Partial Synthesis of the Tetrasaccharide Fragment of Arugomycin

A Senior Thesis in the Department of Chemistry at Tufts University

Research Conducted in the laboratory of Dr. Clay Bennett

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Abstract

Arugomycin is a biologically active natural product, showing both antibacterial and antitumor activities. It intercalates in DNA, binding to both the major and minor grooves. The two oligosaccharide chains play important roles in stabilizing the arugomycin-DNA complex. In recent years the Bennett lab has pioneered a strategy for cyclopropenium cation promoted dehydrative glycosylations of 2-deoxy sugars. With that in mind, a synthetic strategy was outlined for the synthesis of the tetrasaccharide fragment of arugomycin. This structure would be difficult to access using traditional glycosylation methods, highlighting the utility of the cyclopropenium cation methodology.

The synthetic strategy revealed a common intermediate in the synthesis of all four monomers. The common intermediate was successfully synthesized. Three of the four monomers are functionalized with a methyl ether at C-3. Regioselective alkylation of the diol intermediate proved difficult, delaying the synthesis considerably. After several attempts at methylating the fucal, a new synthetic strategy was devised. Regioselective methylation was successfully completed on a 2-deoxy-fucopyranosyl derivative.

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Chapter 1: Introduction

1.1 Biopolymers

The chemistry of life is complex and varied, but polymers play an important role in all living systems. There are several classes of polymers found in nature, often referred to as biopolymers. Deoxyribonucleic acid, also known as DNA, is a polymer composed of nucleotide monomers. Proteins are composed of polypeptides, polymers of amino acids. Finally, oligosaccharides are polymers of sugar molecules joined by glycosidic linkages.

All three classes of biopolymers are composed of specific building blocks used to construct a diverse array of heterogeneous polymers. DNA is primarily composed of four nucleosides—adenosine, guanosine, thymidine, and cytidine—linked by a phosphodiester bond. Proteins are composed of 20 amino acids, which are assembled into linear chains and folded into a diverse array of functional compounds. Oligosaccharides are composed of a much larger library of monomers, adding diversity and complexity to their structure.

Oligosaccharides are commonly found as glycoconjugates, in which polysaccharides are linked to non-carbohydrate molecules such as peptides, proteins, or lipids. Adding to the complexity, many oligosaccharides are found as branched structures; in contrast, polypeptides and oligonucleotides are generally found as linear polymers[1]. Because of these complexities, many of the advances made in proteomics and genomics have not been easily extended to glycomics. For example, both genomics and proteomics have amplification techniques—polymerase chain reaction (PCR) for gene amplification and bacterial expression systems for protein production—that allow for the rapid isolation of large quantities of desired material. Comparable amplification techniques for glycomics are currently unavailable, limiting the availability of carbohydrates for further research.

1.2 Biological Importance of Oligosaccharides

Carbohydrates compose the majority of organic matter on Earth, serving many functions in all forms of life[2]. As a major class of biopolymers, carbohydrates are essential components in many biological processes. Oligosaccharides are used to provide conformational stability to proteins, modulate the function of proteins, store energy, provide binding sites for various microorganisms, toxins, and antibodies, and provide binding sites for cell-cell interactions and cell-matrix interactions[3].

One of the most prevalent roles of carbohydrates is as structural elements. The most abundant structural polysaccharide is cellulose, which is also the most abundant material on Earth. It is a linear chain of glucose molecules connected by β-1,4-*O*-glycosidic linkages[4]. Cellulose maintains structural stability in plants, bacteria, fungi, algae, and even some animals. Oligosaccharides can also be found as negatively charged polysaccharides composed of repeating disaccharide units, known as glycosaminoglycans[5]. The negative charge is derived from a carboxylate anion (COO^-) or sulfate group (SO_3^-) attached to one or more sugars in the disaccharide. Polysaccharides composed of glycosaminoglycans function as structural components in connective tissue and as lubricants in joints[2]. Other structural carbohydrates function as support in extracellular collagen, fibronectin, and heparan sulfate proteoglycans.

Carbohydrates also serve as folding aids for various polypeptides. Some proteins will not fold correctly unless an oligosaccharide is attached to the polypeptide in the rough endoplasmic reticulum. These *N*-linked oligosaccharides also help increase the stability and solubility of the fully folded protein.

Oligosaccharides function as recognition molecules. Specifically, oligosaccharides serve as receptors for viruses, bacteria, parasites, and various plant and bacterial toxins. They function as antigens for autoimmune reactions. Specific polysaccharide sequences are used to differentiate between 'self' and 'non-self'. For example, the human ABO blood type system is built around carbohydrates, where the A and B blood types each correspond to a slightly

different carbohydrate chain appended to the surface of red blood cells. In certain instances, specific carbohydrate chains are more prevalent on the surface of tumor cells than healthy cells, which is the foundation for a variety of carbohydrate vaccines and detection systems[1]. Often, these functions are highly specific and interactions only occur in the presence of a well-defined sequence of sugars[3].

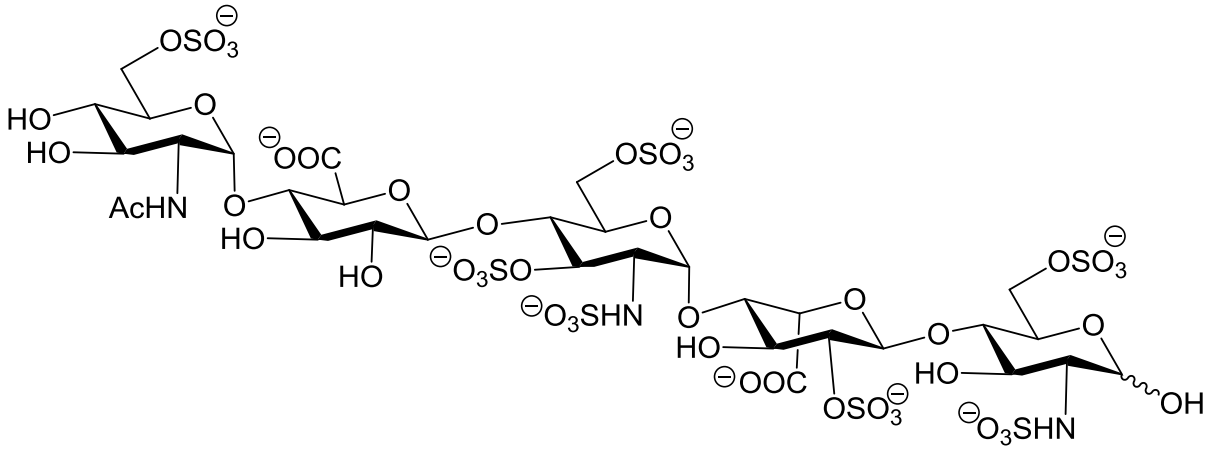


Figure 1. Pentasaccharide portion of heparin responsible for the antithrombin binding action.

Oligosaccharides have found a limited role as therapeutics and diagnostics despite the wide range of biological processes they are involved in. Notable examples are acarbose and heparin. Both are oligosaccharides originally obtained through isolation techniques. Heparin is the oldest carbohydrate-based drug, and is a heterogeneous mixture of carbohydrates[1]. The pentasaccharide responsible for the anticoagulant properties of heparin was elucidated (Figure 1)[6] and marketed as Arixtra by GlaxoSmithKline. However, low molecular weight heparins (LMWHs), a less heterogeneous mixture of saccharides than normal heparin, is still a more effective treatment than the isolated pentasaccharide. Synthesis of heparin analogues would help elucidate the functions of individual heparin components.

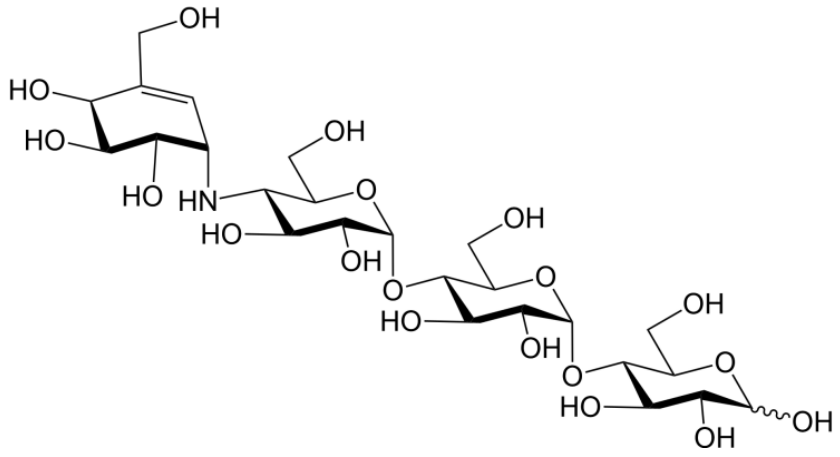


Figure 2. Structure of acarbose.

Acarbose is a pseudo-oligosaccharide produced through fermentation. It has shown activity as an α-glucosidase and α-amylase inhibitor (Figure 2)[7]. Acarbose has been used to treat type 2 diabetes.

A more prominent role of oligosaccharides is as vaccines; carbohydrate based vaccinations have been used for a wide range of diseases for decades[8]. These vaccines take advantage of the specific carbohydrate sequences expressed on the cell surfaces of bacteria, parasites and viruses, which are absent from host cells.

It has been estimated that over half the world's drugs are derived from natural products[9]. Many of the known natural products are secondary metabolites functionalized with carbohydrates. Changing the structure of the carbohydrate moieties of natural products modifies the biological activity of the molecule. For example, when a Chromomycin A_3 precursor is acetylated the biological activity increases greatly[10]. Furthermore, Langenhan and coworkers found that the cytotoxicity of digitoxin derivatives—a class of cardiac glycosides—increased with increasing oligosaccharide chain length, and increased when equivalent MeON-neoglycosides were tested[11]. Kahne et al. revealed that altering the glycosylation pattern of the vancomycin and teicoplanin aglycones restores efficacy against resistant bacterial strains[12].

Altering the glycosylation pattern of natural products has diverse biological impacts. To harness this potential, glycorandomization techniques have been developed. In glycorandomization, libraries of compounds differing only in glycosyl substituents are generated and screened for activity. Currently, glycorandomization is largely an enzyme-mediated process because of the difficulties with carbohydrate synthesis. Naturally occurring anomeric kinases have a narrow substrate range, requiring directed evolution to allow catalysis on a diverse array of sugars[13]. Direct chemical synthesis would be advantageous, allowing for the production of a wider array of variants. Unfortunately, synthetic methods are currently unable to efficiently prepare diverse libraries of glycosylated natural products.

1.3 The Glycosylation Reaction

One of the biggest obstacles to studying carbohydrates is accessing adequate amounts of pure material. Isolation techniques have been employed, but polysaccharides and glycoconjugates are present at low concentrations in nature, making isolation of usable quantities difficult and time consuming. Synthetic methods could allow for the construction of large amounts of pure and well-defined material, but there are several challenges that make carbohydrate synthesis more difficult than other biopolymers.

The linear molecules of polypeptides and nucleic acids lend themselves to simple synthetic and amplification techniques. Large quantities of homogenous oligonucleotides are easily accessed through the polymerase chain reaction (PCR), or through a simple automated synthetic route developed several decades ago. Silica or controlled pore glass supports are used in automated oligonucleotide synthesis, and have been reported to obtain yields in excess of 99 percent for a single transformation[14]. Polypeptides have been efficiently synthesized since 1963, when Robert Bruce Merrifield introduced solid phase peptide synthesis (SPPS)[15]. SPPS has since been automated, further simplifying peptide synthesis. However, the synthesis of oligosaccharides has not been as easily streamlined.

The polyhydroxyl nature of carbohydrates complicates synthetic routes. In order to obtain regioselectivity many protecting group manipulations are required. Orthogonal protection of hydroxyl groups is often the most time consuming aspect of synthesis. Significant time is spent functionalizing the carbohydrate, leaving one unprotected hydroxyl to act as an acceptor/nucleophile.

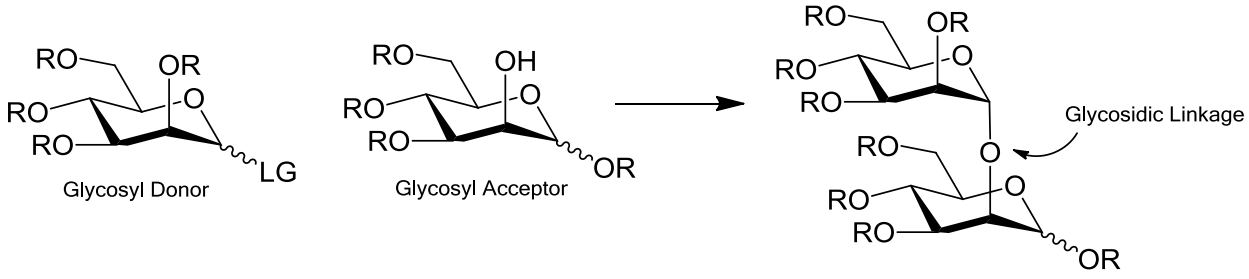


Figure 3. General glycosylation reaction between a glycosyl donor and a saccharide glycosyl acceptor. The glycosidic linkage in this scheme is an α-linkage between two $_D$-mannose monomers.

Much of carbohydrate chemistry revolves around glycosylation reactions, in which a glycosidic linkage is created. A glycosidic linkage is a covalent bond between a sugar molecule and another species, often another carbohydrate molecule. If the sugar is linked to a non-carbohydrate moiety, that group is referred to as an aglycone. There are several types of glycosidic linkages, the most common are O-glycosidic linkages, as carbohydrates are rich in hydroxyl groups. Other forms exist, such as N-glycosidic bonds, S-glycosidic bonds, and C-glycosidic bonds.

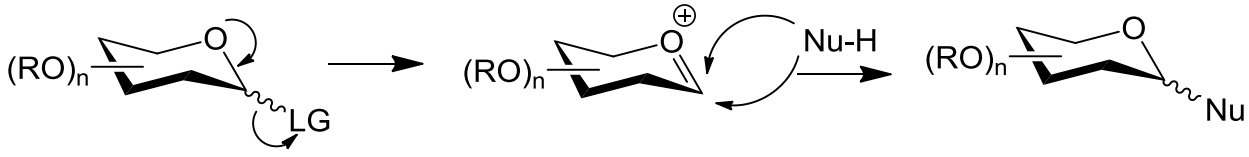


Figure 4. Proposed reaction mechanism for glycosylation.

The glycosylation procedure involves a glycosyl donor and a glycosyl acceptor (Figure 3). The glycosyl donor is a saccharide functionalized with a leaving group at the anomeric

position, functioning as an electrophile. The glycosyl acceptor has a nucleophilic functionality. A detailed mechanism for glycosylation has not been elucidated, but the general assumption is that an oxocarbenium cation intermediate forms, and the reaction proceeds through an S_N1 type mechanism (Figure 4)[16]. The nature of the leaving group, among other effects, determines how much the reaction resembles an S_N1 mechanism vs. an S_N2 mechanism. In low to moderate polarity solvents, the oxocarbenium cation is thought to be stabilized by a tightly coordinated counterion. The counterion can be the anion of the leaving group, or a specific counterion activator such as a triflate anion (TfO^-). The reaction produces two anomers. Assuming the most stable chair conformation, the α anomer specifies the axial glycosidic linkage and the β anomer specifies the linkage that is equatorial (Figure 5). The α/β designation also applies when the saccharide is linked to an aglycone if the linkage is at the anomeric position. The designations 1,2-*cis* and 1,2-*trans* are also used to describe the relationship between the anomeric substituent and the substituent at C-2.

