ar), 130.0 (4 × Ar), 129.9(7) (2 × Ar), 129.9(1) (4 × Ar), 129.8 (2 × Ar), 129.7 (2 × Ar) 129.2 (4 × Ar), 129.1 (4 × Ar), 129.0 (2 × Ar), 128.7 (2 × Ar), 107.1 (C-1β), 101.9 (C-1α), 83.1 (C-4β), 82.6 (C-4α), 81.7 (C-2β), 78.3 (C-3β), 78.0 (C-2α), 76.4 (C-3), 69.3 (C-5α), 69.2 (C-5β), 66.3 (C-6β), 65.7 (C-6α), 57.0 (α O<u>C</u>H$_3$), 55.2 (β O<u>C</u>H$_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{28}H_{26}O_9Na$: 529.1471. Found: 529.1469.

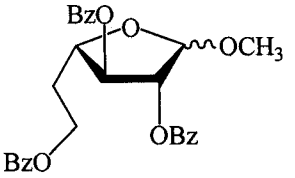
Methyl 2,3,6-Tri-*O*-benzoyl-5-deoxy-5-iodo-α/β-L-altrofuranoside (64)



To a solution of **63** (575 mg, 1.14 mmol) in toluene (46 mL) was added PPh_3 (1.20 g, 4.54 mmol), imidazole (310 mg, 4.54 mmol) and I_2 (868 mg, 3.42 mmol). The reaction mixture was heated at reflux for 4 h. After cooling and concentration of the solution, the resulting residue was dissolved in EtOAc (50 mL), which was washed with satd. aq. $Na_2S_2O_3$ solution (30 mL × 2), brine (30 mL) and the organic layer was dried ($MgSO_4$). The organic layer was concentrated to give a syrup, which was purified by column chromatography (12:1 hexane–EtOAc) to give **64** (674 mg, 96%, α:β = 0.15:1) as a foam. R_f 0.5 (9:1 hexane–EtOAc); only β isomer was assigned: ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.12–7.98 (m, 6 H, Ar), 7.62–7.38 (m, 9 H, Ar), 5.66 (dd, 1 H, J = 5.1, 1.2 Hz, H-3), 5.45 (d, 1 H, J = 1.2 Hz, H-2), 5.17 (s, 1 H, H-1), 4.84–4.72 (m, 3 H, H-5, 2 × H-6), 4.52 (t, 1

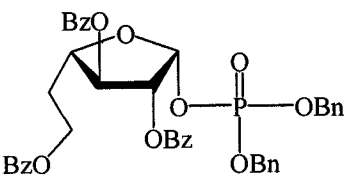
H, J = 5.1 Hz, H-4), 3.58 (s, 3 H, O$\underline{C}H_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 165.6 ($\underline{C}$=O), 165.4 ($\underline{C}$=O), 165.3 ($\underline{C}$=O), 133.5(4) (Ar), 133.5(0) (Ar), 133.3 (Ar), 130.0 (Ar), 129.9(8) (Ar), 129.9(5) (Ar), 129.9(2) (Ar), 129.8 (Ar), 129.7 (Ar), 129.4 (Ar), 129.1 (Ar), 129.0 (Ar), 128.5 (2 × Ar), 128.4 (4 × Ar), 106.9 (C-1), 83.2 (C-4), 82.1 (C-2), 79.9 (C-3), 66.4 (C-6), 55.1 (O$\underline{C}H_3$), 27.5 (C-5); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{28}H_{25}O_8INa$: 639.0488. Found: 639.0486.

Methyl 2,3,6-Tri-*O*-benzoyl-5-deoxy-α/β-L-*arabino*-hexofuranoside (49)



To a solution of **64** (2.82 g, 4.58 mmol) in dry toluene (50 mL) was added AIBN (752 mg, 4.58 mmol) and *n*-Bu_3SnH (1.23 mL, 4.58 mmol) at 50 °C. After the solution was heated to 90 °C, *n*-Bu_3SnH (1.23 mL, 4.58 mmol) was added every 30 min. After 2 h, the solution was cooled, and then concentrated to afford a crude compound, which was purified by column chromatography (9:1 hexane–EtOAc) to give **11** (2 g, 98%, α:β = 0.3:1) as a foam. R_f 0.61 (4:1 hexane–EtOAc); Data Assignment (see P 99)

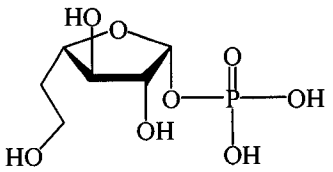
2,3,6-Tri-*O*-benzoyl-5-deoxy-β-L-*arabino*-hexofuranosyl phosphate (65)



To a solution of **49** (130 mg, 0.26 mmol) in dry CH_2Cl_2 (3 mL) was slowly added 44% HBr in HOAc (1 mL) at 0 °C. The solution was stirred at room temperature for 1 h, and TLC (15:1 toluene–EtOAc) indicated that there were two intermediates (presumably an

α/β mixture of glycosyl bromides) whose R_f values were 0.45 and 0.35. After the solution was co-evaporated with toluene, the resulting syrup was dissolved in dry toluene (3 mL), which was cooled to 0 °C. To the solution was added dibenzyl phosphate (80 mg, 0.28 mmol) and Et_3N (0.5 mL) at 0 °C. After 3 h, the solution was concentrated to give a crude compound that was purified by chromatography (2:1 hexane–EtOAc) to afford **65** (93 mg, 48%) as a foam; $[\alpha]_D$ +71.9 (*c* 0.2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.13–7.97 (m, 6 H, Ar), 7.31–7.17 (m, 19 H, Ar), 6.31 (dd, 1 H, J = 4.3, 3.5 Hz, H-1), 5.84 (dd, 1 H, J = 6.8, 5.4 Hz, H-3), 5.67 (ddd, 1 H, J = 6.8, 4.3, 2.3 Hz, H-2), 5.08–4.86 (m, 4 H, 2 × $PhC\underline{H}_2$), 4.50–4.42 (m, 2 H, H-4, 2 × H-6), 2.51–2.25 (m, 2 H, 2 × H-5); ^{13}C (125 MHz, $CDCl_3$, δ_C) 166.2 ($\underline{C}$=O), 165.8 ($\underline{C}$=O), 165.5 ($\underline{C}$=O), 135.4(8) (d, 1 C, J = 7.7 Hz, Ar), 135.4 (3) (d, 1 C, J = 7.7 Hz, Ar), 133.6(2) (Ar), 133.6(1) (Ar), 132.9 (Ar), 130.1 (Ar), 130.0 (2 × Ar), 139.9 (2 × Ar), 129.6 (2 × Ar), 128.9 (Ar), 128.7 (Ar), 128.5(4) (5 × Ar), 128.5(2) (2 × Ar), 128.5(0) (2 × Ar), 128.4 (Ar), 128.3 (2 × Ar), 127.8 (2 × Ar), 127.7 (2 × Ar), 98.4 (d, 1 C, J = 5.8 Hz, C-1), 79.8 (C-4), 78.2 (d, 1 C, C-3), 77.0 (d, 1 C, J = 9.8 Hz, C-2), 69.4 (d, 1 C, J = 5.0 Hz, $Ph\underline{C}H_2$), 69.3 (d, 1 C, J = 5.0 Hz, $Ph\underline{C}H_2$), 61.3 (C-6), 34.4 (C-5); ^{31}P (162 MHz, CD_2Cl_2, δ_C) -1.6 (P); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{41}H_{37}O_{11}PNa$: 759.1962. Found: 759.1965.

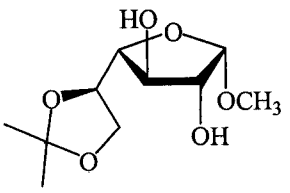
5-Deoxy-β-L-*arabino*-hexofuranosyl phosphate (66)



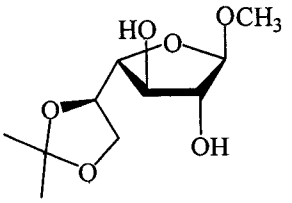
To a solution of **65** (93 mg, 0.13 mmol) in EtOAc (2 mL) was added Et_3N (0.4 mL) and 10% Pd–C (12 mg). The reaction mixture was stirred for 12 h under H_2 (1 atm), filtered

and concentrated to afford a syrup, which was dissolved in MeOH–H_2O–Et_3N (5:2:1, 5 mL) and stirred for 4 days. Concentration of the solution gave a crude residue that was purified by a reverse phase chromatography (10:2:1 EtOH–NH_4OH–H_2O) to give **66** (16 mg, 53%) as a solid. R_f 0.26 (10:2:1 EtOH–$NH_3.H_2O$–H_2O); $[\alpha]_D$ +10。92 (*c* 0.13, $CHCl_3$); ^1H NMR (500 MHz, D_2O , δ_H) 5.50 (dd, 1 H, *J* = 6.4, 4.3 Hz, H-1), 4.06 (dd, 1 H, *J* = 7.4, 4.3 Hz, H-2), 4.02 (dd, 1 H, *J* = 7.5, 7.4 Hz, H-3), 3.86 (ddd, 1 H, *J* = 7.5, 5.1, 5.1 Hz, H-4), 3.77–3.71 (m, 2 H, 2 × H-6), 2.00–1.90 (m, 2 H, 2 × H-5); ^{13}C (125 MHz, $CDCl_3$, δ_C) 97.5 (d, 1 C, *J* = 5.2 Hz, C-1), 80.0 (C-4), 78.8 (C-3), 77.5 (d, 1 C, *J* = 6.1 Hz, C-2), 59.4 (C-6), 37.3 (C-5); ^{31}P (162 MHz, D_2O, δ_C) 4.4 (P); HRMS (ESI) *m/z* Calc. for (M-H) $C_6H_{12}O_8P$: 243.0264. Found: 243.0264.

Methyl 5,6-*O*-Isopropylidine-α-D-galactofuranoside (68) and Methyl 5,6-*O*-Isopropylidene-β-D-galactofuranoside (69)



68

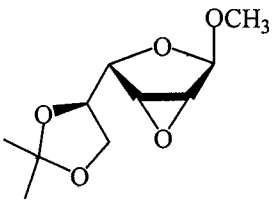


69

To a solution of **3** (2.78 g, 14.3 mmol) in dry acetone (60 mL) was added (±)-camphorsulfonic acid (162 mg, 0.05 mmol) and 2,2-dimethyoxypropane (2.70 mL, 1.50 mmol). After 2 h, the solution was neutralized by the addition of Et_3N (2 mL), and then concentrated to afford a syrup, which was purified by column chromatography (1:2 hexane–EtOAc) to give **68** (0.76) and **69** (2.05 g) as an oil in overall yield of 84%. Data of α-isomer **68** are shown as following: R_f 0.25 (20:1 CH_2Cl_2–MeOH), $[\alpha]_D$ +6.70 (*c*

3.1, CH_3OH); ^1H NMR (600 MHz, $CDCl_3$, δ_H) 4.84 (d, 1 H, J = 4.50 Hz, H-1), 4.19 (t, 1 H, J = 6.6 Hz, H-5), 4.10–4.00 (m, 3 H, H-2, H-3, H-6), 3.93 (dd, 1 H, J = 8.5, 6.9 Hz, H-6), 3.83 (t, 1 H, J = 6.6 Hz, H-4), 3.49 (s, 3 H, $OC\underline{H}_3$), 2.64–2.60 (m, 2 H, 2 × $O\underline{H}$), 1.47 (s, 3 H, isopropylidene $C\underline{H}_3$), 1.40 (s, 3 H, isopropylidene CH_3); ^{13}C (125 MHz, $CDCl_3$, δ_C) 109.7 (isopropylidene $\underline{C}$), 101.9 (C-1), 81.3 (C-4), 78.1 (C-2/C-3), 77.2 (C-5), 76.7 (C-3/C-2), 64.9 (C-6), 55.6 ($O\underline{C}H_3$), 25.5 (isopropylidene $\underline{C}H_3$), 25.2 (isopropylidene $\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{10}H_{18}O_6Na$: 257.0994. Found: 257.0995. Data of β-isomer **69** are shown as following: R_f 0.36 (20:1 CH_2Cl_2–MeOH); $[\alpha]_D$ -84.01 (*c* 0.8, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 4.9 (s, 1 H, H-1), 4.33 (dt, 1 H, J = 7.2, 1.8 Hz, H-5), 4.15–3.95 (m, 6 H, H-2, H-3, H-4, 2 × H-6, $O\underline{H}$), 3.39 (s, 3 H, $OC\underline{H}_3$), 3.01 (d, 1 H, J = 10.8 Hz, $O\underline{H}$), 1.41 (s, 3 H, isopropylidene $C\underline{H}_3$), 1.39 (s, 3 H, isopropylidene $C\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C), 110.1 (isopropylidene $\underline{C}$), 109.6 (C-1), 85.5 (C-2), 78.5 (C-3/C-4), 78.3 (C-4/C-3), 75.7 (C-5), 65.7 (C-6), 55.0 ($O\underline{C}H_3$), 25.6 (2 × isopropylidene $\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{10}H_{18}O_6Na$: 257.0998. Found: 257.0996.

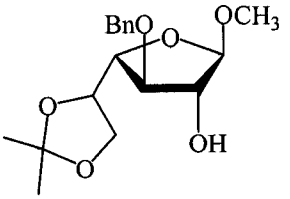
Methyl 2,3-Anhydro-5,6-*O*-isopropylidene-β-D-galactofuranoside (70)



To a solution of **69** (1.97 g, 8.4 mmol) in THF (60 mL) was added PPh_3 (2.87 g, 10.9 mmol) and DIAD (2.11 mL, 10.9 mmol) at 0 °C. The solution stirred at room temperature for 1 h, and then concentrated by evaporation under reduced pressure. The resulting crude residue was dissolved in Et_2O, which was cooled to precipitate the solid PPh_3=O. After

filtration, the organic layer was concentrated, and the resulting pale yellow syrup was purified by column chromatography (6:1 hexane–EtOAc) to yield **70** (1.91 g, 100%) as an oil. R_f 0.29 (5:1 hexane–EtOAc); $[\alpha]_D$ -51.9 (*c* 1.3, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 4.95 (s, 1 H, H-1), 4.25 (ddd, 1 H, *J* = 7.0, 6.6, 6.5 Hz, H-5), 4.08 (dd, 1 H, *J* = 8.3, 6.5 Hz, H-6), 3.99 (d, 1 H, *J* = 7.0 Hz, H-4), 3.88 (dd, 1 H, *J* = 8.3, 6.6 Hz, H-6), 3.61 (d, 1 H, *J* = 2.8 Hz, H-2), 3.59 (dd, 1 H, *J* = 7.0, 2.8 Hz, H-3), 3.42 (s, 3 H, O$\underline{C}H_3$) 1.45 (s, 3 H, isopropylidene C$\underline{H}_3$), 1.35 (s, 3 H, isopropylidene C$\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 109.8 (isopropylidene $\underline{C}$), 102.4 (C-1), 77.3 (C-4), 77.2 (C-5), 65.7 (C-6), 55.8 (C-2), 55.6 (C-3), 53.3 (O$\underline{C}H_3$), 26.6 (isopropylidene $\underline{C}H_3$), 25.2 (isopropylidene $\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{10}H_{16}O_5Na$: 239.0890. Found: 239.0890.

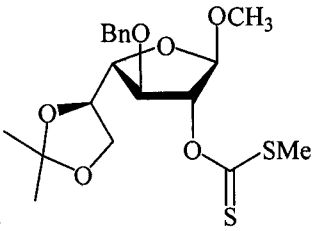
Methyl 3-*O*-Benzyl-5,6-*O*-isopropylidene-β-D-galactofuranoside (71)



Compound **70** (8.0 g, 37 mmol) was dissolved in 1 M NaOBn in BnOH (100 mL). The solution was heated to reflux at a bath temperature of 90 °C for 12 h. Reduced pressure distillation was applied to remove BnOH, and the resulting residue was diluted with EtOAc (60 mL). The solution was washed with distilled water (30 mL × 3), brine (30 mL) and dried (Na_2SO_4). The solution was concentrated to give a pale yellow syrup that was purified by column chromatography (2:1 hexane–EtOAc) to yield **71** (9.5 g, 79%) as an oil. R_f 0.31 (2:1 hexane–EtOAc); $[\alpha]_D$ -102.2 (*c* 2.0, CH_2Cl_2);); ^1H NMR (600 MHz, $CDCl_3$, δ_H) 7.39–7.25 (m, 5 H, Ar), 4.91 (s, 1 H, H-1), 4.72 (d, 1 H, *J* = 12.3 Hz, PhC$\underline{H}_2$) 4.54 (d, 1 H, *J* = 12.3 Hz, PhC$\underline{H}_2$), 4.14–4.10 (m, 3 H, H-2, H-4, H-5), 4.10–3.95 (m, 2 H,

2 × H-6), 3.82 (d, 1 H, J = 2.8 Hz, H-3), 3.41 (s, 3 H, OC<u>H</u>$_3$), 3.83 (d, 1 H, J = 10.8 Hz, 2-O<u>H</u>), 1.41 (s, 3 H, isopropylidene C<u>H</u>$_3$), 1.36 (s, 3 H, isopropylidene C<u>H</u>$_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 137.6 (Ar), 128.5 (2 × Ar), 127.9 (Ar), 127.9 (2 × Ar), 110.6 (C-1), 109.9 (isopropylidene <u>C</u>), 85.7 (C-3), 82.8 (C-5), 77.6 (C-2/C-4), 76.3 (C-4/C-2), 72.1 (Ph<u>C</u>H$_2$), 65.6 (C-6), 55.3 (OC<u>H</u>$_3$), 25.8 (isopropylidene <u>C</u>H$_3$), 25.6 (isopropylidene <u>C</u>H$_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{17}H_{24}O_6Na$: 347.1467. Found: 347.1465.