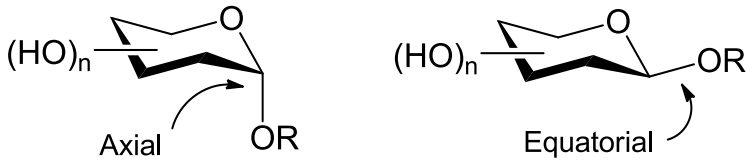


Figure 5. Axial vs. equatorial conformations of a generic carboohydrate.

Whether the linkage is α or β has important implications on the activity of the species. For example, there are two polymeric forms of glucose with 1,4-linkages, cellulose and glycogen. The major difference between these two compounds is the orientation of the glycosidic linkages, in cellulose the monomers are joined by β-1,4 linkages, whereas in glycogen the monomers are joined by α-1,4 linkages. The properties of these two compounds are vastly different. Glycogen acts as an energy storage molecule in animals, while cellulose serves as the primary structural component of cell walls in plants. They are different enough that

the human stomach can easily break down glycogen, but is completely unable to break down cellulose.

1.4 Anomeric & Solvent Effects

The general conformational rule in six-membered ring systems is that the chair conformation is energetically favored. Conformational analysis also predicts that the most stable conformation has the maximum number of equatorial substituents[17]. This preference stems from the unfavorable 1,3-diaxial interactions that occur between axial substituents. In contrast, electronegative substituents (such as OH, OR, SR, Cl, F, etc.) at the anomeric position tend to favor the axial conformation. The origin of this preference largely stems from the stabilization provided by hyperconjugation, in which the lone pair electrons of the O-5 atom, n_o, interact with the antibonding orbital of the C-X bond, $\sigma^*_{C\text{-}X}$, where X is the electronegative substituent at C-1. Hyperconjugation can only take place when the C-1 substituent is in the axial conformation; when the lone pair and X are antiperiplanar. In the equatorial conformation, the n_o electrons and σ* orbital are not correctly aligned (Figure 6). The anomeric effect is central to stereoselectivity in glycoside bond formation, as the conformation of the leaving group helps determine whether a reaction is α- or β-selective.

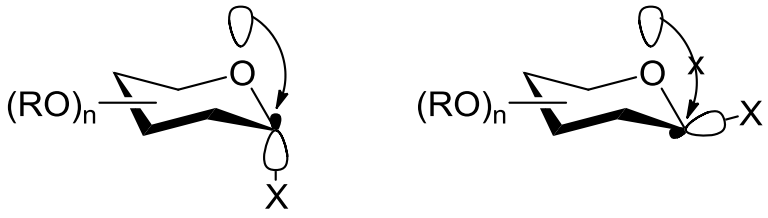


Figure 6. Hyperconjugation between the lone pairs of the ring oxygen, n_o (O-5) and the antibonding orbital of the anomeric substituent, $\sigma^*_{C\text{-}X}$. The interaction is strongest when X is an electronegative group such as an oxygen, halide, or nitrogen containing compound. On the right, hyperconjugation is possible when the anomeric substituent is axial, and it is impossible when the anomeric substituent is equatorial, as depicted on the left.

Solvents also play an important role in glycoside bond formation. It has been proposed that the rate of β-glycoside formation is increased in polar solvents due to the charge separation between the ring oxygen and the anomeric β-oxygen[16]. It follows from this argument that the rate of α-glycoside formation is increased in nonpolar solvents. There are also solvents which are thought to participate in glycoside bond formation, namely, acetonitrile and various ethers. Empirically, glycosylations conducted with acetonitrile tend to favor the production of β-linkages, while reactions conducted in ethers favor the production of α-linkages[18]. The dominant theory is that acetonitrile preferentially coordinates to the oxocarbenium cation from below the plane of the ring (α-coordination), blocking nucleophilic attack from the α-face and predominantly forming the β-linkage. The ethers do the opposite, coordinating to the β-face of the sugar, promoting the formation of α-linkages. Many factors affect the selectivity besides the solvent, so these trends are not absolute and depend on the system in question.

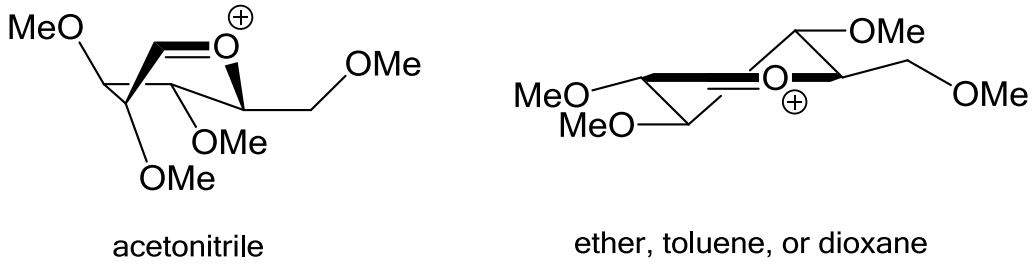


Figure 7. The conformations of a model carbohydrate in various solvents. The model saccharide is a 2,3,4,6-tetra-*O*-metyl-$_D$-glucopyranosyl triflate. In acetonitrile (left) the oxocarbenium ion exists primarily in a $B_{2,5}$ boat conformation, with the counterion preferentially coordinated to the α-face, predominately forming the β-product. In ether, toluene, or dioxane, the saccharide primarily adopts a $^4H_3$ half-chair conformation, with the counterion preferentially coordinating to the β-face, predominantly forming the α-product.

Recent evidence from computational studies has suggested that the solvent effects in glycosylations stem from the conformation of the ring and the location of the counterion[18]. Hünenberger and coworkers have suggested that in acetonitrile the oxocarbenium ion adopts a boat conformation (Figure 7), in which the counterion (the triflate anion in this study) is on the α side. In ether, toluene, or dioxane, the cation preferentially exists in a half-chair conformation

where the counterion is located on the β side. These calculations were carried out using density functional theory using Gaussian 03 software.

1.5 General Synthetic Strategies

Chemists have been interested in glycosylation reactions for over a century. Many strategies have been employed to link carbohydrates to other molecules. One of the earliest strategies was developed by Emil Fischer and consisted of creating a glycosidic linkage to an alcohol (aglycone). The alcohol acceptor was used as the solvent, and the reaction was conducted in the presence of excess acid. The reversibility of this process makes it undesirable for many applications, and in 1901 Wilhelm Koenigs and Edward Knorr introduced a method that functionalizes the anomeric position with a bromine leaving group, using silver or mercury salts as activators. The salts precipitate liberated bromine atoms, and push the equilibrium in favor of product formation[19]. The advantage of the Koenigs-Knorr method is in its irreversibility. Unfortunately, large amounts of promoter are necessary, requiring between 1-4 equivalents of mercuric bromide or silver triflate, for example. These reactions often call for a sterically hindered base as an acid scavenger to prevent acid hydrolysis of the glycosidic bond[20]. Glycosylations with poorly nucleophillic acceptors, such as carbohydrate hydroxyls, were found to be slow and inefficient using this method[16].

Other glycosyl halides have been explored. The Koenigs-Knorr methodology was extended to chlorine with similar results. In 1929, glycosyl iodides were prepared from glycosyl bromides in the presence of sodium iodide in acetone[21]. In basic conditions, glycosyl iodides are generated from the anomeric acylated or silylated derivative using trimethylsilyl iodide (TMSI), forming β-glycosides in an S_N2-type displacement of the α-iodide[22]. When glycosyl iodides are allowed to undergo in situ anomerization in the presence of TBAI and *N,N*-diisopropylethylamine (DIPEA), α-glycosidic linkages can be formed through S_N2-like attack of the highly-reactive β-iodide intermediate[23].

A common class of glycosyl donors are thioglycosides. Thioglycosides were first prepared in 1909 by Emil Fischer[24], and have been actively researched since. An advantage of thioglycosides is their high stability in many conditions, allowing them to survive most protecting group manipulations[20]. Thioglycosides are generally formed from peracetylated sugars in the presence of a thiol and a lewis acid (usually BF_3•OEt)[25]. Thioglycosides can be activated by several promoters, all of which require stoichiometric quantities. The most frequently used activator combination is *N*-iodosuccinimide (NIS) in the presence of AgOTf[26], a variation on the original conditions employing NIS and triflic acid[27]. Many other electrophilic activator systems have been introduced since the NIS/triflic acid system was first reported. One difficulty when using thioglycosides is stereoselectivity. Obtaining α or β linkages often requires fine-tuning each individual reaction, as the selectivity is not general.

Glycosyl thioimidates are another common class of glycosyl donors. First introduced as glycosyl donors in the late 1970s by Woodward et al., thioimidates are highly stable. They can withstand the harsh conditions used for many protecting group manipulations[20]. They can even withstand activation conditions for other glycosyl donors, such as thioglycosides, glycosyl bromides, and trichloroacetimidates. Thioimidates are prepared through Lewis-acid catalyzed displacement of the anomeric *O*-acetyl group, or by reacting anomeric halides with thiolate ions[28]. Thioimidate donors can be activated under a variety of conditions due to their activity as both a thioglycoside and an imidate. The stability of thioimidates makes them useful in the development of rapid syntheses of oligosaccharides (Figure 8) [29].

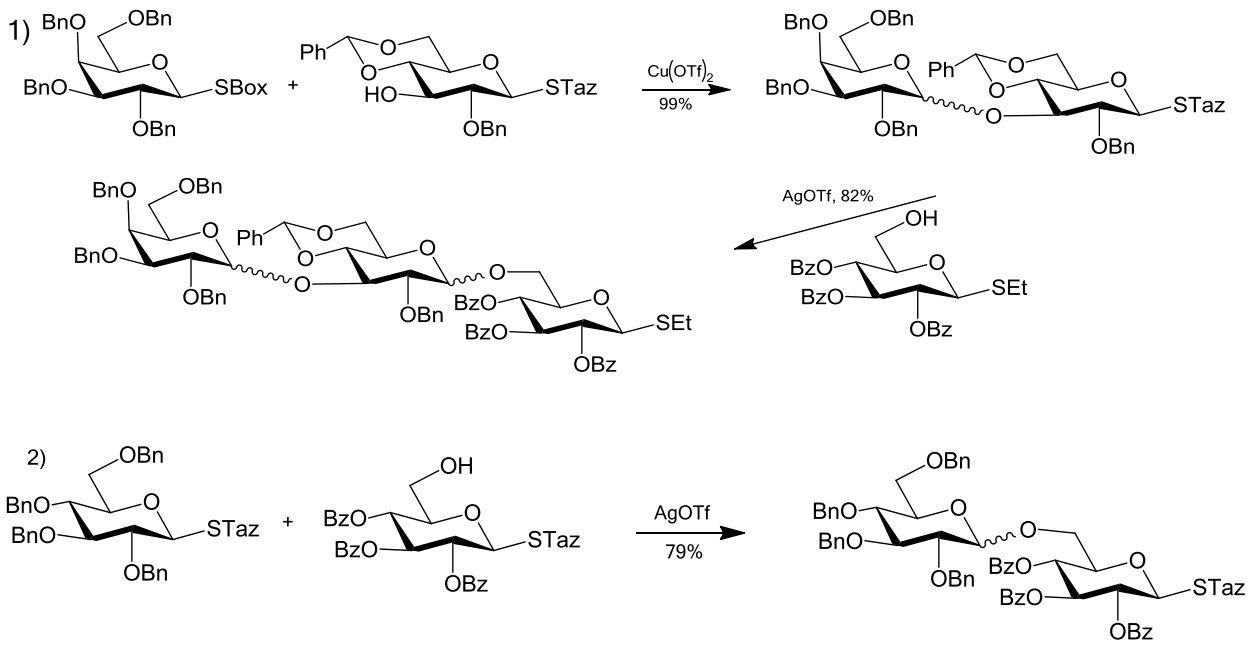


Figure 8. Two chemoselective schemes utilizing thioimidates as both glycosyl donors and acceptors.

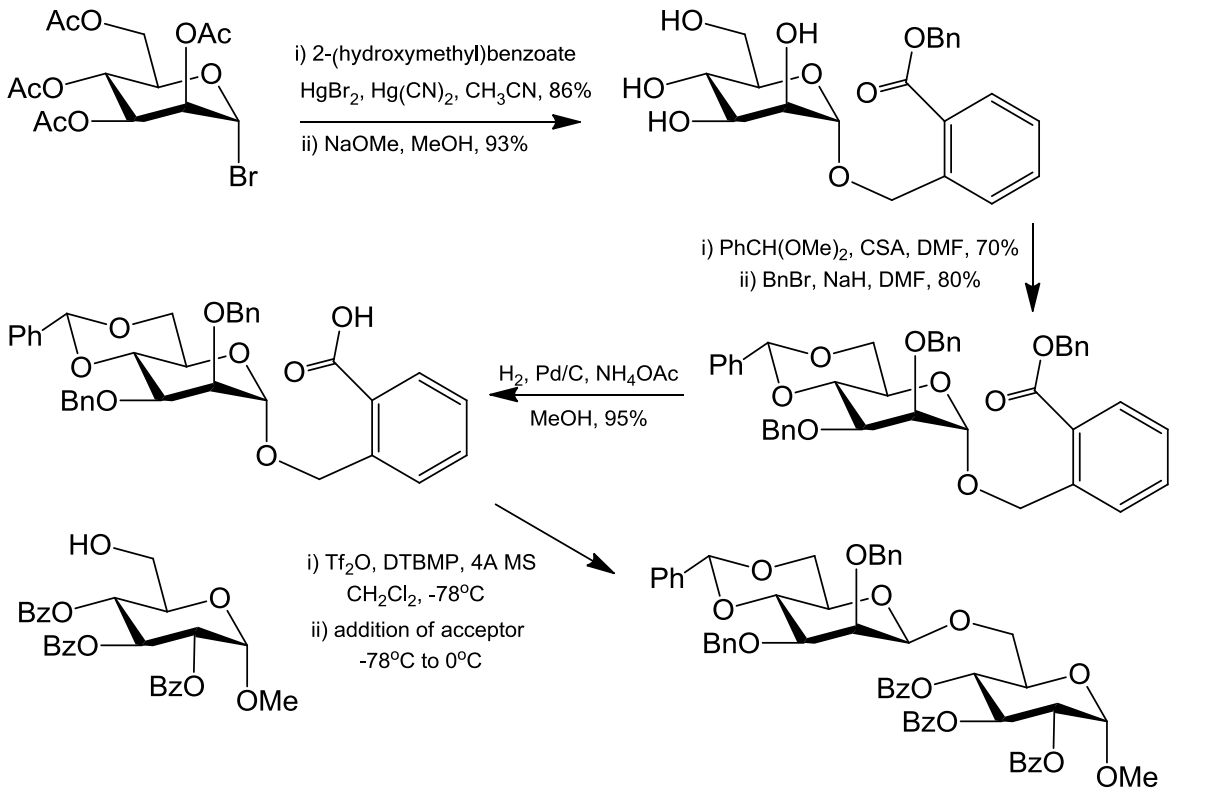
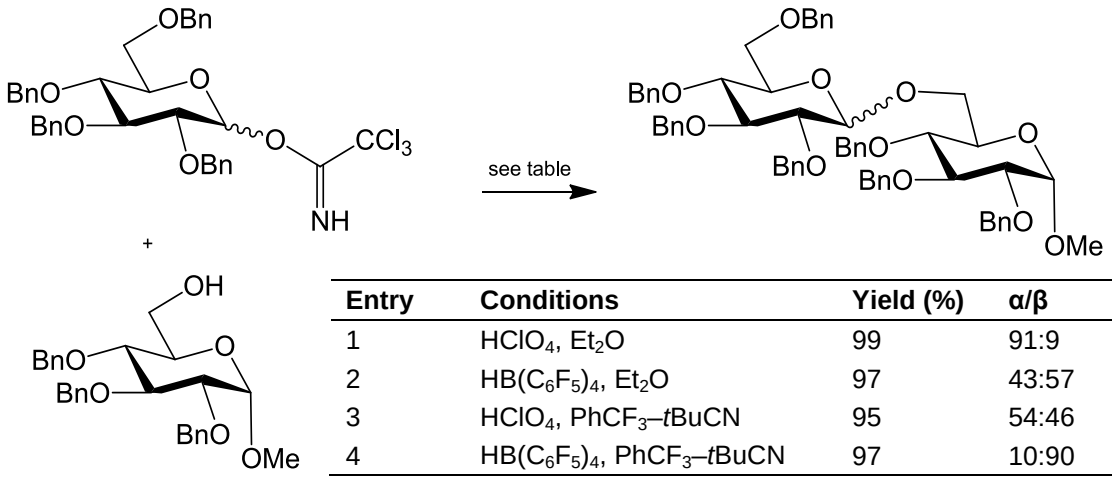


Figure 9. Synthesis of a disaccharide using an activated CB glycosyl donor.