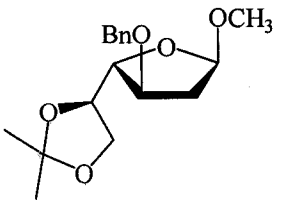
Methyl 3-*O*-Benzyl-2-*O*-(methylthio)thiocarbonyl-5,6-*O*-isopropylidene-β-D-galactofuranoside (72)



To a solution of **71** (100 mg, 0.37 mmol) in THF (4 mL) was added 60% NaH (31 mg, 0.78 mmol). The reaction mixture was stirred for 30 min at room temperature, and cooled to 0 °C followed by the addition of CS_2 (47 μL, 0.78 mmol). After 3 h, MeOH (2 mL) was used to quench the excess NaH. Solvent removal by evaporation under reduced pressure afforded a crude compound, which was purified by column chromatography (9:1 hexane–EtOAc) to give **72** (105 mg, 88%) as an oil. R_f 0.25 (9:1 hexane–EtOAc); $[\alpha]_D$ -81.5 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.39–7.28 (m, 5 H, Ar), 5.88 (s, 1 H, H-2), 5.02 (s, 1 H, H-1), 4.76 (d, 1 H, J = 11.9 Hz, PhC<u>H</u>$_2$), 4.54 (d, 1 H, J = 11.9 Hz, PhC<u>H</u>$_2$), 4.18 (ddd, 1 H, J = 6.7, 6.6, 6.5 Hz, H-5), 4.15 (dd, 1 H, J = 6.5, 5.4 Hz, H-4), 3.86 (d, 1 H, J = 5.4 Hz, H-3), 3.84 (dd, 1 H, J = 8.5, 6.6 Hz, H-6), 3.75 (dd, 1 H, J = 8.5, 6.7 Hz, H-6), 3.43 (s, 3 H, OC<u>H</u>$_3$), 2.60 (s, 3 H, SC<u>H</u>$_3$), 1.41 (s, 3 H, isopropylidene C<u>H</u>$_3$),

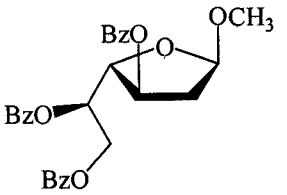
1.38 (s, 3 H, isopropylidene C$\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 214.5 ($\underline{C}$=S), 136.9 (Ar), 128.5 (2 × Ar), 128.3 (2 × Ar), 128.1 (Ar), 109.9 (isopropylidene $\underline{C}$), 106.6 (C-1), 88.6 (C-2), 84.4 (C-4), 82.9 (C-3), 75.9 (C-5), 72.7 (Ph$\underline{C}H_2$), 65.3 (C-6), 55.1 (O$\underline{C}H_3$), 26.4 (isopropylidene $\underline{C}H_3$), 25.3 (isopropylidene $\underline{C}H_3$), 19.3 (S$\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{19}H_{26}O_6S_2Na$: 437.1065. Found: 437.1063.

Methyl 3-*O*-Benzyl-2-deoxy-5,6-*O*-isopropylidene-β-D-*lyxo*-hexofuranoside (73)



To a solution of **72** (97 mg, 0.23 mmol) in dry toluene (5 mL) was added AIBN (46 mg, 0.28 mmol) and *n*-Bu_3SnH (0.55 mL, 1.56 mmol) at 50 °C, and then the solution continued to be heated to 110 °C. AIBN (46 mg, 0.28 mmol) was added in portions every 30 min. After 2 h, the solution was cooled to room temperature, concentrated, and resulting residue purified by column chromatography (6:1 hexane–EtOAc) to give **73** (41 mg, 59%) as an oil. R_f 0.25 (6:1 hexane–EtOAc); $[\alpha]_D$ -101.8 (*c* 1.6, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.38–7.42 (m, 5 H, Ar), 5.09 (s, 1 H, H-1), 4.60 (d, 1 H, J = 12.2 Hz, Ph$\underline{C}H_2$), 4.45 (d, 1 H, J = 12.2 Hz, Ph$\underline{C}H_2$), 4.10–4.05 (m, 2 H, H-4, H-5), 3.92–3.50 (m, 3 H, H-3, H-6), 3.40 (s, 3 H, O$\underline{C}H_3$), 2.25–2.02 (m, 2 H, H-2), 1.40 (s, 3 H, isopropylidene C$\underline{H}_3$), 1.33 (s, 3 H, isopropylidene C$\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 137.8 (Ar), 128.4 (2 × Ar), 127.9 (2 × Ar), 127.8 (Ar), 109.5 (isopropylidene $\underline{C}$), 105.3 (C-1), 82.9 (C-4), 78.8 (C-3), 76.6 (C-5), 71.6 (Ph$\underline{C}H_2$), 65.6 (C-6), 55.2 (O$\underline{C}H_3$), 38.7 (C-2), 26.3 (isopropylidene $\underline{C}H_3$), 25.5 (isopropylidene $\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{17}H_{24}O_5Na$: 331.1517. Found: 331.1516.

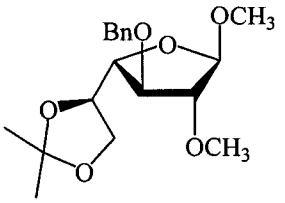
Methyl 3,5,6-Tri-*O*-benzoyl-2-deoxy-β-D-*lyxo*-hexofuranoside (74)



To a solution of **73** (271 mg, 0.88 mmol) in CH_2Cl_2 (15 mL) was added 10% HCl in MeOH (1 mL). The reaction mixture was stirred for 1 h at room temperature, neutralized with Et_3N (2 mL) and concentrated by solvent evaporation to give a crude compound, which was purified by a flash column (1:2 hexane–EtOAc) to produce a pale yellow syrup. To the syrup was added 3:1 EtOAc–MeOH solution (16 mL) and 10% Pd–C (30 mg), and the mixture was stirred under H_2 (1 atm) for 12 h. After the waste Pd–C was filtered, the solution was concentrated to give a pale yellow syrup. The resulting syrup was dissolved in pyridine (20 mL), and then benzoyl chloride (0.4 mL, 3.2 mmol) was added dropwise under 0 °C. The solution was slowly warmed to 25 °C. After 12 h, the solution was concentrated by evaporation under reduced pressure. To the resulting residue was added EtOAc (30 mL). The solution was sequentially washed with 1 M HCl (20 mL), satd aq. $NaHCO_3$ solution (20 mL), brine (20 mL), and dried (Na_2SO_4). The concentrated crude compound was purified by column chromatography (7:1 hexane–EtOAc) to give **74** (325 mg, 84%) as a solid. R_f 0.5 (4:1 hexane–EtOAc); $[\alpha]_D$ -84.97 (*c* 1.3, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–7.98 (m, 6 H, Ar), 7.60–7.18 (m, 9 H, Ar), 5.92 (ddd, *J* = 7.2, 4.5, 3.6 Hz, H-5), 5.42 (ddd, 1 H, *J* = 8.3, 3.6, 2.1 Hz, H-3), 5.26 (d, 1 H, *J* = 5.4 Hz, H-1), 4.72 (dd, 1 H, *J* = 11.8, 4.5 Hz, H-6), 4.69 (dd, 1 H, *J* =11.8, 7.2 Hz, H-6), 4.63 (t, 1 H, *J* = 3.6 Hz, H-4), 3.41 (s, 3 H, $OC\underline{H}_3$) 2.51 (ddd, *J* = 14.5, 8.3, 5.4 Hz, H-2), 2.16 (dd, 1 H, *J* = 14.5, 2.1 Hz, H-2); ^{13}C (125 MHz, $CDCl_3$, δ_C) 166.2 ($\underline{C}$=O), 166.1 ($\underline{C}$=O),

165.6 (C=O), 133.3 (Ar), 133.1 (Ar), 133.0 (Ar), 129.8 (2 × Ar), 129.7 (2 × Ar), 129.7 (2 × Ar), 129.6 (2 × Ar), 129.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (2 × Ar), 128.3 (2 × Ar), 105.1 (C-1), 81.5 (C-4), 74.7 (C-3), 71.1 (C-5), 63.5 (C-6), 55.1 ($O\underline{C}H_3$), 39.3 (C-2); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{28}H_{26}O_8Na$: 513.1518. Found: 513.1520.

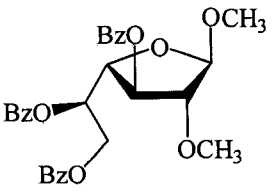
Methyl 3-*O*-Benzyl-5,6-*O*-isopropylidene-2-*O*-methyl-β-D-galactofuranoside (75)



To a solution of **71** (1.15 g, 3.55 mmol) in DMF (35 mL) was added NaH (284 mg, 1.10 mmol) under 0 °C. After 5 min, MeI (0.33 mL, 5.33 mmol) was slowly added. This reaction mixture was stirred at room temperature for 6 h, and then concentrated. The resulting crude compound was dissolved in EtOAc (60 mL), which was washed with water (30 mL × 2), brine (30 mL) and dried (Na_2SO_4). After concentration, the crude residue was purified by a chromatography (6:1 hexane–EtOAc) to give **75** (1.15 g, 95%) as an oil. R*f* 0.30 (6:1 hexane–EtOAc); $[\alpha]_D$ -102.5 (*c* 2.1, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.39–7.28 (m, 5 H, Ar), 4.90 (s, 1 H, H-1), 4.65 (d, 1 H, *J* = 12.0 Hz, $PhC\underline{H}_2$), 4.49 (d, 1 H, *J* = 12.0 Hz, $PhC\underline{H}_2$), 4.15 (q, 1 H, *J* = 6.5 Hz, H-5), 4.02 (t, 1 H, *J* = 6.5 Hz, H-4), 3.84 (dd, 1 H, *J* = 8.4, 6.5 Hz, H-6), 3.80–3.76 (m, 2 H, H-2, H-6), 3.67 (dd, 1 H, *J* = 6.7, 2.3 Hz, H-3), 3.44 (s, 3 H, 1-$OC\underline{H}_3$), 3.38 (s, 3 H, 2-$OC\underline{H}_3$), 1.41 (s, 3 H, isopropylidene $C\underline{H}_3$), 1.38 (s, 3 H, isopropylidene $C\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 137.4 (Ar), 128.4 (2 × Ar), 128.1 (2 × Ar), 127.9 (Ar), 109.7 (isopropylidene $\underline{C}$), 106.8 (C-1), 89.8 (C-2), 83.7 (C-3), 82.3 (C-4), 76.4 (C-5), 72.2 ($Ph\underline{C}H_2$), 65.3 (C-6), 57.6 (2-$OC\underline{H}_3$),

54.9 (1-OC$\underline{H}_3$), 26.5 (isopropylidene $\underline{C}H_3$), 25.3 (isopropylidene $\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{18}H_{26}O_6Na$: 361.1620. Found: 361.1621.

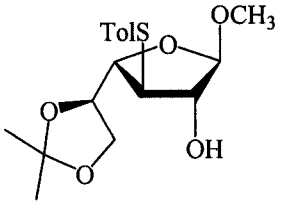
Methyl 3,5,6-Tri-*O*-benzoyl-2-*O*-methyl-β-D-galactofuranoside (76)



To a solution of **75** (1.16 g, 3.44 mmol) in 3:1 EtOAc–MeOH (40 mL) was added 20% $Pd(OH)_2$–C (116 mg). The mixture was stirred under H_2 (1 atm) for 12 h, and the $Pd(OH)_2$ was removed by filtration. The filtrate was concentrated to give a syrup, which was dissolved in CH_2Cl_2–MeOH (1:1 40 mL). To the solution was added 10% HCl in MeOH (1 mL), and the solution was stirred at room temperature for 1 h. The solution was then concentrated to afford a crude residue that was dissolved in dry pyridine (35 mL). Benzoyl chloride (1.55 mL, 12.4 mmol) was added dropwise under 0 °C. The solution was kept for 12 h at room temperature, and concentrated. The resulting residue was dissolved in EtOAc (60 mL), which was washed with 1 M HCl (30 mL × 2), satd aq. $NaHCO_3$ solution (30 mL), brine (30 mL) and dried (Na_2SO_4). The crude residue produced from concentration of the solution was purified by chromatography (6:1 hexane–EtOAc) to give **76** (1.56 g, 84%) as a foam. R_f 0.4 (4:1 hexane–EtOAc); $[\alpha]_D$ -74.4 (*c* 1.2, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.15–7.95 (m, 6 H, Ar), 7.61–7.35 (m, 9 H, Ar), 5.90 (ddd, 1 H, J = 7.0, 4.4, 3.8 Hz, H-5), 5.42 (d, 1 H, J = 4.7 Hz, H-3), 5.09 (s, 1 H, H-1), 4.74 (dd, 1 H, J = 11.8, 4.4 Hz, H-6), 4.68 (dd, 1 H, J = 11.8, 7.0 Hz, H-6), 4.60 (dd, 1 H, J = 4.7, 3.8 Hz, H-4), 3.89 (s, 1 H, H-2), 3.43 (s, 3 H, 1-OC$\underline{H}_3$), 3.37 (s, 3 H, 2-OC$\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 166.1 ($\underline{C}$=O), 165.8 ($\underline{C}$=O), 165.7 ($\underline{C}$=O),

133.3 (Ar), 133.1 (Ar), 133.0 (Ar), 129.9 (2 × Ar), 129.8 (2 × Ar), 129.7 (2 × Ar), 129.6 (2 × Ar), 129.3 (Ar), 128.3 (2 × Ar), 128.3 (2 × Ar), 128.2 (2 × Ar), 107.4 (C-1), 89.1 (C-2), 81.3 (C-4), 76.9 (C-3), 70.6 (C-5), 63.4 (C-6), 57.6 (2-O<u>C</u>H$_3$), 54.9 (1-O<u>C</u>H$_3$); HRMS (ESI) *m/z* calc. for (M+Na) $C_{29}H_{28}O_9Na$: 543.1625. Found: 543.1625.

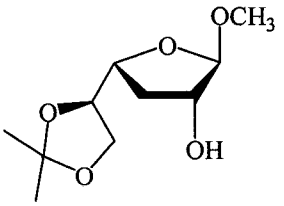
Methyl 5,6-Isopropylidene-3-deoxy-3-thiotolyl-β-D-galactofuranoside (77)



To a solution of **70** (1.38 g, 6.38 mmol) in DMF (65 mL) was added 60% NaH (382 mg, 9.57 mmol) and thiocresol (2.38 g, 19.1 mmol) at 0 °C. After 10 min, the solution was heated to 90 °C, and then stirred for 1.5 h. After cooling the solution, MeOH was added to quench the excess NaH. The solution was concentrated, and resulting residue was dissolved in EtOAc (100 mL). The solution was washed with distilled water (50 mL × 2), brine (30 mL), and the organic layer dried (Na_2SO_4). After concentration of the organic solution, the crude compound was purified by column chromatography (3:1 hexane–EtOAc) to give **77** (1.86 g, 87%) as an oil. R_f 0.27 (3:1 hexane–EtOAc); $[\alpha]_D$ -98.0 (*c* 2.4, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.35–7.31 (m, 2 H, Ar), 7.18–7.13 (m, 2 H, Ar), 4.94 (s, 1 H, H-1), 4.18–4.12 (m, 3 H, H-2, H-4, H-6), 4.07–4.00 (m, 2 H, H-5, H-6), 3.47 (d, 1 H, J = 10.4 Hz, O<u>H</u>), 3.44 (d, 1 H, J = 4.2 Hz, H-3), 3.40 (s, 3 H, OC<u>H</u>$_3$), 2.33 (s, 3 H, SC<u>H</u>$_3$), 1.42 (s, 3 H, isopropylidene C<u>H</u>$_3$), 1.38 (s, 3 H, isopropylidene C<u>H</u>$_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 137.1 (Ar), 131.7 (Ar), 130.9 (2 × Ar), 129.9 (2 × Ar), 110.5 (C-1), 109.9 (isopropylidene <u>C</u>), 83.3 (C-4), 80.2 (C-2), 76.6 (C-5), 65.6 (C-6), 55.1 (O<u>C</u>H$_3$), 54.0 (C-3), 25.8 (isopropylidene <u>C</u>H$_3$), 25.5 (isopropylidene

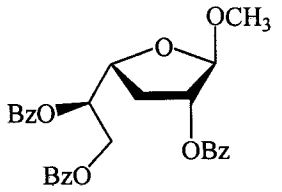
$\underline{C}H_3$), 21.0 (STol $\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{17}H_{24}O_5SNa$: 363.1235. Found: 363.1236.