A recently developed class of glycosyl donors are 2-(benzyloxycarbonyl)benzyl (BCB) glycosides, first introduced in 2001[30]. Selective hydrogenolysis of the benzyl ether leads to formation of the active 2-carboxybenzyl (CB) glycoside (Figure 9). The hydrogenolysis of the BCB moiety occurs selectively even in the presence of benzyl and benzylidine groups. Another advantage of this method is that BCB glycosides are stable under most glycosylation conditions, allowing rapid sequential glycosylations through activation of BCB glycosides. Furthermore, CB glycosides can be activated in either acidic or basic conditions. These glycosides have been used in the formation of β-mannoside and β-arabinofuranoside bonds.

The glycosyl donors discussed thus far are generated through exchange of anomeric oxygen for various leaving groups, activating the carbohydrate as a donor. An alternative method involves the direct *O*-alkylation at the anomeric position[31]. Base activation of the anomeric hydroxyl group, using sodium hydride or *tert*-butoxide, followed by addition of an *O*-trifyl functionalized saccharide, leads to glycosidic bond formation. The stereoselectivity stems from metal chelation, depending on the protection pattern of the base-activated carbohydrate.

A widely used variation involves creation of the activated glycosyl donor species through base activation of the anomeric hydroxyl group, followed by addition of trichloroacetonitrile (Cl_3CCN). The *O*-glycosyl imidate formed is an excellent leaving group[32]. This method uses sodium hydride (NaH) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the base, and trimethylsilyl trifluoromethanesulfonate (TMSOTf) or boron trifluoride complexed with diethyl ether (BF_3•OEt_2) as the catalyst. For armed carbohydrates, $Sm(OTf)_3$ can be used under mild conditions[33] while disarmed carbohydrates can be catalyzed by $Yb(OTf)_3$[34]. Both $Sm(OTf)_3$ and $Yb(OTf)_3$ are stable salts that are easily stored.



Entry	Conditions	Yield (%)	α/β
1	$HClO_4$, Et_2O	99	91:9
2	$HB(C_6F_5)_4$, Et_2O	97	43:57
3	$HClO_4$, $PhCF_3$–*t*BuCN	95	54:46
4	$HB(C_6F_5)_4$, $PhCF_3$–*t*BuCN	97	10:90

Figure 10/Table 1. The effect of different conditions on the synthesis of a disaccharide using a trichloroacetimidate donor. The reaction can be tuned to produce either α- or β- selective products depending on the reaction conditions used.

The reaction conditions for trichloroacetimidate glycosylations can be tuned to alter the configuration of the glycosidic bond. The solvent has been shown to play an important role (see section 1.4) as has the counterion of the catalyst used (Figure 10/Table 1)[35]. The effects of the counterion on selectivity are not well understood.

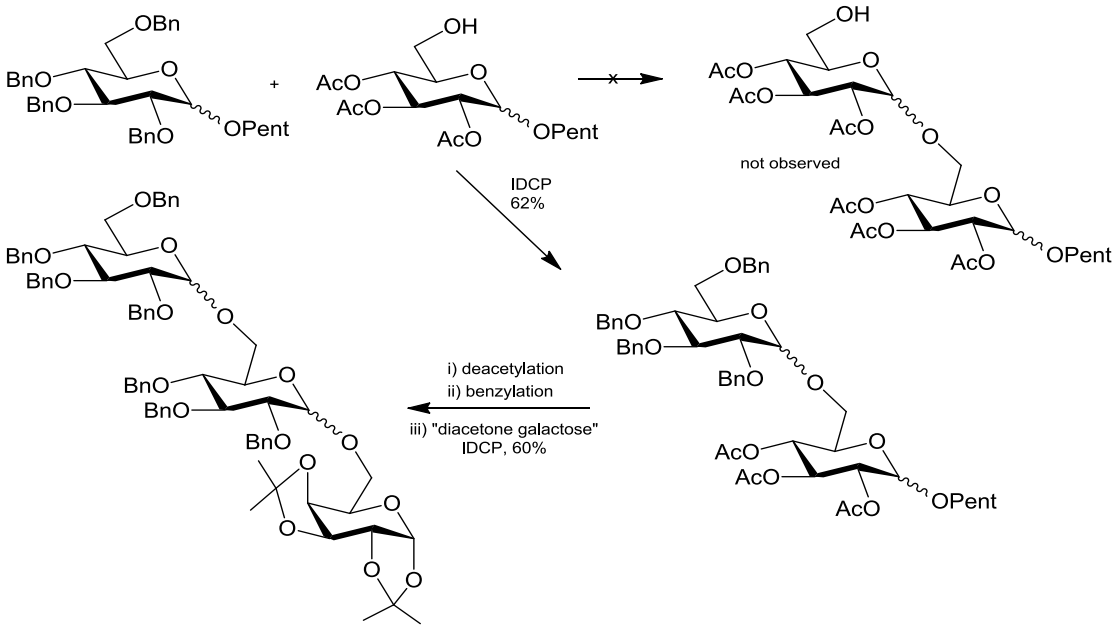


Figure 11. Synthesis of a trisaccharide using the armed/disarmed strategy to selectively perform a single glycosylation in the presence of two glycosyl donors.

To improve the efficiency of glycoside bond formation, 'one pot sequential glycosylation' oligosaccharide synthesis was developed. A concept based on the observation that carbohydrates with different protection patterns have varying reactivities within a class of glycosyl donors (Figure 11)[36]. The terms 'armed' and 'disarmed' were introduced to describe carbohydrates with different donor reactivities, where armed sugars react more rapidly than disarmed sugars. If a sugar is protected with electron-donating groups (benzyl ether), especially at C-2, then it is said to be armed, and if it is protected with electron-withdrawing groups (acetyl ester) it is described as disarmed. Several laboratories have used the armed-disarmed strategy to obtain sequence selectivity when multiple donors are present in the same reaction. The synthesis is designed such that the donor reactivity is decreased with each sequential glycosylation. Wong and coworkers assigned numerical values to a library of thioglycosides indicating their relative reactivity. They also designed a computer program that searches the database of donors and select sets of donors to maximize yield (Figure 12)[37].

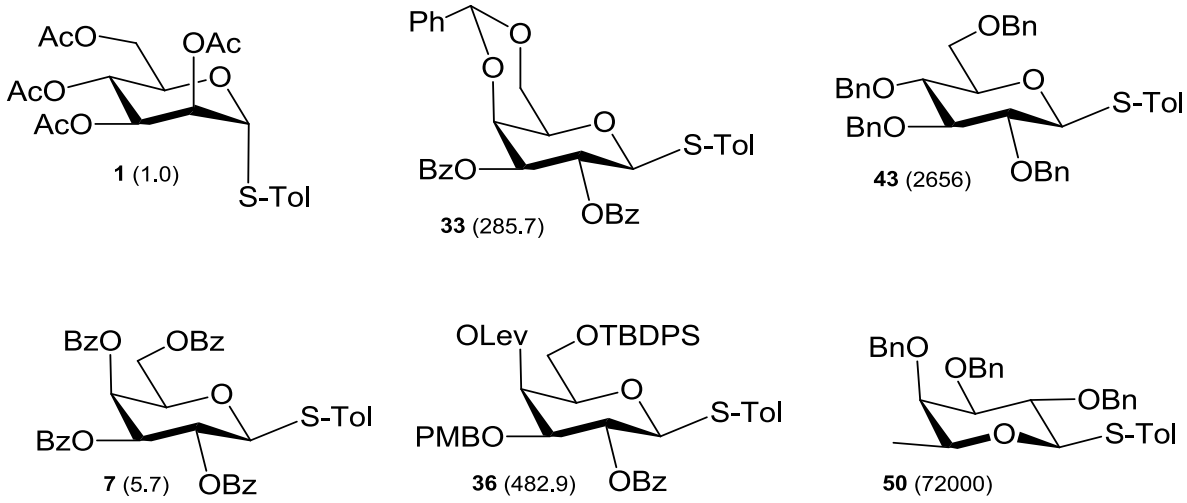


Figure 12. Relative reactivities of selected thioglycosides. The lower the number in parentheses, the lower the reactivity of the compound.

Often, the most time consuming aspect of oligosaccharide synthesis is regioselective protection of the hydroxyl groups. To obtain regioselective glycosylations between two

saccharides, the glycosyl donor should not have any nucleophilic functionality (to avoid dimerization), and the glycosyl acceptor must have a single free hydroxyl group. Obtaining this configuration is not trivial and often involves a multiple step protection-deprotection sequence with several purification steps. Hung et al. reported in 2007 a one-pot protection scheme for D-glucose (Figure 13) [38]. They developed a combinatorial, regioselective, orthogonal, and one-pot protection of per-O-silylated α-glucosides and β-thioglucosides to produce fully protected monosaccharides, 2-alcohols, 3-alcohols, 4-alcohols, and 6-alcohols.

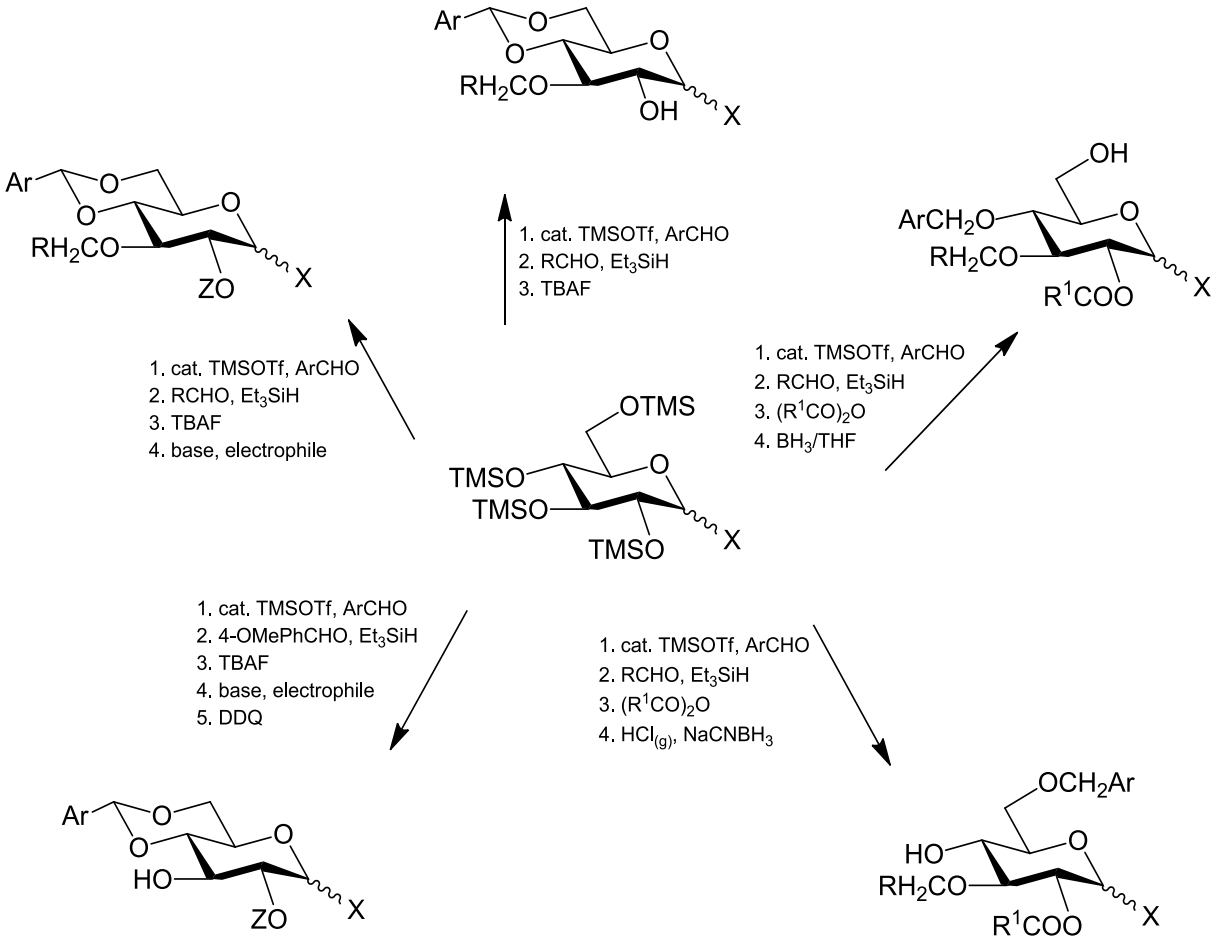


Figure 13. One-pot regioselective protection of glucose. The X substituent is either the α-methoxy or β-thiophenoxy group. The Z substituent depends on the electrophile used.

1.6 Neighboring Group Participation and 2-Deoxy Sugars

A common way to obtain selectivity in glycosidic bond formation is through anchimeric assistance. When there is an acyl protecting group at C-2 of the donor, such as an *O*-acetyl (Ac), *O*-benzoyl (Bz), or 2-phthalimido (NPhth), anchimeric assistance favors the formation of a 1,2-*trans* glycosidic bond[39]. The C-2 functionality attacks the anomeric carbon, forming a stabilized acyloxonium ion intermediate. The formation of the acyloxonium ion is favored over oxocarbenium cation formation, and the glycosyl acceptor reacts preferentially from the opposite face to form the 1,2-*trans* isomer (Figure 14). When the C-2 hydroxyl is functionalized with ether protecting groups, the anchimeric effect is no longer effective, and the anomeric effect favors the formation of 1,2-*cis* glycosides in gluco- or galactopyranosides[20].