Methyl 3-Deoxy-5,6-isopropylidene-β-D-*xylo*-hexofuranoside (78)



To a solution of **77** (1.86 g, 5.46 mmol) in toluene (55 mL) was added AIBN (0.90 g, 5.46 mmol) and *n*-Bu_3SnH (8.80 mL, 32.8 mmol) at 50 °C. The solution continued to be heated to 110 °C, and AIBN was added (0.90 g, 5.46 mmol) every 30 min. After 2 h, the solution was concentrated to afford a crude compound, which was purified by chromatography (3:1 hexane–EtOAc) to give **78** (957 mg, 81%) as an oil. R_f 0.31 (2:1 hexane–EtOAc); $[\alpha]_D$ -83.2 (*c* 2.0, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 4.86 (s, 1 H, H-1), 4.20 (dt, 1 H, J = 9.2, 2.5 Hz, H-4), 4.14–4.06 (m, 3 H, H-5, 2 × H-6), 4.03 (dd, 1 H, J = 11.2, 5.5 Hz, H-2), 3.88 (d, 1 H, J = 11.2 Hz, O$\underline{H}$), 3.33 (s, 3 H, OC$\underline{H}_3$), 2.45 (ddd, 1 H, J = 13.8, 9.2, 5.5 Hz, H-3), 1.81 (dd, 1 H, J = 13.8, 2.5 Hz, H-3); 1.46 (s, 3 H, isopropylidene C$\underline{H}_3$), 1.40 (s, 3 H, isopropylidene C$\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C), 109.9 (C-1), 109.7 (isopropylidene $\underline{C}$), 77.6 (C-5), 76.0 (C-4), 73.7 (C-2), 65.7 (C-6), 54.5 (O$\underline{C}H_3$), 34.4 (C-3), 25.7 (isopropylidene $\underline{C}H_3$), 25.6 (isopropylidene $\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{10}H_{18}O_5Na$: 241.1047. Found: 241.1046.

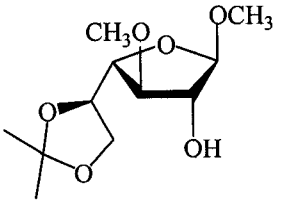
Methyl 3-Deoxy-2,5,6-tri-*O*-benzoyl-β-D-*xylo*-hexofuranoside (79)



To a solution of **78** (957 mg, 4.39 mmol) in CH_2Cl_2 (50 mL) was added 10% HCl in MeOH (1 mL). The solution was stirred at room temperature for 1 h, and then neutralized with Et_3N (1 mL). After concentration of the reaction mixture, the resulting syrup was purified by a chromatography (1:2 hexane–EtOAc). The intermediate was dissolved in 3:1 EtOAc–MeOH (40 mL), and then 10% Pd–C (80 mg) was added. The mixture was stirred under H_2 (1 atm) for 12 h, followed by removal of the catalyst through filtration. Solvent evaporation afforded a pale yellow syrup, which was dissolved in dry pyridine (40 mL), and then benzoyl chloride (1.98 mL, 15.8 mmol) was added dropwise under 0 °C. After 12 h, pyridine was removed by reduced evaporation, and the resulting residue was dissolved in EtOAc (40 mL). The solution was washed with 1 M HCl (30 ml), satd aq. $NaHCO_3$ solution (30 mL), brine (30 mL), and dried ($MgSO_4$). The dried organic layer was concentrated to give a pale yellow syrup that was purified by column chromatography (7:1 hexane–EtOAc) to yield **79** (1.80 g, 84%) as a foam. R_f 0.5 (4:1 hexane–EtOAc); $[\alpha]_D$ -14.9 (*c* 3.2, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.13–7.92 (m, 6 H, Ar), 7.56–7.30 (m, 9 H, Ar), 5.76 (ddd, 1 H, J = 7.2, 3.9, 3.2 Hz, H-5), 5.36 (dd, 1 H, J = 7.0, 1.8 Hz, H-2), 5.17 (s, 1 H, H-1), 4.76 (dd, 1 H, J = 12.0, 3.9 Hz, H-6), 4.69 (dd, 1 H, J = 12.0, 7.2 Hz, H-6), 4.62 (ddd, 1 H, J = 7.5, 6.5, 3.2 Hz, H-4), 3.41 (s, 3 H, $OC\underline{H}_3$), 2.70 (ddd, 1 H, J = 14.6, 7.5, 7.0 Hz, H-3), 2.08 (ddd, 1 H, J = 14.6, 6.5, 1.8 Hz, H-3); ^{13}C (125 MHz, $CDCl_3$, δ_C) 166.1(7) ($\underline{C}$=O), 166.1(2) ($\underline{C}$=O), 165.9(2) ($\underline{C}$=O), 133.2

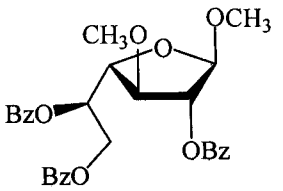
(Ar), 133.1 (Ar), 133.1 (Ar), 129.9 (2 × Ar), 129.6 (5 × Ar), 129.6 (Ar), 129.4 (2 × Ar), 128.4 (5 × Ar), 107.2 (C-1), 77.9 (C-2), 76.3 (C-4), 72.0 (C-5), 63.8 (C-6), 54.7 ($O\underline{C}H_3$), 32.1 (C-3); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{28}H_{26}O_8Na$: 513.1521. Found: 513.1520.

Methyl 5, 6-*O*-Isopropylidene-3-*O*-methyl-β-D-galactofuranoside (80)



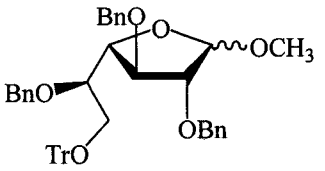
To a solution of **70** (70 mg, 0.32 mmol) in DMF (3.0 mL) was added CH_3OH (0.26 mL, 6.48 mmol) and NaH (129 mg, 3.24 mmol) at 0 °C, and the solution was then heated to 90 °C. After 1 h, the solution was cooled, and MeOH (1 mL) was added to quench the excess NaH. DMF was removed by evaporation to afford a crude residue that was dissolved in EtOAc (10 mL). The solution was washed with water (2 × 10 mL), brine (10 mL) and dried ($MgSO_4$). The organic layer was concentrated to afford a crude syrup that was purified by chromatography (3:2 hexane–EtOAc) to yield **80** (50 mg, 63%) as an oil. R_f 0.33 (1:1 hexane–EtOAc); $[\alpha]_D$ -107.3 (*c* 0.98, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 4.91 (s, 1 H, H-1), 4.29 (dt, 1 H, J = 7.2, 2.1 Hz, H-5), 4.12–4.00 (m, 4 H, H-2, H-4, 2 × H-6), 3.68 (d, 1 H, J = 2.5 Hz, H-3), 3.45 (d, 1 H, J = 11.0 Hz, 2-O$\underline{H}$), 3.42 (s, 3 H, 3-O$\underline{C}H_3$), 3.39 (s, 3 H, 1-O$\underline{C}H_3$), 1.42 (s, 3 H, isopropylidene $C\underline{H}_3$), 1.39 (s, 3 H, isopropylidene $C\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 110.7 (C-1), 109.9 (isopropylidene $\underline{C}$), 88.4 (C-3), 82.7 (C-4), 76.5 (C-2/C-5), 76.4 (C-5/C-2), 65.7 (C-6), 58.1 (3-O$\underline{C}H_3$), 55.5 (1-O$\underline{C}H_3$), 25.7 (isopropylidene $\underline{C}H_3$), 25.6 (isopropylidene $\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{11}H_{20}O_6Na$: 271.1150. Found: 271.1152.

Methyl 2,5,6-Tri-*O*-benzoyl-3-*O*-methyl-β-D-galactofuranoside (81)



To a solution of **80** (550 mg, 2.22 mmol) in 1:1 CH_2Cl_2–MeOH (30 mL) was added 10% HCl in MeOH (0.5 mL). The solution was stirred at room temperature for 1 h. and then concentrated. The resulting residue was dissolved in dry pyridine (30 mL) and then benzoyl chloride (1.0 mL, 8.0 mmol) was added dropwise under 0 °C. After 12 h, the pyridine was removed by evaporation, and the resulting residue was dissolved in EtOAc (50 mL). The solution was washed with 1 M HCl (30 mL), satd aq. $NaHCO_3$ solution (30 mL), brine (30 mL) and dried ($MgSO_4$). The organic layer was concentrated to afford a yellow syrup, which was purified by column chromatography (6:1 hexane–EtOAc) to give **81** (1.0 g, 83%) as a white foam. R_f 0.4 (4:1 hexane–EtOAc); $[\alpha]_D$ -11.42 (*c* 1.6, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.08–7.88 (m, 6 H, Ar), 7.56 –7.29 (m, 9 H, Ar), 5.87 (ddd, 1 H, J = 7.5, 4.7, 3.5 Hz, H-5), 5.30 (s, 1 H, H-2), 5.14 (s, 1 H, H-1), 4.73 (dd, 1 H, J = 11.7, 4.7 Hz, H-6), 4.69 (dd, 1 H, J = 11.7, 7.5 Hz, H-6), 4.44 (dd, 1 H, J = 5.5, 3.5 Hz, H-4), 3.90 (d, 1 H, J = 5.5 Hz, H-3), 3.51 (s, 3 H, 3-OC$\underline{H}_3$), 3.44 (s, 3 H, 1-OC$\underline{H}_3$); ^{3}C (125 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 166.0 (C=O), 165.3 (C=O), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 129.8 (2 × Ar), 129.7 (4 × Ar), 129.6 (Ar), 129.5 (Ar), 129.1 (Ar), 128.4 (4 × Ar), 128.3 (2 × Ar), 107.1 (C-1), 86.0 (C-3), 81.6 (C-4), 80.9 (C-2), 70.5 (C-5), 63.5 (C-6), 58.8 (3-O$\underline{C}H_3$), 54.9 (1-OC$\underline{H}_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{29}H_{28}O_9Na$: 543.1625. Found: 543.1625.