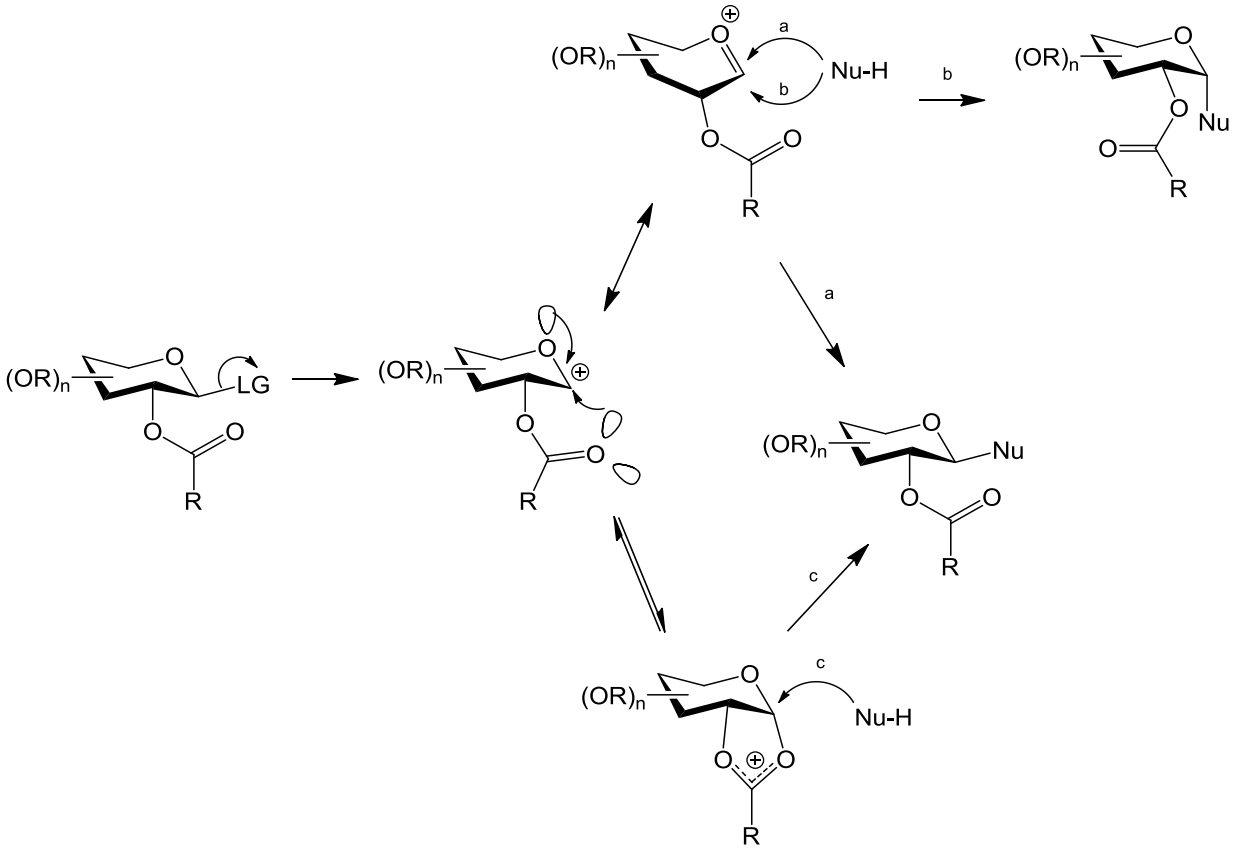


Figure 14. General mechanism for neighboring group assistance. Generally, the 1,2-*trans* product is favored (in this scheme, the β-product).

For 2-deoxy sugars, the possibility for anchimeric assistance is eliminated, and stereocontrol of glycoside bond formation is more difficult. The tendency for 2-deoxy glycosides to undergo elimination further complicates synthesis, especially considering that glycosyl donors are functionalized with an excellent leaving group. Biologically, 2-deoxy sugars are important components of steroidal glycosides, antibiotics, and antitumor compounds[40], and interest in their efficient synthesis has steadily grown in recent years. There are two common methods for glycosidic bonds formation in 2-deoxy sugars: direct synthesis and indirect synthesis. Indirect synthesis involves using the 2-deoxy sugar as a donor after addition of a directing group at C-2. After the linkage is formed the directing group is removed. Direct synthesis involves directly using the 2-deoxy sugar as a donor. Direct synthesis eliminates the extra steps required to functionalize the carbohydrate and then remove the directing group.

Thioglycosides have been used as 2-deoxy donors for stereoselective glycosylations. Various promoters have been used for α-selective reactions, including $AgPF_6$[41]. Another study used a pre-activation strategy to obtain α-selectivity, in which benzenesulfonyl morpholine (BSM) and triflic anhydride (Tf_2O) were used prior to addition of the acceptor[42]. The pre-activation is thought to form the reactive β-glycosyl triflate, which undergoes S_N2-like displacement upon addition of the donor, forming the α-glycoside.

From a common thioglycoside donor, Paul and Jayaraman were able to prepare both α- and β-glycosidic linkages using different conditions[43]. To obtain α-selectivity, *N*-iodosuccinimide and triflic acid (NIS-TfOH) were employed. Obtaining β-selectivity involved treating the thioglycoside with bromine in dichloromethane, followed by treatment of the crude bromide with the acceptor, using lithium bis(trimethylsilyl)amide (LHMDS) as the base.

Direct synthesis using activated oxygen derivatives has also been performed on 2-deoxy glycosides. Using a CB glycoside donor activated with Tf_2O and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) at -78^{o}C, Kim et al. reported excellent yield (91%) of pure α product[44]. However, this result only held for secondary carbohydrate alcohols, as primary alcohols formed

a mixture of anomers. Changing protecting groups at O-4 and O-6 from benzyl ethers to benzylidine acetals completely reverses selectivity, forming pure β product.

Takahashi and coworkers devised a highly β selective synthesis (β:α > 95:5) using 2-deoxy glycosyl trichloroacetimidates which works with primary and secondary alcohol acceptors[45]. This procedure uses 1.5 equivalents of iodine (I_2) and a catalytic amount of triethylsilane (Et_3SiH) to promote the reaction, which takes place at -94^{o}C in toluene.

Another procedure using 2-deoxy trichloroacetimidate donors was developed by Boons et al. and involves a participating (*S*)-(phenylthiomethyl)benzyl group at C-6 to efficiently generate α-linkages with a variety of alcohol acceptors (Figure 15) [46].

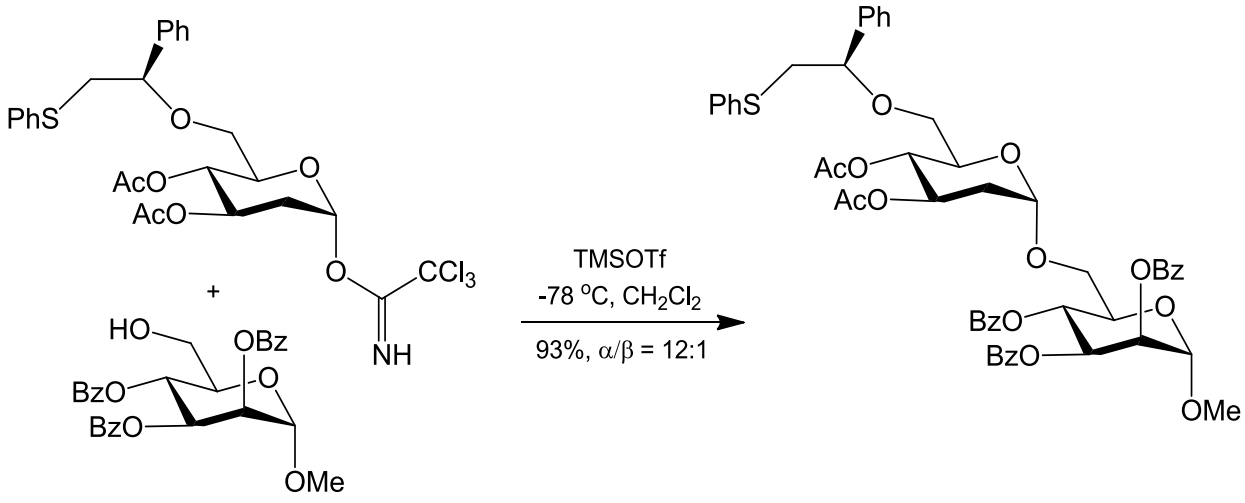


Figure 15. Efficient generation of α-glycosidic linkages using 2-deoxy donors.

Glycosyl phosphites have been used for β-selective glycosylations with 2-deoxy donors, as reported by Toshima and coworkers[47]. Primary and secondary acceptors, both aglycones and carbohydrates, were reacted with the phoshpite donors in diethyl ether at -78^{o}C in the presence of 100 wt % monmorillonite K-10 to form the β-linkage in high yields (70% - 97%) and good stereoselectivity, with α:β ratios ranging from 29:71 to 10:90.

One of the oldest classes of donors, glycosyl halides, have been used to prepare 2-deoxy glycosides. 2-Deoxy glycosyl halides are difficult to use because they are prone to

hydrolysis. Glycosyl fluorides are more stable than other halides, and are the donor of choice for 2-deoxy glycosyl halides[48]. Toshima et al. utilized glycosyl fluorides to form α linkages[49]. The 2-deoxy donors were treated with cyclohexylmethanol in the presence of 5 wt % heterogeneous solid acid SO_4-ZrO_2 at room temperature. Excellent yield (98%) and good stereoselectivity (α:β = 88:12) were observed. Several other alcohol acceptors were used, including secondary carbohydrates, affording moderate to excellent yields and good selectivity (α:β > 80:20). Using diethyl ether instead of DCM or MeCN yielded primarily the β anomer when 100 wt % of heterogeneous acid SO_4-ZrO_2 and 500 wt % 5 Å molecular sieves were used.

Glycals—unsaturated carbohydrates bearing a double bond between C-1 and C-2—have also been used for the preparation of 2-deoxy glycosides. Acid can be used on glycals to form the oxocarbenium ion, often forming α-glycosides from anomeric effect stabilization[48]. Using $CeCl_3$•$7H_2O$-NaI, α-selectivity was obtained by Yadav et al. with triacetyled glucals, galactals, and xylals as donors and simple alcohol acceptors (allyl alcohol, *n*-octanol, phenyl allyl alcohol, and (*E*)-hex-2-en-1-ol)[50]. The same group later reported the synthesis of 2-deoxy thioglycosides from glycals using gallium (III) trichloride as a catalyst in yields ranging from 75-95% and good stereoselectivity (α:β > 8:2) [51].

A rhenium-oxo complex was used by Toste et al. to synthesize 2-deoxy-α-glycosides from various glucals and galactals[52]. The reaction was carried out with 1 mol % of the catalyst, [$ReOCl_3(SMe_2)(Ph_3PO)$], in toluene, using several protecting groups on the glycals (benzyl, silyl ether, acetate, benzoate, and isopropylidene acetal) and using several acceptors (Figure 16). When the galactals were used as donors, the reaction produced pure α-product, and when glucals were used a mixture of products was formed favoring the α-product (α:β > 3:1).

Figure 16. [$ReOCl_3(SMe_2)(Ph_3PO)$]-catalyzed formation of 2-deoxy-glycosides from glycals.

1.7 Dehydrative Glycosylation

All the methodologies discussed to this point have focused on forming glycoside bonds with isolatable glycosyl donors, such as thioglycosides and trichloroacetimidates. Dehydrative glycosylation involves 1-hydroxy glycosyl donors, which are activated *in situ* and reacted with an acceptor in a one-pot fashion. The synthesis and isolation of unstable sugar donors is not necessary. In 1997, Gin and coworkers described a dehydrative glycosylation procedure, using diphenyl sulfoxide and triflic anhydride to form a reactive oxosulfonium intermediate (Figure 17)[53]. The oxosulfonium donor is exposed to an acceptor, forming a glycosidic bond. The reaction liberates triflic acid (TfOH), so an acid scavenger is added to prevent acid hydrolysis of the glycosidic bond; 2,4,6-tri-*tert*-butylpyridine (TTBP) has been employed, as this bulky base reduces elimination. Gin and coworkers extended this methodology to a sulfide-mediated dehydrative glycosylation in which a dialkyl sulfide (R_2S) and triflic anhydride are added to a 1-hydroxy carbohydrate[54]. Most of the reactions conducted using these procedures lead to a mixture of anomers, generally favoring the formation of the α product.

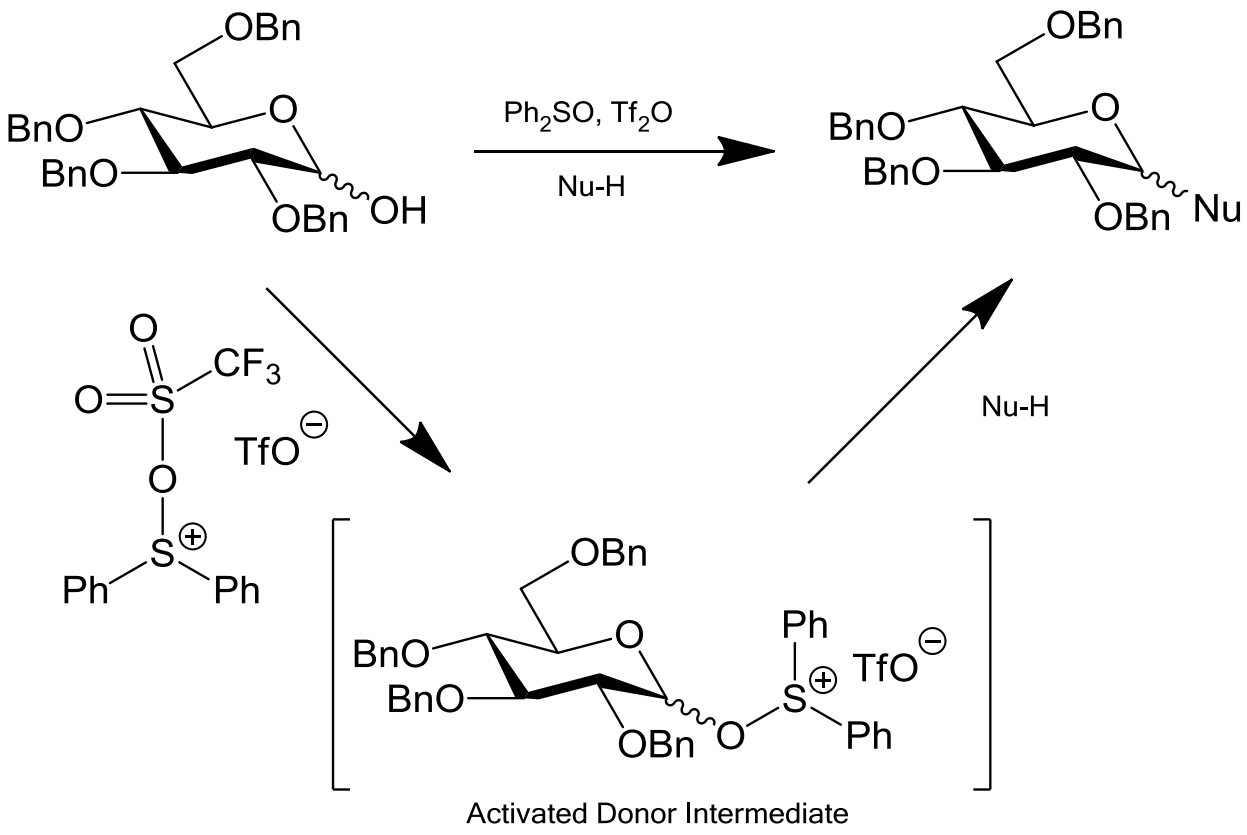


Figure 17. Sulfoxide-mediated dehydrative glycosylation.

Dehydrative glycosylations have proven difficult when using 2-deoxy donors. Using the Mitsunobu reaction, Roush et al. described the formation of Aryl 2-deoxy-β-glycosides[55]. They utilized 2α-(thiophenyl)- and 2α-(selenophenyl)-α-$_D$-sugars in the presence of triphenyl phosphine, diethyl azodicarboxylate, and an aromatic acceptor to generate the β-linkage. This method is not general, as aliphatic acceptors require the use of mercury salts[56].