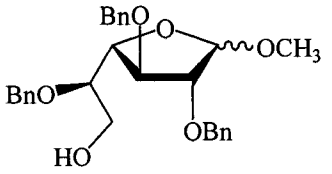
Methyl 2,3,5-Tri-*O*-benzyl-6-*O*-trityl-β-D-galactofuranoside (82)



To a solution of **67** (2.20 g, 11.4 mmol) in dry pyridine (70 mL) was added trityl chloride (3.8 g, 13.6 mmol) and DMAP (140 mg, 1.2 mmol). The solution was stirred for 12 h at 40 °C and quenched with the addition of MeOH (5 mL). After concentration, the resulting residue was dissolved in dry DMF (60 mL) and 60% NaH (1.63 g, 41.0 mmol) was added at 0 °C. After 15 min, benzyl bromide (4.8 mL, 41.0 mmol) was added and the solution was stirred at room temperature for 12 h. Then, MeOH was added to quench the excess NaH, and DMF was evaporated to give a crude compound that was dissolved in EtOAc (100 mL). The solution was washed with water (40 mL × 2), brine (40 mL), and dried ($MgSO_4$). The dried organic layer was concentrated to afford a syrup that was purified by column chromatography (9:1 hexane–EtOAc) to give **82** (3.74 g, 46% α:β 0.3:1) as a foam. R_f 0.5 (6:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.50–7.10 (m, 39 H, Ar), 4.93 (s, 1 H, H-1β), 4.88–4.26 (m, 8.22 H, 3 × α PhC$\underline{H}_2$, 3 × β PhC$\underline{H}_2$), 4.72 (d, 0.37 H, H-1α), 4.20–4.13 (m, 1.37 H, H-3α, H-4β), 4.02 (dd, 0.37 H, J = 7.4, 4.3 Hz, H-2α), 3.98–3.94 (m, 2.37 H, H-4α, H-3β, H-2β), 3.74–3.70 (m, 1.37 H, H-5α, H-5β), 3.45 (dd, 1 H, J = 9.9, 6.6 Hz, H-6β), 3.40–3.32 (m, 4.74 H, 2 × H-6α, H-6β, β-O$\underline{C}H_3$), 3.32 (s, 3 H, α-O$\underline{C}H_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 144.1 (3 × Ar), 144.0 (3 × Ar), 138.9 (Ar), 138.4 (Ar), 138.2 (Ar), 137.9 (Ar), 137.7 (β Ar), 137.6 (Ar), 129.1 (Ar), 128.8 (Ar), 128.7 (Ar), 128.4 (Ar), 128.3(8) (Ar), 128.3(2) (Ar), 128.2(8) (Ar), 128.2(5) (Ar), 128.2(4) (Ar), 128.2(1) (Ar), 128.1 (Ar), 127.9 (Ar), 127.8(5) (Ar), 127.8(3) (Ar), 127.8(0) (Ar), 127.7(6) (Ar), 127.7(0) (Ar), 127.6(4) (Ar), 127.6(2) (Ar), 127.5(2) (Ar), 127.4(7) (Ar),

127.4(3) (Ar), 127.3 (Ar), 127.1 (Ar), 127.0 (Ar), 106.9 (C-1β), 101.1 (C-1α), 88.4 (C-2β), 87.0 (2 × trityl C), 84.1 (C-2α), 82.9 (C-3β), 81.5 (C-3α), 81.1 (C-4β), 80.5 (C-4α), 80.5 (C-5α), 76.9 (C-5β), 73.6 (α $PhCH_2$), 73.3 (β $PhCH_2$), 72.5 (α $PhCH_2$), 72.2 (α $PhCH_2$), 72.1 (β $PhCH_2$), 71.8 (β $PhCH_2$), 63.2 (C-6α), 63.8 (C-6β), 54.9 (α OCH_3), 54.7 (β OCH_3); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{47}H_{46}O_6Na$: 729.3184. Found: 729.3186.

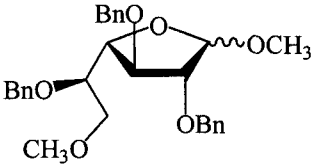
Methyl 2,3,5-Tri-*O*-benzyl-β-D-galactofuranoside (83)



To a solution of **82** (1.22 g, 1.73 mmol) in 1:1 MeOH–CH_2Cl_2 (30 mL) was added 10% HCl in MeOH (1 mL). The solution was stirred at room temperature for 1 h, and then Et_3N (2 mL) was added to neutralize the solution. After concentration of the reaction mixture, the resulting residue was dissolved in EtOAc (60 mL). The solution was washed with water (40 mL), brine (40 mL) and dried ($MgSO_4$). The organic layer was concentrated to give a syrup, which was purified by chromatography (5:2 hexane–EtOAc) to give **83** (701 mg, 87%, α:β = 0.3:1) as a solid. R_f 0.74 (1:1 hexane–EtOAc); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.42–7.24 (m, 19.5 H, Ar), 4.90 (s, 1 H, H-1β), 4.82–4.42 (m, 9.22 H, H-1α, 3 × α $PhCH_2$, 3 × β $PhCH_2$), 4.31–4.20 (m, 1.38 H, H-3α, H-4β), 4.10–3.98 (m, 2.74 H, H-2α, H-4α, H-2β, H-3β), 3.80–3.64 (m, 3.37 H, H-5β, H-6α, 2 × H-6β), 3.63–3.53 (m, 0.74 H, H-5α, H-6α), 3.39 (s, 3 H, β OCH_3), 3.38 (s, 1.11 H, α OCH_3); ^{13}C (125 MHz, $CDCl_3$, δ_C) 138.4 (Ar), 138.2 (Ar), 137.7 (Ar), 137.4 (2 × Ar), 137.3 (Ar), 128.5 (3 × Ar), 128.4 (Ar), 128.4 (6 × Ar), 128.3 (2 × Ar), 128.1 (2 × Ar), 128.1 (2 × Ar),

128.1 (Ar), 128.0 (2 × Ar), 127.9 (3 × Ar), 127.9 (2 × Ar), 127.9 (Ar), 127.8 (2 × Ar), 127.7 (2 × Ar), 127.7 (Ar), 107.0 (C-1β), 101.4 (C-1α), 87.7 (C-2β), 83.9 (C-2α), 83.1 (C-3β), 82.1 (C-4β), 81.1 (C-3α), 80.8 (C-4α/C-5α), 80.8 (C-5α/C-4α), 78.2 (C-5β), 72.8 (α $PhCH_2$), 72.8 (β $PhCH_2$), 72.6 (α $PhCH_2$), 72.3 (α $PhCH_2$), 72.2 (β $PhCH_2$), 72.0 (β $PhCH_2$); 62.2 (C-6β), 61.5 (C-6α), 55.3 (α-OCH_3), 54.8 (β-OCH_3); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{28}H_{32}O_6Na$: 487.2092. Found: 487.2091.

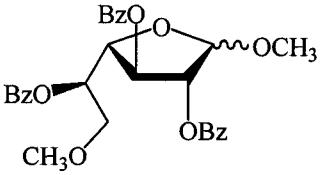
Methyl 2,3,5-Tri-*O*-benzyl-6-*O*-methyl-β-D-galactofuranoside (84)



To a solution of **83** (914 mg, 1.97 mmol) in dry DMF (20 mL) was added 60% NaH (160 mg, 3.96 mmol) under 0 °C. After 10 min, CH_3I (160 μL, 2.56 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 3 h, and MeOH was added to quench the excess NaH. The DMF was removed, and the resulting crude compound was dissolved in EtOAc (50 mL), which was washed with water (30 mL × 2), brine (30 mL × 2) and dried ($MgSO_4$). The organic layer was concentrated to give a syrup that was purified by chromatography (7:1 hexane–EtOAc) to give **84** (930 mg, 98%, α:β = 0.3:1) as a foam. R_f 0.34 (6:1 hexane–EtOAc); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.41–7.24 (m, 19.5 H, Ar), 4.98 (s, 1 H, H-1β), 4.78–4.30 (m, 8.1 H, H-1α, 3 × α $PhCH_2$, 3 × β $PhCH_2$), 4.32 (dd, 0.3 H, J = 7.5, 7.3 Hz, H-3α), 4.13 (dd, 1 H, J = 6.8, 3.5 Hz, H-4β), 4.06 (dd, 0.3 H, J = 7.5, 4.3 Hz, H-2α), 4.01 (dd, 1 H, J = 6.8, 2.8 Hz, H-3β), 4.00–3.97 (m, 1.3 H, H-4α, H-2β), 3.74 (ddd, 1 H, J = 6.4, 4.9, 3.5 Hz, H-5β), 3.63–3.43 (m, 2.9 H, H-5α, 2 × H-6α, 2 × H-6β), 3.38 (s, 3 H, 1-OCH_3β), 3.36 (s, 3.9 H, 1-OCH_3α,

6-OC$\underline{H}_3\beta$), 3.31 (s, 0.9 H, 6-OC$\underline{H}_3\alpha$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 138.8 (Ar), 138.5 (Ar), 138.2 (Ar), 137.8 (Ar), 137.7 (Ar), 137.6 (Ar), 128.4(4) (2 × Ar), 128.4(0) (2 × Ar), 128.3 (2 × Ar), 128.2(4) (3 × Ar), 128.2(0) (4 × Ar), 128.0(2) (2 × Ar), 128.0(0) (2 × Ar), 127.8(3) (3 × Ar), 127.7(8) (2 × Ar), 127.7(6) (Ar), 127.7(3) (2 × Ar), 127.7(0) (Ar), 127.6 (3 × Ar), 127.4 (Ar), 106.9 (C-1β), 101.0 (C-1α), 88.2 (C-4β), 84.2 (C-2α), 82.7 (C-3β), 81.3 (C-2β), 80.7 (C-3α/C-4α), 80.4 (C-4α/C-3α), 79.1 (C-5α), 76.4 (C-5β), 73.3 (C-6β/β Ph $\underline{C}H_2$), 73.2 (β Ph$\underline{C}H_2$/C-6β), 72.9 (α Ph$\underline{C}H_2$), 72.6 (2 × C, C-6α, α Ph$\underline{C}H_2$), 72.2 (α Ph$\underline{C}H_2$), 72.1 (β Ph$\underline{C}H_2$), 71.8 (β Ph$\underline{C}H_2$), 59.1(4) (6-O$\underline{C}H_3\beta$), 59.1(2) (6-O$\underline{C}H_3\alpha$), 55.0 (1-O$\underline{C}H_3\alpha$), 54.7 (1-O$\underline{C}H_3\beta$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{29}H_{34}O_6Na$: 501.2250. Found: 501.2248.

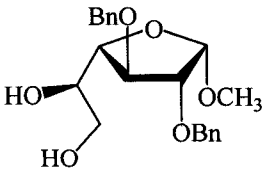
Methyl 2,3,5-Tri-*O*-benzoyl-6-*O*-methyl-β-D-galactofuranoside (85)



To a solution of **84** (2.81 g, 5.90 mmol) in 3:1 EtOAc–MeOH (60 mL) was added 20% $Pd(OH)_2$ (350 mg). The solution was stirred under H_2 (1 atm) for 12 h. After filtration of the catalyst, the filtrate was concentrated to afford a syrup, which was dissolved in pyridine (60 mL) and cooled to 0 °C. Benzoyl chloride (2.67 mL, 21.2 mmol) was slowly added into the solution. After 12 h, MeOH (5 mL) was used to quench the excess benzoyl chloride, and the mixture was concentrated. The resulting residue was dissolved in EtOAc (100 mL), and then washed with 1 M HCl solution (50 mL), satd. aq. $NaHCO_3$ solution (50 mL), brine (50 mL) and dried ($MgSO_4$). The dried organic layer was concentrated to give a syrup that was purified by column chromatography (6:1 hexane–EtOAc) to yield

85 (2.76 g, 85%, α:β = 0.3:1) as a foam. R_f 0.25 (6:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.20–7.95 (m, 7.8 H, Ar), 7.61–7.25 (m, 11.7 H, Ar), 6.20 (dd, 0.3 H, *J* = 7.4, 6.2 Hz, H-3α), 5.78 (ddd, 1 H, *J* = 6.0, 5.9, 4.0 Hz, H-5β), 5.60 (d, 1 H, *J* = 5.5 Hz, H-3β), 5.54 (m, 0.3 H, H-5α), 5.46 (m, 1.3 H, H-2α, H-2β), 5.36 (d, 0.3 H, *J* = 4.6 Hz, H-1α), 5.20 (s, 1 H, H-1β), 4.66 (dd, 1 H, *J* = 5.5, 4.0 Hz, H-4β), 4.61 (t, 0.3 H, *J* = 6.2 Hz, H-4α), 3.84 (dd, 1 H, *J* = 10.2, 6.0 Hz, H-6β), 3.82-3.77 (m, 1.3 H, H-6α, H-6β), 3.74 (dd, 0.3 H, *J* = 10.4, 5.3 Hz, H-6α), 3.50 (s, 3 H, β 1-OC$\underline{H}_3$), 3.44 (s, 0.9 H, α 1-OC$\underline{H}_3$), 3.38 (s, 3 H, β 6-OC$\underline{H}_3$), 3.27 (s, 0.9 H, α 1-OC$\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 165.9 (C=O), 165.9 (C=O), 165.8 (C=O), 165.6 (C=O), 165.5 (C=O), 165.4 (C=O), 133.4 (2 × Ar), 133.3(7) (Ar), 133.3(3) (Ar), 133.1 (Ar), 133.0 (Ar), 130.0 (2 × Ar), 129.9 (6 × Ar), 129.8(5) (2 × Ar), 129.8(3) (4 × Ar), 129.2(6) (Ar), 129.2(1) (2 × Ar), 129.2(0) (Ar), 129.1 (2 × Ar), 128.4(3) (4 × Ar), 128.4(0) (4 × Ar), 128.3 (4 × Ar), 106.6 (C-1β), 101.1 (C-1α), 82.4 (C-2β), 80.8 (C-4β), 78.4 (C-4α), 77.8 (C-2α), 77.5 (C-3β), 74.5 (C-3α), 73.1 (C-5α), 71.1 (C-5β), 70.9 (C-6β), 70.4 (C-6α), 59.2(3) (6-OC$\underline{H}_3$α/6-OC$\underline{H}_3$β), 59.2(1) (6-OC$\underline{H}_3$β/6-OC$\underline{H}_3$-α), 55.6 (1-OC$\underline{H}_3$α), 54.8 (1-OC$\underline{H}_3$β); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{29}H_{28}O_9Na$: 543.1623. Found: 543.1625.

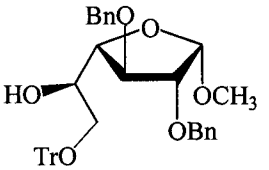
Methyl 2,3-Di-*O*-benzyl-α-D-galactofuranoside (86)



To a solution of **68** (511 mg, 2.18 mmol) in DMF (20 mL) was added BnBr (0.65 mL, 5.46 mmol) and 60% NaH (262 mg, 6.54 mmol) at 0 °C. After 12 h, the solution was quenched by the addition of MeOH (3 mL), and then concentrated. The resulting residue

was dissolved in CH_2Cl_2–MeOH (1:1, 30 mL). To this mixture was added 10% HCl in MeOH (1 mL), and then the solution was stirred at room temperature for 1 h before being neutralized with Et_3N (2 mL). After the solution was concentrated, the resulting crude residue was dissolved in EtOAc (30 mL) and washed with distilled water (20 mL), brine (20 mL), and dried ($MgSO_4$). After concentration of the organic layer, the resulting syrup was purified by column chromatography to give **86** (709 mg, 87%) as an oil. R_f 0.46 (25:1 CH_2Cl_2–MeOH); $[\alpha]_D$ +49.9 (*c* 1.6, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.40–7.24 (m, 10 H, Ar), 4.78–4.60 (m, 4 H, 2 × PhC<u>H</u>$_2$), 4.23 (s, 1 H, H-1), 4.35 (dd, 1 H, J = 7.2, 6.3 Hz, H-3), 4.07 (dd, 1 H, J = 7.2, 4.4 Hz, H-2), 4.03 (dd, 1 H, J = 6.3, 3.9 Hz, H-4), 3.68–3.60 (m, 3 H, H-5, 2 × H-6), 3.42 (s, 3 H, OC<u>H</u>$_3$), 2.82 (d, 1 H, J = 6.8 Hz, O<u>H</u>), 2.16 (d, J = 12.1 Hz, O<u>H</u>); ^{13}C (125 MHz, $CDCl_3$, δ_C) 137.7 (Ar), 137.3 (Ar), 128.5 (2 × Ar), 128.4 (2 × Ar), 128.2 (2 × Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (2 × Ar), 102.0 (C-1), 84.1 (C-2), 81.9 (C-4), 81.1 (C-3), 72.8 (Ph<u>C</u>H$_2$), 72.5 (Ph<u>C</u>H$_2$), 72.2 (C-5), 64.2 (C-6), 56.0 (O<u>C</u>H$_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{21}H_{26}O_6Na$: 397.1621. Found: 397.1621.