1.8 Cyclopropenium Cations

Direct nucleophilic substitution of alcohols is inefficient due to the poor leaving-group potential of hydroxide ions. Methods have been developed to convert the hydroxyl to a viable leaving group, including protonation, conversion to sulfonate, sulfite, or phosphite esters, or Mitsunobu inversion using phosphonium ethers[57]. Lambert et al. proposed cyclopropenium

cation activated alcohols as alternatives to traditional methods. The mechanism involves a cyclopropene with two geminal halide substituents that exists in equilibrium with the cyclopropenium salt. When exposed to an alcohol, the cyclopropenyl ether forms, and ionizes through loss of the second halide. The alkoxycyclopropenium species is prone to nucleophilic substitution. This method has been extended to the cyclization of diols (Figure 18). When converting hydroxyl groups to chlorides, 1.1 equivalents of 1,2-diphenylcyclopropenone was added to the desired alcohol at room temperature in dichloromethane to produce >80% of the desired product in all cases (except when a tertiary alcohol was used, then the yield was 45%). When a chiral alcohol was used the reaction proceeded with inversion of configuration, implying an S_N2 mechanism.

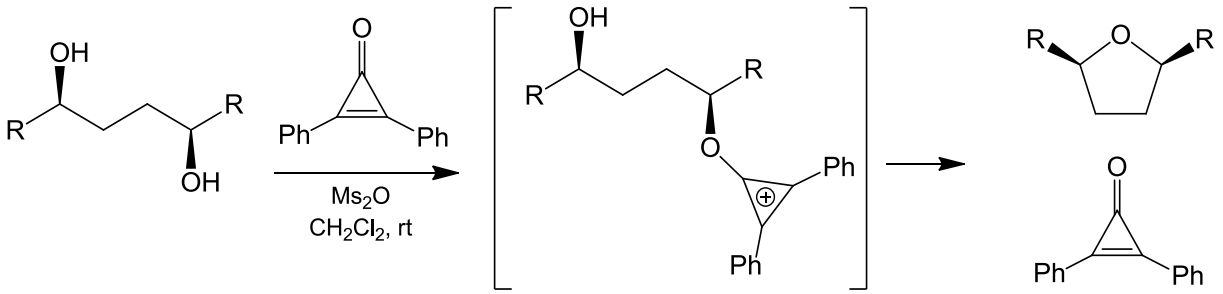


Figure 18. Cyclopropenium-activated cyclodehydration of diols[58].

1.9 Arugomycin

Arugomycin was characterized in 1983 by Ōtake and coworkers at the University of Tokyo (Figure 19)[59]. Arugomycin was extracted from the cultured broth of organism 1098-AV_2, identified as a strain of *Streptomyces violochromogenes*. The strain was isolated from a soil sample collected at Motoyama, Saga, Japan. The organism was cultured for 6 days in a medium containing 2% starch, 1% soybean oil, 0.2% yeast extract, and 0.4% $CaCO_3$ (as a buffer to pH 7.0) at 27^{o}C. After some purification, the active fraction was further purified to yield a pure orange powder, named arugomycin by the investigators. It was shown that Arugomycin

inhibited the growth of Gram-positive bacteria, with an LD_{50} in mice of 1.75mg/kg by intraperitoneal injection. Antibacterial tests on several strains were performed with arugomycin and the results are summarized in Table 2.

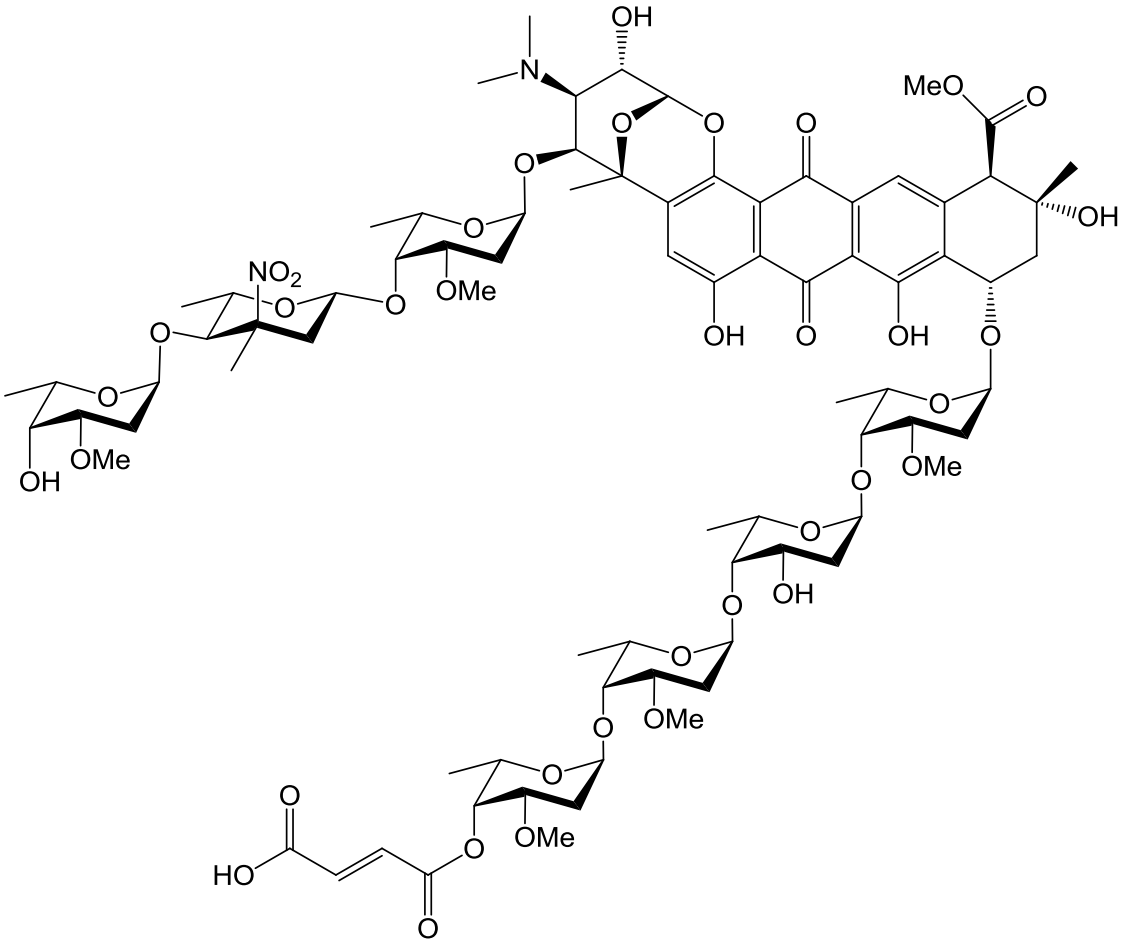


Figure 19. Arugomycin.

Arugomycin is composed of an aglycone linked to two oligosaccharide chains; a trisaccharide and a tetrasaccharide. There are four carbohydrate monomers that constitute both oligosaccharides in arugomycin (Figure 20)[60]. The aglycone portion, arugorol, is the 4'-epimer of nogalarol, which is the aglycone of nogalamycin[61].

Arugomycin has been shown to interact with both the major and minor grooves of DNA simultaneously (Figure 21)[62]. Molecular modelling studies have suggested that the trinucleotide sequence binds in the major groove, with stabilization from both electrostatic and hydrogen bonding interactions with the trinucleotide sequence 5'-GCA (5'-TGC). The tetrasaccharide binds to the minor groove through several van der Waals interactions, most likely contributing to the stability but not the specificity of the complex. The arugomycin-DNA complex has been predicted from several ^{1}H NMR studies to exist as the dimeric species (arugomycin)$_2$-d(GCATGC)$_2$, in which two arugomycin molecules bind to the d(GCATGC)$_2$ DNA duplex. Both nogalamycin and arugomycin produce similar DNASE I cleavage inhibition patterns, as both drugs bind preferentially to alternating sequences of purines and pyrimidines.

Organism	MIC (μg/mL)
Bacillus subtilis PCI 219	12.5
Staphylococcus aureus FDA 209 P	12.5
Micrococcus luteus ATCC 9341	12.5
Pseudomonas aeruginosa NCTC 10490	> 100
Salmonella typhimurium IFO 12529	> 100
Escherichia coli NIHJ JC-2	> 100
Saccharomyces cerevisiae No. Yu 1200	> 100
Candida albicans IFO 0396	> 100
Aspergillus fiunigatus IFO 4400	> 100
Penicillium chrysogenum ATCC 10002	> 100

Table 2. Antibacterial activity of arugomycin. Minimum inhibitory concentration (MIC) is given on the right.

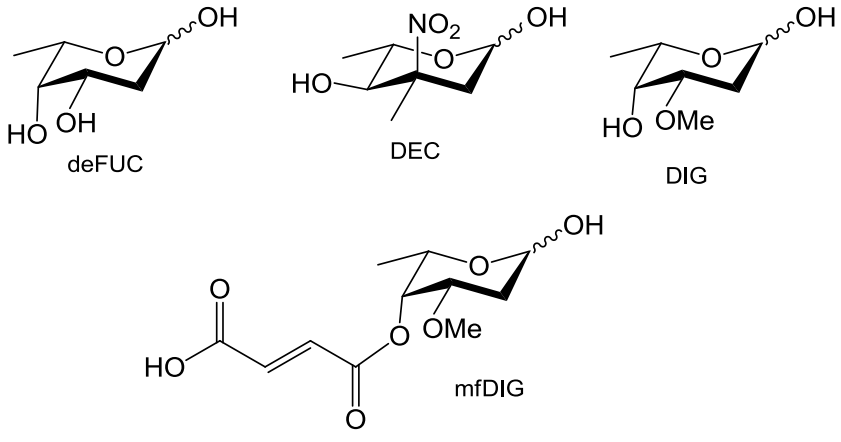


Figure 20. Carbohydrate monomers of Arugomycin. deFUC = 2-deoxy-$_L$-fucose, DEC = $_L$-decilonitrose, DIG = $_L$-diginose, mfDIG = methyl fumaryl-$_L$-diginoside.

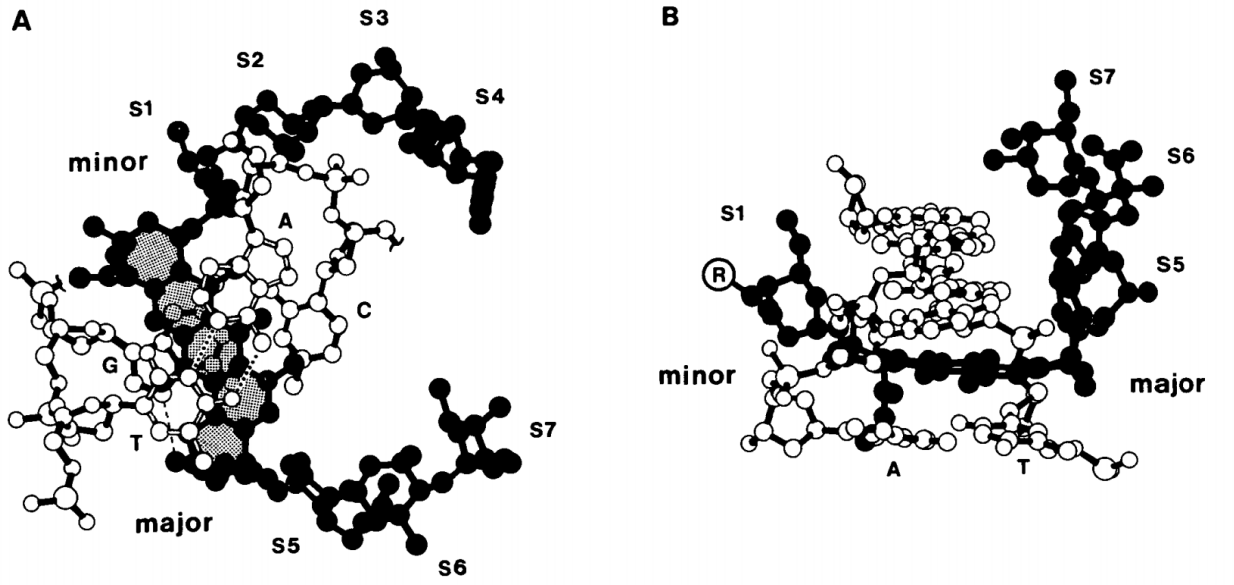


Figure 21. A) Interaction of arugomycin with d(CA).d(TG) and **B)** and with d(GCA).d(TGC). In **A)** the tetrasaccharide fragment is pointing up and archs to the left, while in **B)** the tetrasaccharide is pointing into the page[61].

Chapter 2: Synthesis of Monomers

2.1 Cyclopropenium Cation Promoted Dehydrative Glycosylation

In recent years, the Bennett lab has pioneered a new synthetic strategy for glycosidic bond formation[56]. This strategy utilizes a 1-hydroxy glycosyl donor activated *in situ* to a highly reactive donor species. First, a cyclopropene derivative with two geminal halides is generated, forming an equilibrium with the ionized species (Figure 1). Both chlorine and bromine have been used for this procedure, chlorine provided good initial results, but investigations with bromine have afforded higher yields. Upon addition of the lactol, the cyclopropenyl ether forms at the anomeric position, transforming the hydroxyl group into an excellent leaving group. A halide ion attacks the anomeric position, displacing the cyclopropenyl ether and forming the glycosyl halide. In the presence of a soluble source of iodide, such as tetrabutylammonium iodide (TBAI), the glycosyl bromide is in equilibrium with the glycosyl iodide. Figure 1 shows the favorable α-selective pathway, in which the α-bromide is generated from the cyclopropenyl ether, and the highly reactive β-iodide is generated forming the α product upon treatment with an acceptor. The α-iodide and β-iodide species are in rapid equilibrium, with the formation of the α-iodide favored. The β-iodide is the

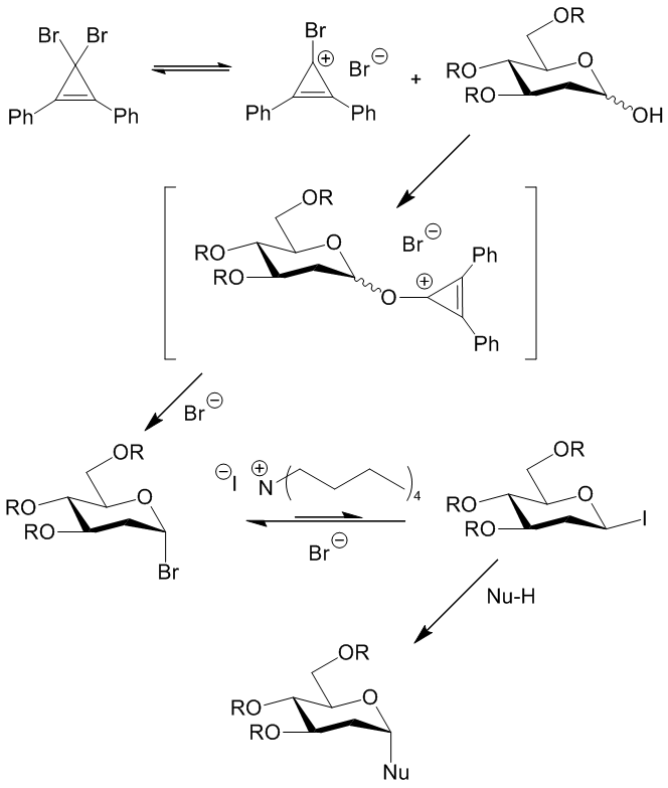


Figure 1. Proposed mechanism for the formation of α-linkages using cyclopropenium cations in dehydrative glycosylations. The formation of β-products is also observed in most cases, the mechanism is not shown.

more reactive species, and so the formation of the α-linkage is favored over the β-linkage. The results confirm that the major product is the α-glycoside[56]. Most glycosylations using this methodology have shown high degrees of α-selectivity. The reaction liberates hydrochloric acid, so an acid scavenger is added to prevent acid hydrolysis of the glycosidic bond. A commonly used acid scavenger is the bulky 2,4,6-tri-*tert*-butylpyridine (TTBP), used because it tends not to form elimination products and is easy to handle.

The utility of this methodology stems from its ability to promote lactol donors in mild conditions without the use of toxic and/or hazardous materials. Furthermore, the activation of lactols *in situ* removes the need to isolate reactive donor intermediates. Several other dehydrative glycosylation methods have been described, such as Gin's sulfide-mediated dehydrative glycosylations[53], and Roush's work using the Mitsunobu reaction[55]. Extension to 2-deoxy carbohydrates has not been successful for the sulfide-mediated reaction, and has required toxic mercury salts for the Mitsunobu reaction[56], implying that a general, powerful dehydrative method is still needed.

2.2 Synthetic Strategy 1

The tetrasaccharide fragment of arugomycin was selected as a synthetic target for several reasons: (1) arugomycin has been shown to be a biologically active natural product; it has shown antibacterial activity as well as antitumor activity[59], (2) all four glycosidic linkages in the tetrasaccharide are α-glycoside linkages, allowing for the efficient use of the cyclopropenium cation promoted dehydrative glycosylation protocol, (3) all four saccharides are 2-deoxy sugars, highlighting the utility of the cyclopropenium methodology, as this oligosaccharide would be difficult to access using other dehydrative glycosylation techniques, and (4) all four carbohydrates in the tetrasaccharide can be synthesized from a common intermediate.

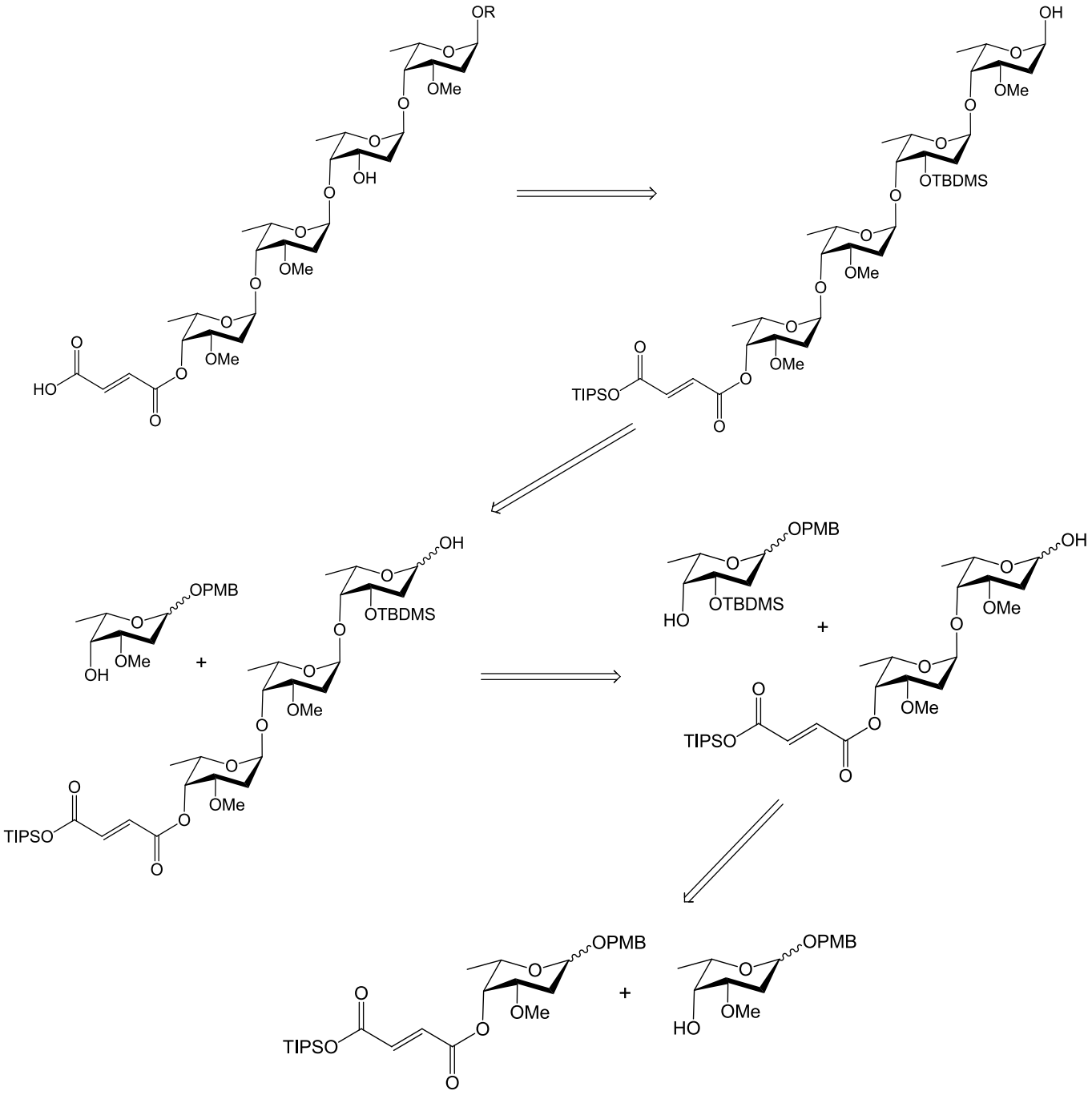


Figure 2. Retrosynthetic analysis of the tetrasaccharide fragment of arugomycin. This retrosynthesis breaks up the tetrasaccharide into the four monomers (three different monomers).

A preliminary pair of retrosynthetic analyses were developed for the tetrasaccharide fragment (Figures 2 & 3). The first analysis breaks apart the oligosaccharide into its four regioselectively protected monomers, and the second describes the synthesis of each monomer

from readily available starting material. The synthesis of all four monomers starts from $_{L}$-(-)-Fucose , as each monomer shares a common derivative (Figure 3).

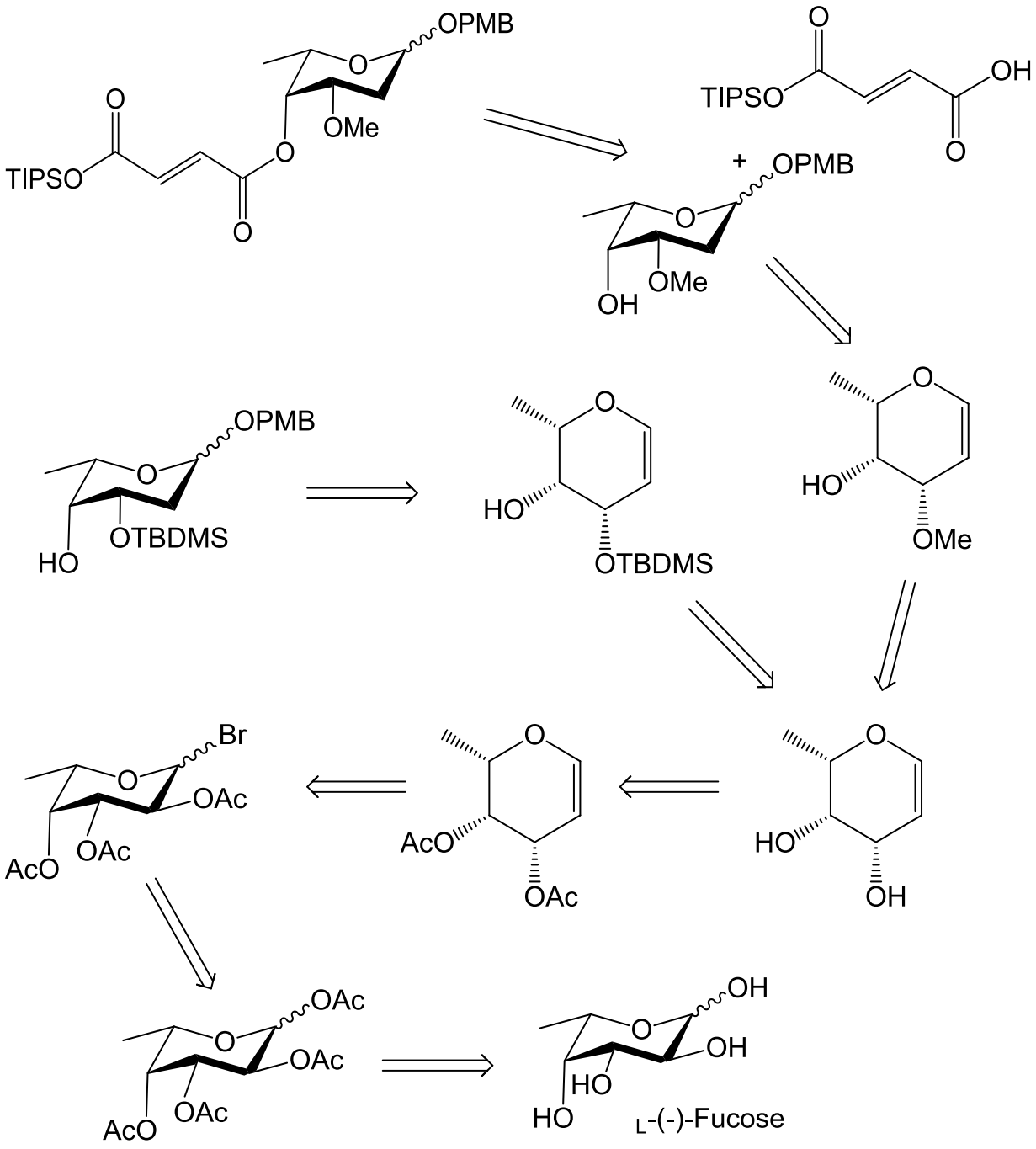
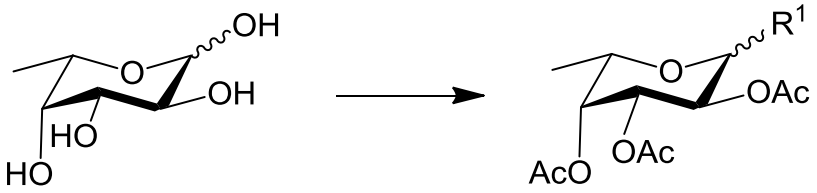


Figure 3. Retrosynthetic analysis of the monomers of the tetrasaccharide fragment of arugomycin. All three monomers have a single common derivative, which can be synthesized from $_{L}$-(-)-Fucose.

2.3 Synthesis: Part I

Before beginning the synthesis of the tetrasaccharide, each reaction in the retrosynthetic analysis was researched. The literature was searched for similar or identical reactions to determine ideal conditions for each transformation.

Many reactions involving carbohydrates are water sensitive, and it is common practice to flame dry a flask before use, removing the volatile material from the interior walls of the glassware. To further isolate the reactions from reactive elements, all reactions were conducted under an argon atmosphere to prevent moisture in the air from entering the vessel.



L-(-)-Fucose

1 R^1=OAc
2 R^1=Br

Figure 4. Conditions: Fucose to **1**, pyridine, acetic anhydride, 25°C, 12h, **1** to **2,** acetic acid, 33% HBr in acetic acid, 25°C, 20 min, ~75% over two steps

The first transformation involves the addition of four acetyl ester protecting groups on Fucose. This is a common reaction, and has been performed in our laboratory prior to this synthesis. Using a precedent from our lab, Fucose was mixed with acetic anhydride (1.15mL per mmol of Fucose) and pyridine (1.65mL per mmol) and stirred at room temperature under Argon overnight. The pyridine deprotonates hydroxyl groups, increasing the nucleophilicity of the oxygen atom. The alkoxide attacks the carbonyl carbon of a pyridine-acetic anhydride intermediate, displacing the pyridine in the process (Appendix, Figure 1). Upon completion, the solvent was evacuated under vacuum, and the resulting material was washed several times with toluene to remove excess pyridine. Proton nucluear magnetic resonance (^{1}H NMR) spectroscopy was performed, revealing the desired product **1** alongside several impurities.

Initially, it was thought that the acetylation had failed to occur cleanly, and the reaction was performed again with the same results. The crude product was exposed to bromination conditions and pure bromide **2** was obtained in good yield.

The bromination of crude **1** was carried out according to precedent[63], in which the tetraacetate **1** was dissolved in a minimal amount of glacial acetic acid (0.65mL/mmol) and then treated with three portions of 33% hydrobromic acid (HBr) in acetic acid (0.325mL/mmol for each portion) over the course of 5min. The reaction was then allowed to proceed for 15min without being monitored by thin layer chromatography (TLC). After the allotted time, the reaction mixture was added to dichloromethane and washed with water, neutralized with sodium bicarbonate ($NaHCO_3$), dried with sodium sulfate (Na_2SO_4), filtered and condensed under vacuum, affording pure bromide **2** in 75% yield over two steps. The acid protonates the ester oxygen on the anomeric acetyl group, allowing the acetic acid to act as a leaving group, forming the oxocarbenium cation. The bromide anion attacks the oxocarbenium species, forming the bromide (Appendix, Figure 2).

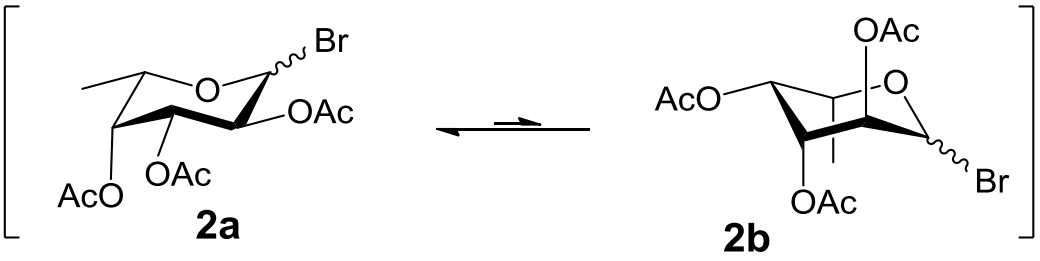


Figure 5. The equilibrium between the chair conformations of bromide **2**. The conformation in which more substituents are equatorial (**2a**) is more stable, and more prevalent, than the conformation in which the majority of substituents are axial (**2b**).

The diacetyl fucal **3** was prepared as reported in the literature using Fischer-Zach conditions[64]. The bromide **2** was dissolved in ethyl acetate (0.85mL/mmol) and added dropwise, over the span of 1.5h to a refluxing solution of ethyl acetate (3.45mL/mmol) with pre-washed Zinc dust (washed with 10% HCl, then water, acetone, and ether in a Büchener funnel) and 1-methylimidazole (1eq.). Upon completion (~2h), the mixture was allowed to reach room

temperature, filtered through a column of celite, washed with 10% HCl solution, neutralized with $NaHCO_3$, dried with Na_2SO_4, filtered, and condensed under vacuum to afford a fluid yellow oil in 90% yield. Initially, a recrystalization was attempted using methanol and water, but the yields were low, and the product **3** was taken on crude to the next step with good results.

The mechanism for the Fischer-Zach reaction involves a single electron transfer from the zinc metal to the anomeric carbon, forming radical anion species **3a** (Figure 6). The bromide anion leaves, forming the radical species **3b**, to which another electron is transferred to form carbanion **3c**. An elimination occurs, in which the acetate group leaves, forming fucal **3**. The nitrogenous base, in this case 1-methylimidazole, functions to coordinatively stabilize an intermediate, probably the reactive zinc species. Without the N-base, the reaction does not proceed[64]. The mechanism was elucidated by Somsák et. al in 2006[65].