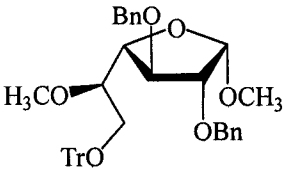
Methyl 2,3-di-*O*-benzyl-6-*O*-trityl-α-D-galactofuranoside (87)



To a solution of **86** (101 mg, 0.27 mmol) in dry pyridine (3 mL) was added trityl chloride (102 mg, 0.36 mmol) and DMAP (11 mg, 0.06 mmol). The solution was stirred at 40 °C for 12 h, and then concentrated to afford a crude compound, which was dissolved in EtOAc (10 mL). The solution was washed with 1 M HCl (5 ml), satd aq. $NaHCO_3$

solution (5 mL), brine (5 mL) and dried ($MgSO_4$). After the organic layer was concentrated, the resulting syrup was purified by column chromatography (6:1 hexane–EtOAc) to give **87** (163 mg, 98%) as an oil. R_f 0.39 (4:1 hexane–EtOAc); $[\alpha]_D$ +27.7 (*c* 2.1, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.50–7.20 (m, 25 H, Ar), 4.70–4.49 (m, 4 H, 2 × PhC<u>H</u>$_2$), 4.69 (d, 1 H, J = 4.4 Hz, H-1), 4.33 (dd, 1 H, J = 7.1, 6.0 Hz, H-3), 4.20 (dd, 1 H, J = 6.0, 3.8 Hz, H-4), 4.18 (dd, 1 H, J = 7.1, 4.4 Hz, H-2), 3.71 (ddd, 1 H, J = 6.5, 6.2, 3.8 Hz, H-5), 3.36 (s, 3 H, OC<u>H</u>$_3$), 3.24 (dd, 1 H, J = 9.4, 6.5 Hz, H-6), 3.18 (dd, 1 H, J = 9.4, 6.2 Hz, H-6); ^{13}C (125 MHz, $CDCl_3$, δ_C) 143.9 (3 × Ar), 138.0 (Ar), 137.5 (Ar), 128.7 (5 × Ar), 128.5 (2 × Ar), 128.4 (2 × Ar), 128.2 (2 × Ar), 128.0 (Ar), 127.9 (Ar), 127.9 (Ar), 127.7 (5× Ar), 127.7 (3 × Ar), 126.9 (3 × Ar), 101.8 (C-1), 86.8 (trityl <u>C</u>), 84.8 (C-2), 81.8 (C-4), 81.5 (C-3), 72.7 (Ph<u>C</u>H$_2$), 77.6 (Ph<u>C</u>H$_2$), 71.1 (C-5), 64.6 (C-6), 55.9 (O<u>C</u>H$_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{40}H_{40}O_6Na$: 639.2714. Found: 639.2717.

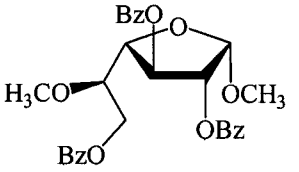
Methyl 2,3-di-*O*-benzyl-5-*O*-methyl-6-*O*-trityl-α-D-galactofuranoside (88)



To a solution of **87** (2.93 g, 4.75 mmol) in DMF (50 mL) was added 60% NaH (380 mg, 9.50 mmol) under 0 °C. After 5 min, CH_3I (354 μL, 5.70 mmol) was added to the solution, which was stirred for 3 h at room temperature and quenched by the addition of MeOH (5 mL). The solution mixture was then concentrated to afford a crude compound that was dissolved in EtOAc (100 mL). The solution was washed with water (60 mL × 2), brine (60 mL) and dried ($MgSO_4$), and the organic layer was concentrated. The resulting syrup

was purified by column chromatography (6:1 hexane–EtOAc) to give **88** (2.84 g, 97%) as an amorphous solid. R_f 0.44 (4:1 hexane–EtOAc); $[\alpha]_D$ +47.5 (*c* 0.4, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.47–7.07 (m, 25 H, Ar), 4.73 (d, 1 H, J = 4.2 Hz, H-1), 4.63–4.25 (m, 4 H, 2 × PhC$\underline{H}_2$), 4.06 (dd, 1 H, J = 7.2, 6.7 Hz, H-3), 4.01 (dd, 1 H, J = 7.2, 4.2 Hz, H-2), 3.86 (dd, 1 H, J = 6.7, 6.7 Hz, H-4), 3.57 (s, 3 H, 5-OC$\underline{H}_3$), 3.42 (ddd, 1 H, J = 6.7, 6.7, 3.5 Hz, H-5), 3.37 (s, 3 H, 1-OC$\underline{H}_3$), 3.26 (dd, 1 H, J = 10.4, 3.5 Hz, H-6), 3.22 (dd, 1 H, J = 10.4, 6.7 Hz, H-6); ^{13}C (125 MHz, $CDCl_3$, δ_C) 144.1 (3 × Ar), 138.2 (Ar), 137.6 (Ar), 128.8 (6 × Ar), 128.4 (2 × Ar), 128.3 (2 × Ar), 128.2 (2 × Ar), 128.0 (Ar), 127.8 (6 × Ar), 127.6 (2 × Ar), 127.5 (Ar), 126.9 (3 × Ar), 101.3 (C-1), 87.0 (trityl $\underline{C}$), 84.4 (C-2), 82.8 (C-5), 81.9 (C-3), 80.5 (C-4), 72.5 (Ph$\underline{C}H_2$), 72.2 (Ph$\underline{C}H_2$), 63.9 (C-6), 59.6 (5-O$\underline{C}H_3$), 54.9 (1-O$\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{41}H_{42}O_6Na$: 653.2875. Found: 653.2874.

Methyl 2,3,6-tri-*O*-bezoyl-5-*O*-methyl-α-D-galactofuranoside (89)



To a solution of **88** (2.81 g, 4.45 mmol) in EtOAc–MeOH (3:1, 50 mL) was added 20% $Pd(OH)_2$ (280 mg). The solution was stirred under H_2 (1 atm) for 12 h. The catalyst was filtered, and the filtrate was concentrated to afford a syrup that was dissolved in 1:1 CH_2Cl_2–MeOH (50 mL). To the solution was added 10% HCl in MeOH (0.5 mL) and the mixture was stirred at room temperature for 1 h. After being neutralized by Et_3N (2 mL), the reaction mixture was concentrated to afford a crude compound that was dissolved in pyridine (60 mL) at 0 °C. To the solution was added benzoyl chloride (2.24 mL, 16.02

mmol) dropwise. The reaction was stirred for 12 h while warming to room temperature and then MeOH was added to quench the excess benzoyl chloride. The solvent was evaporated to give a residue that was dissolved in EtOAc (100 mL). The solution was washed with 1 M HCl (50 mL), satd aq. $NaHCO_3$ (50 mL), brine (50 mL), and dried ($MgSO_4$). After the solution was concentrated, the resulting syrup was purified by column chromatography (6:1 hexane–EtOAc) to give **89** (2.03 g, 88%) as an amorphous solid. R_f 0.36 (4:1 hexane–EtOAc); $[\alpha]_D$ +120.43 (*c* 0.6, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$, δ_H), 8.14–7.94 (m, 6 H, Ar), 7.63–7.34 (m, 9 H, Ar), 6.23 (dd, 1 H, J = 7.3, 6.0 Hz, H-3), 5.45 (dd, 1 H, J = 7.3, 4.5 Hz, H-2), 5.34 (d, 1 H, J = 4.5 Hz, H-1), 4.60 (dd, 1 H, J = 12.0, 4.2 Hz, H-6), 4.51 (dd, 1 H, J = 12.0, 5.0 Hz, H-6), 4.37 (t, 1 H, J = 6.0 Hz, H-4), 3.80 (ddd, 1 H, J = 6.0, 5.0, 4.2 Hz, H-5), 3.66 (5-OC$\underline{H}_3$), 3.47 (1-OC$\underline{H}_3$); ^{13}C (125 MHz, $CDCl_3$, δ_C) 166.1 ($\underline{C}$=O), 165.9 ($\underline{C}$=O), 165.6 ($\underline{C}$=O), 133.7 (Ar), 133.3 (Ar), 132.9 (Ar), 130.1 (Ar), 130.0 (2 × Ar), 129.8 (2 × Ar), 129.7 (Ar), 129.6 (2 × Ar), 129.1 (2 × Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (2 × Ar), 100.9 (C-1), 79.7 (C-4/C-5), 79.6 (C-5/C-4), 77.6 (C-2), 74.5 (C-3), 63.0 (C-6), 59.2 (5-O$\underline{C}H_3$), 55.5 (1-O$\underline{C}H_3$); HRMS (ESI) *m/z* Calc. for (M+Na) $C_{29}H_{28}O_9Na$: 543.1627. Found: 543.1626.

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