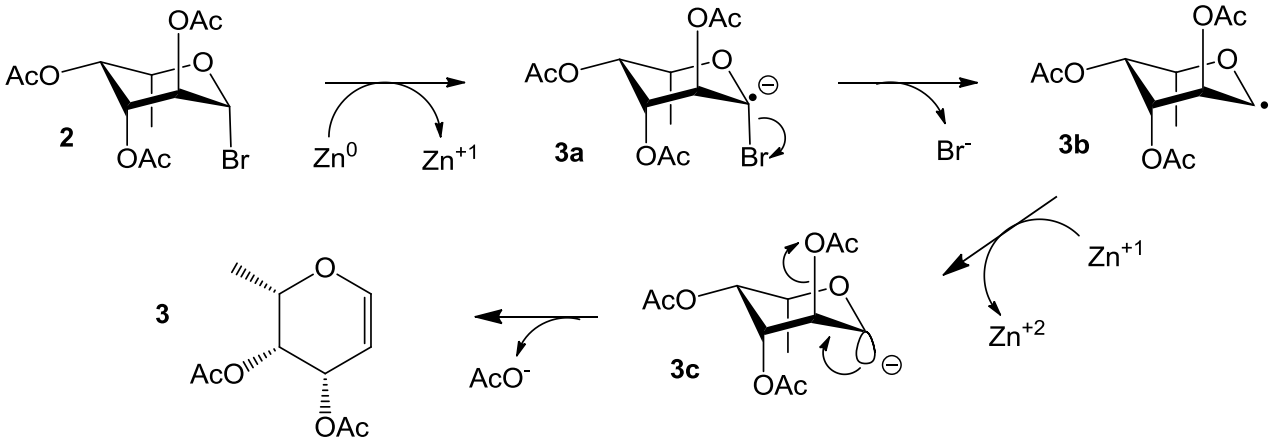
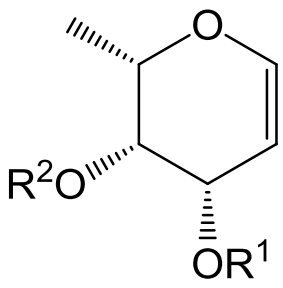


Figure 6. Fischer-Zach mechanism.

Next, a deacetylation reaction is required. This reaction has previously been performed in our lab using two different procedures. The first is a methanolysis, in which a ~0.25M sodium methoxide solution is freshly prepared and added to the acetate. The other procedure involves dissolving the acetate in methanol and adding a catalytic amount of potassium carbonate (K_2CO_3). The latter method was applied for this synthesis. The acetylated fucal **3** was dissolved in methanol (0.515mL/mmol), to which 0.1 equivalents of K_2CO_3 was added, and the reaction

was stirred at 25°C for 3h. The solvent was evaporated under vacuum, affording the fucal **4** in quantitative yield (Figure 7). The mechanism involves attack by the methoxide anion at the carbonyl center of the acetate ester, which then leaves the carbohydrate molecule as methyl acetate (Appendix, Figure 3).

From the initial retrosynthetic analysis of the monomers (Figure 3), the common intermediate is **4**. The next step in the synthesis depends on which monomer is desired. Two of the three monomers require a regioselective methylation, making that the logical next step in the synthesis.



3 R^1 = Ac R^2 = Ac
4 R^1 = H R^2 = H
5 R^1 = Me R^2 = H

Figure 7. Transformations of the fucal.

2.4 Methylation of the Fucal

After synthesis of **4**, a regioselective methylation of the fucal was necessary. The C-3 hydroxyl needed to be converted to a methyl ether in the presence of unprotected hydroxyl C-4. The use of organotin derivatives to regioselectively alkylate diols in nucleoside derivatives was first described by Moffatt and coworkers in 1974[66], and the procedure was later extended to six-membered ring *cis*-1,2-diols (Figure 8)[67]. Results from several other experiments have confirmed that the equatorial hydroxyl is preferentially alkylated (with rare exceptions[68]).

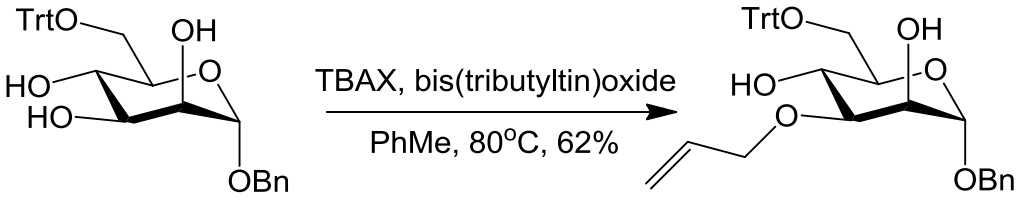


Figure 8. Equatorial allylation using organotin derivatives, where X is a halide.

Most regioselective alkylations were performed on saturated six-membered ring systems, but there is precedent for a regioselective methylation of a glycal[69] (Figure 9). The glycal used by Ogihara et al. is not identical to the fucal in our scheme, but both have one axial

and one equatorial hydroxyl group, which is thought to be the minimum requirement for obtaining regioselectivity when using organotin derivatives.

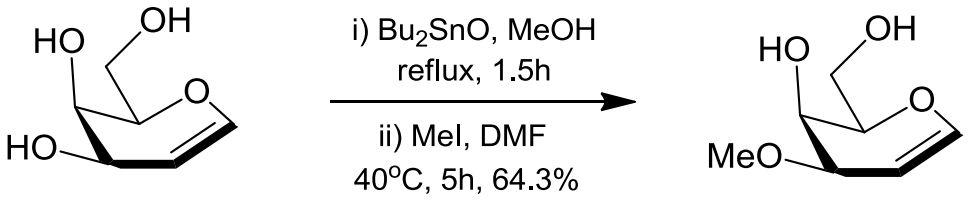


Figure 9. Equatorial methylation using organotin.

It is not clear why reactions using organotin derivatives are regioselective, but it has been proposed that a dimeric stannylene structure forms. Tin occupies the center of a trigonal bipyramidal arrangement with the butyl groups in the equatorial positions (Figure 10)[70]. The less electronegative oxygen is coordinated to two tin atoms, while the more electronegative oxygen is at the apical position and is coordinated to only one tin atom, making it the more reactive of the two.

The procedure from the literature was carried out with minor modification. Fucal **4** was dissolved in methanol (8.9mL/mmol), dibutyltin oxide was added (1.07eq.), and the mixture was stirred for 1.5h at reflux. The solvent was evaporated under vacuum, the resulting solid was dissolved in DMF (6.2mL/mmol) and iodomethane (7.5eq.) was added. The solution was stirred at 40^{o}C for two days in a reflux condenser to prevent iodomethane from escaping. Upon completion, the solution was allowed to reach room temperature, added to ethyl acetate, washed with water, sodium hydroxide (NaOH), and brine. The original procedure passes the solution through a column of hydroxide exchange resin (Amberlite IR-410 OH^{-}) to remove the tin. The sodium hydroxide wash was designed for the same purpose in the absence of hydroxide resin. The organic layer was dried with Na_2SO_4, filtered and condensed under vacuum. After purification using flash chromatography (40% ethyl acetate in hexanes), a small amount of **5** was obtained, with a yield of 10%.

Several other unsuccessful attempts were made to synthesize **5** using variations on the procedure outline above, and the results are summarized in Table 1. It was hypothesized that after formation of the tin acetal in entry 1, the methanol was not fully evaporated, allowing coordination to the tin and disrupting the stannylene dimer (Figure 10). In entry 2, the methanolic solution was allowed to dry overnight under high vacuum to remove all of the methanol. All of the starting material disappeared, but the reaction did not produce **5**, and it was hypothesized that over-methylation may have occurred. The amount of methyl iodide was reduced in entry 3, and a small amount of product **5** was observed.

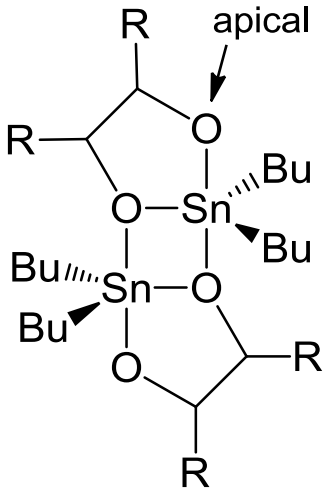


Figure 10. Proposed trigonal bipyramidal structure of stannylene dimer.

Tin Acetal Formation					*Methylation*				
Entry	**Solvent**	**Bu_2SnO**	**Time**	**Temp.**	**Solvent**	**MeI**	**Time**	**Temp.**	**Yield**
1	MeOH	1.0	1.25h	75	DMF	11.25	4d	40	0
2	MeOH	1.0	2.50h	75	DMF	7.5	2d	40	0
3	MeOH	1.07	1.50h	75	DMF	2.0	2d	40	10
4	Benzene	1.1	1week	86	Benzene	2.0	3d	106	N.R.
5	Toluene	1.1	1d	120	DMF	2.0	1d	110	Deg
6	Toluene	1.1	1d	120	DMF	2.0	1h	120	Deg
7	Toluene	1.1	1d	120	DMF	2.0	1h	120	Deg
8	Toluene	1.1	1d	120	DMF	2.0	1h	120	Deg

Table 1. Attempts to form the methyl ether **5** from fucal **4**. Temperatures are reported using the Celsius scale.

It was hypothesized that after tin acetal formation methanol remained in the reaction, disrupting the activation of the equatorial hydroxyl group over the axial hydroxyl group. The procedure was modified based on the literature, in which benzene is used for both the tin acetal formation and the alkylation steps[71]. Fucal **4** was dissolved in benzene and the reaction was

conducted in a Dean-Stark apparatus for continuous removal of water. The fucal was not very soluble in benzene, and after several days TLC revealed that the mixture was mostly starting material. Toluene was used when forming the tin acetal in entries 5 through 8, followed by solvent evaporation and addition of DMF for the methylation step. The reaction proceeded quickly, requiring only one hour for complete disappearance of starting material. TLC was performed with the same eluent in entries 1-8 so that direct comparison was possible, with entry 3 as reference. In entries 6-8, TLC appeared to indicate that a high-yielding reaction was occurring, but after removal of the solvent (DMF) under vacuum and elevated temperature, the crude product degraded. After alkylation in entry 8, the reaction mixture was passed through a column of hydroxide exchange resin (Amberlite IRA 402), but the degradation still occurred upon removal of DMF. Removing solvent under vacuum increases the concentration of all species, and if done while heating degradation may occur. Other methods for removing DMF under milder conditions were suggested but not explored.

2.5 Synthetic Strategy 2

Following failure to produce methylated fucal **5** using the original synthetic strategy, a new strategy was employed (Figure 11). Considering the greater literature presence of saturated ring systems for regioselective alkylations with organotin, it was proposed that the addition of *para*-methoxybenzyl alcohol (PMBOH) to the fucal should occur prior to the regioselective alkylation.

It was theorized that the unsaturated ring system forces a smaller angle between the *cis*-1,2-hydroxyls, potentially destabilizing the stannylene dimer (Figure 10). If the ring system is saturated during methylation, the angle between hydroxyls would increase, restabilizing the stannelyne dimer and restoring the regioselectivity of the reaction. It is also possible that the saturated system is more stable than the fucal and would not undergo degradation upon DMF removal.

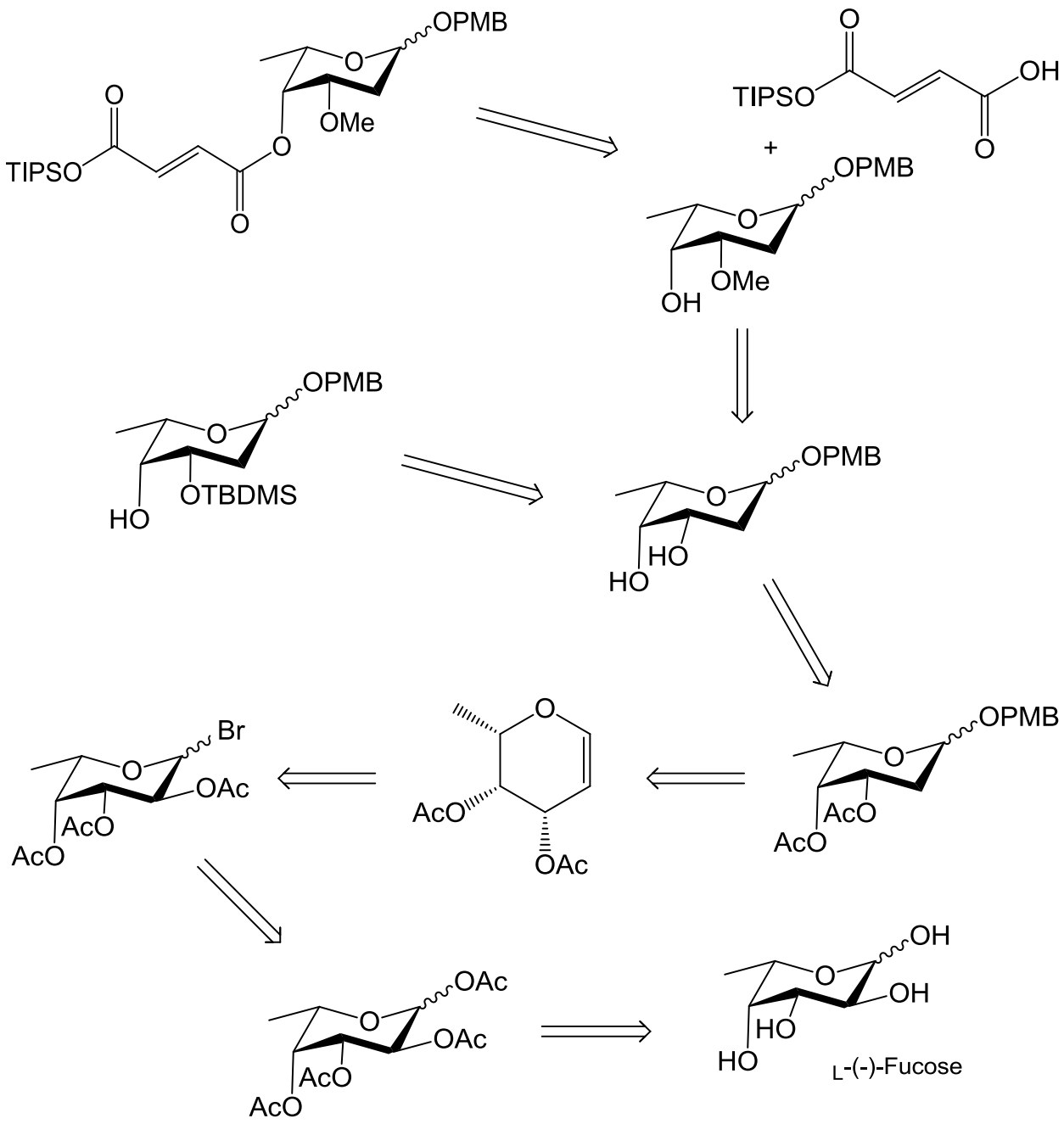


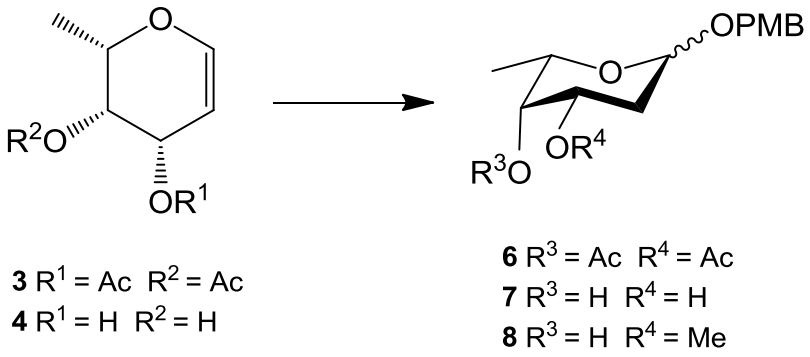
Figure 11. Alternative retrosynthetic analysis of the monomers of the tetrasaccharide fragment of arugomycin. All three monomers have a single common derivative, which can be synthesized from L-(-)-Fucose.

2.6 Synthesis: Part II

Some fucal **4** remained from Part I of the synthesis, and it was dissolved in anhydrous dichloromethane (DCM, 6.5mL/mmol). According to precedent[72], the solution was treated with 3 equivalents of 4-methoxybenzyl alcohol and 5 mol % triphenylphosphine hydrobromide

(TPP•HBr) for 3h at 25°C. TPP•HBr provides a catalytic amount of acid, allowing the alcohol to add to the oxocarbenium cation (Appendix, Figure 4). The fucal was not adequately soluble in DCM, so the starting material **4** was recovered, purified, and subjected to the same conditions using tetrahydrafuran (THF, 6.5mL/mmol) as the solvent. The fucal wasn't soluble in THF either, and the reaction yielded no product.

It was found that the acetylated fucal **3** could be subjected to similar conditions with better results[73]. The fucal **3** was dissolved in dichloromethane (5mL/mmol), to which 5 mol % TPP•HBr and 3 equivalents of PMBOH were added. The alcohol was added at 0°C and the reaction was allowed to reach 25°C overnight. After completion the crude product was purified using flash chromatography (25% ethyl acetate in hexanes) yielding 62% pure **6**.



3 R^1 = Ac R^2 = Ac
4 R^1 = H R^2 = H

6 R^3 = Ac R^4 = Ac
7 R^3 = H R^4 = H
8 R^3 = H R^4 = Me

Figure 12. Several transformations in Part II of the synthesis.

Acetylated 2-deoxy fucose **6** was deprotected using the same procedure as **3** → **4**. After completion, the crude product was purified using flash chromatography (FC, 25% ethyl acetate in hexanes), yielding 80.6% pure **7** (for mechanism see Appendix, Figure 3).

A methylation of **7** was carried out with the same conditions as entry 8 in Table 1, except that the methylation was performed in toluene, not DMF. Upon purification, no **8** was detected by ^{1}H NMR in any of the fractions. A different set of conditions[74] was used in which **7** was dissolved in excess benzene (50mL/mmol), to which dibutyltin oxide (1.2eq.) was added and the mixture was stirred for 12h under reflux. Overnight, the solvent had evaporated, and the

solid was redissolved in half as much benzene, to which 15 equivalents of iodomethane and 0.5 equivalents of tetrabutylammonium iodide (TBAI) were added. The mixture was stirred for three days under reflux, more benzene and another 15 equivalents of MeI were added. The mixture was stirred for another 2d, after which it was cooled, and purified (FC, 40% ethyl acetate in hexanes) to afford **8** in 42% yield.

2.7 Conclusion

The synthesis of the tetrasaccharide fragment of arugomycin was not completed. The synthesis of the common derivative **7** of all four monomers was completed in five steps. Difficulties with the original synthetic route involving the regioselective methylation of fucal diol were encountered, which significantly delayed the project. Eventually, the regioselective methylation of 1-(4-methoxyphenoxy)-2,6-dideox -fucopyranoside was completed in moderate yield.

One monomer has been completed, and only one step is required to reach the other two monomers. Once the monomers are synthesized only three glycosylations remain for completion of the tetrasaccharide fragment.

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Appendix

Arrow Pushing Mechanisms

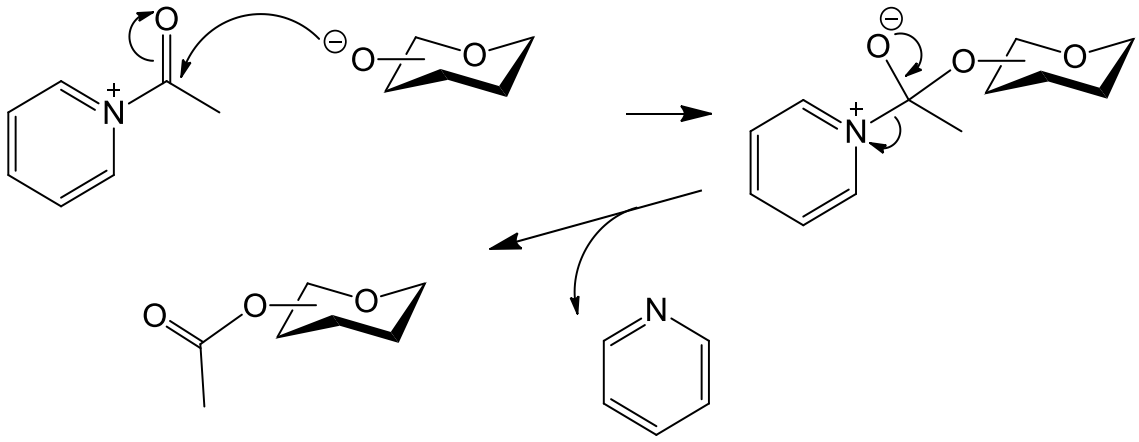

Figure 1. Mechanism for the formation of acetyl ester protecting groups. The deprotonation step is not shown.

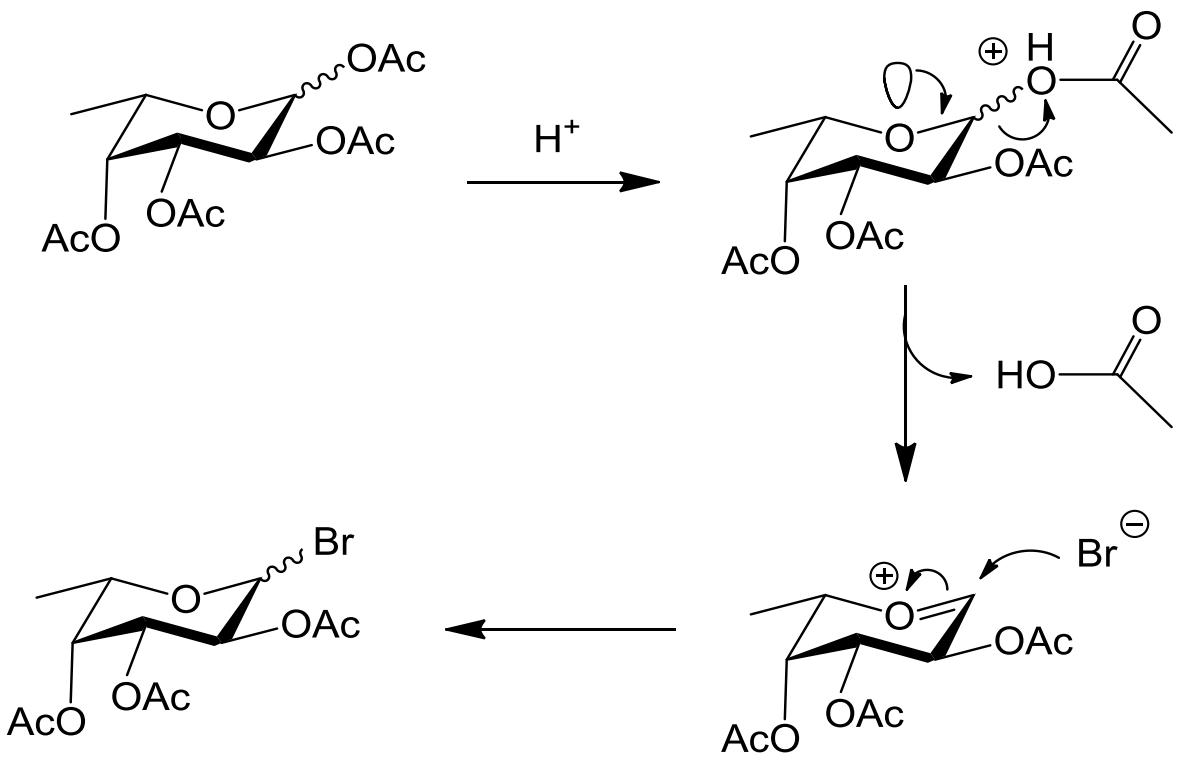

Figure 2. Mechanism for the formation of the bromide **2** from the tetraacetate **1**.

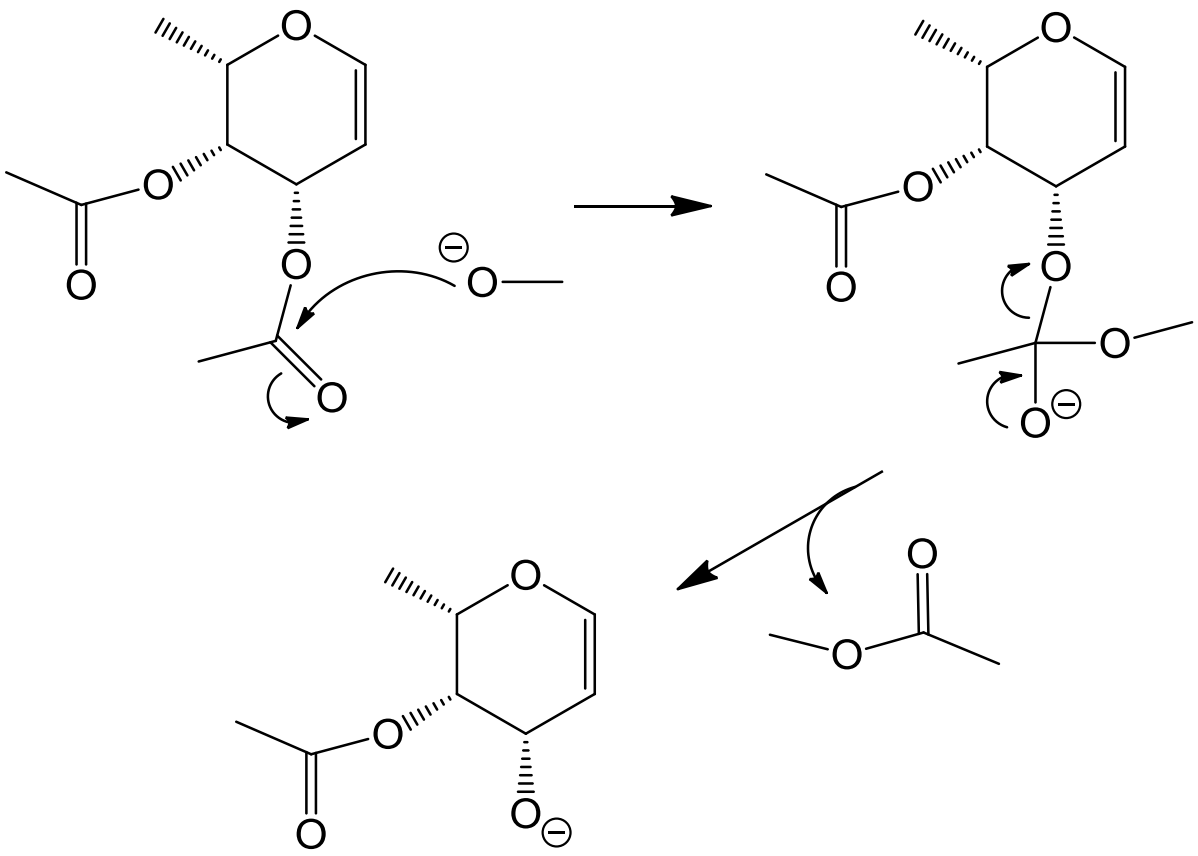

Figure 3. Mechanism for methanolysis; removal of ester protecting groups.

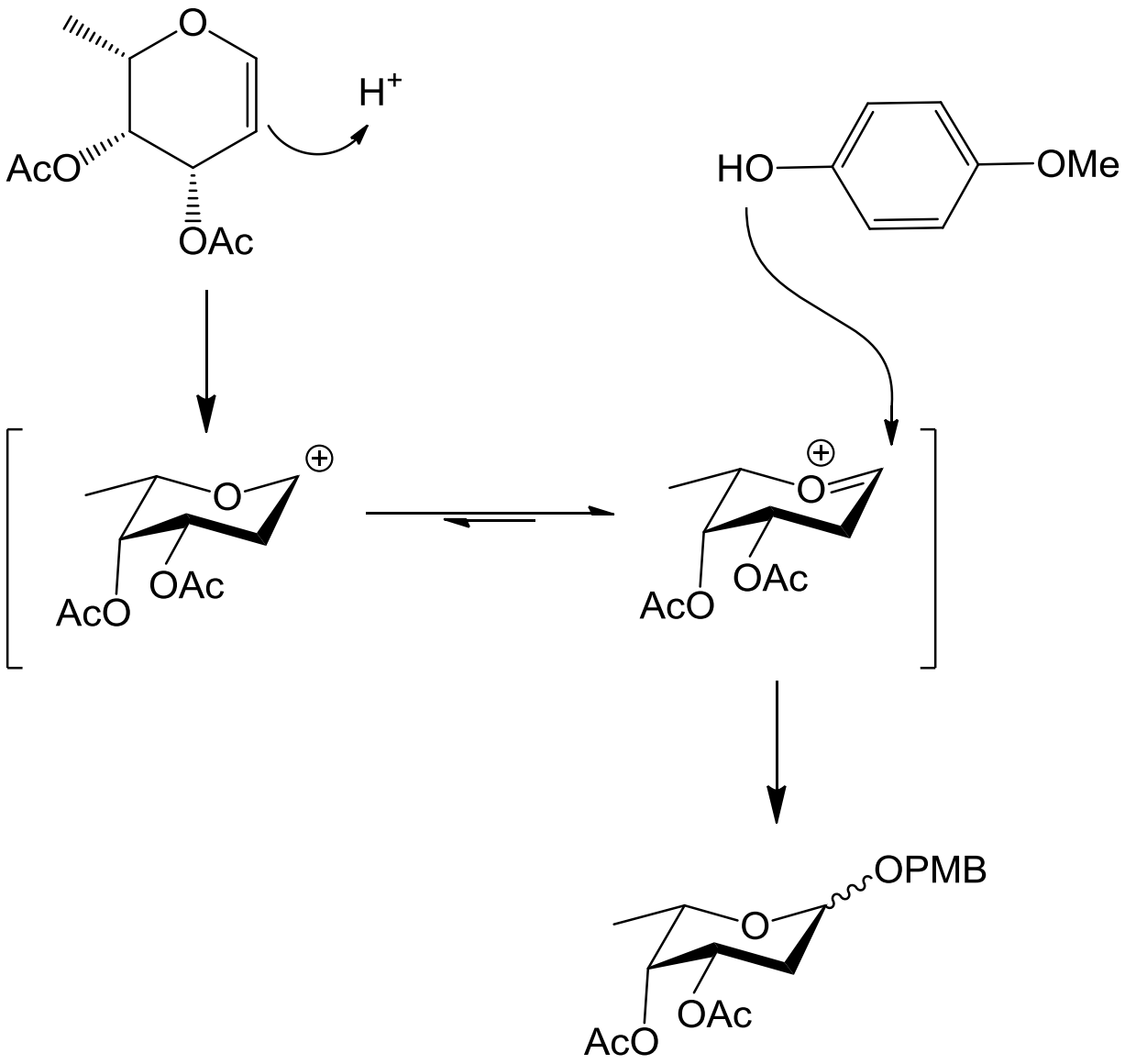

Figure 4. Mechanism for 4-methoxybenzyl alcohol addition to fucal. The acid is catalytic because a proton is released after the alcohol adds to the oxocarbenium cation.