5-2011

The Design and Synthesis of Different α-Linked Galactose Disaccharides

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Chem 408

Carbohydrates have been at the forefront of biological research as well as chemical research for the past few decades. The role of carbohydrates was once believed to be limited to that of a storage source for energy. However as technology progressed and our understanding of biology increased, the scientific community quickly learned that carbohydrates play a very complex and vital role in cellular recognition and homeostasis.

Our current understanding of carbohydrates tells us that cells use mono- and oligosaccharides as a means of recognition and to specify a function to a protein through post-translational modification (PTM). Carbohydrates that are attached to a macromolecule take on a more specified name such as glycolipid or glycoprotein. In the case of glycolipids, a monosaccharide can either be attached to a phospholipid or a sphingolipid. Glycolipids are currently being investigated for their role in lipid rafts, which are regions of membranes that contains a higher concentration of kinases.[1]

Glycoproteins are proteins that are modified by enzymes that attach either N- or O-linked carbohydrates. The N or O signifies that the carbohydrate is either attached to an asparagine (Asn) or serine/threonine (Ser/Thr) amino acid on the protein chain, respectively. (See **1** and **2** in Figure 1)
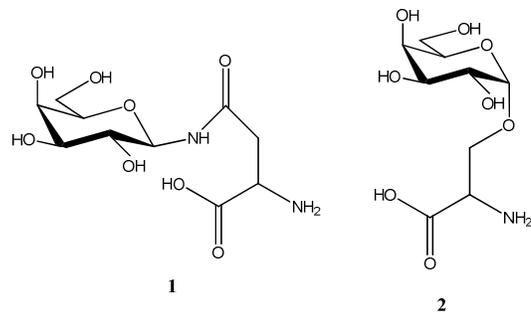
Figure 1. N-linked galactose and O-linked galactose

The recognition of carbohydrates is mediated either through the cooperative binding of a protein and the coordination of a metal cation, through direct hydrogen bonding, or through water-mediated hydrogen bonding. The protein that recognizes a carbohydrate motif is known as a lectin and is categorized by its mechanism of recognition. For example, a lectin that mediates recognition through coordination of a calcium ion is known as a C-type lectin. The ultimate goal of this research is learn more about C-type lectins and the binding events between the lectin and the carbohydrate.[2]

The research that is being presented in this manuscript is the design of several different α-galactose disaccharides. These galactose disaccharides will be used as controls in the current study being performed on C-lectins. Galactose is used as a negative control in this project since the recognition of carbohydrates by this class of lectins is mediated by the coordination of a calcium ion between

the carbohydrate's equatorial hydroxyl groups present on the 3rd and 4th carbon

atoms. Galactose's hydroxy group that is present on the 4th carbon is axial, which

means that it is unable to coordinate the calcium ion, so it is not recognized by C-
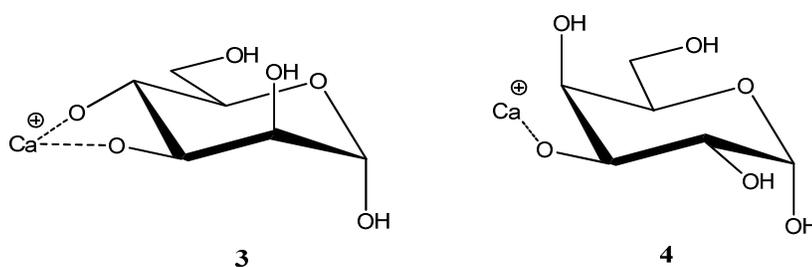
lectins (Figure 2).

Figure 2. Calcium coordinated between two equatorial hydroxy groups (**3**)
and calcium coordinated with galactose (**4**)

The major extrinsic factor in every project is time, and to help increase the

efficiency in using this scarce resource the synthetic design would have to

maximize overlap between different accepting carbohydrate motifs as well as

limiting the number of synthetic steps. Since the main focus of the project is the

understanding of a significant biological event, the design of the synthesis

focused on finding established reactions that would allow for maximum yield

and limit the number of synthetic steps used.

The most important step, around which the synthetic scheme was designed, was the glycosylation step in which the donor and acceptor would be combined to yield a disaccharide. The glycosylation step is notorious for its low yield and difficulty in controlling the production of anomeric diastereomers. The two anomers that can be produced (α and β) are fundamentally different in their geometry. The α-linkage is an axial connection between the carbohydrate units. This leads to one sugar motif sitting on top of the other. The β-linkage is a connection that is equatorial, meaning that the two carbohydrates lie within the same plane. This may seem like a small difference on paper, but when a three-dimensional object is introduced to the environment, the differences between the two structures become more apparent.
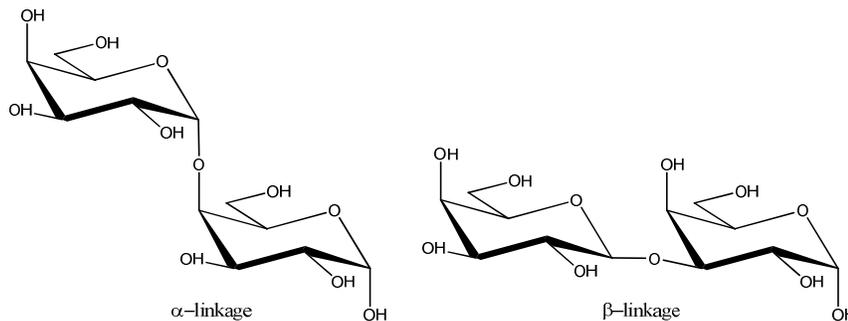

Figure 3. α-Linked and β-linked galactose disaccharides

Design of the Donor

Two major classes of functional groups or leaving groups have been designed to allow for the reaction to be diastereoselective. The first class is a halide atom at the anomeric center (see below). The halide allows for diastereoselectivity based on the strength of the bond between the anomeric carbon and the halide. As the bond between the carbon and the halide lengthens and weakens, the selectivity for the α-connection increases (Figure 4).
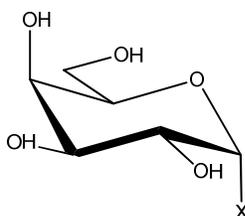

Figure 4. *α*-Galactopyranosyl halide

The reaction mechanism for the halide glycosylation helps explain why selectivity changes with the size of the halide. From the anomeric effect, we know that the oxygen with the ring is able to interact with the anti-bonding orbital of the carbon-halide bond. This interaction causes the halide to leave and form an ionic complex. The halide is quickly able to perform a nucleophilic addition to remake the α-compound **1a,** or if another nucleophile is present it can perform a nuleophilic addition to create the β-compound **1c**. The β-compound **1c** is less stable because it is unable to participate in the anomeric effect. However the β-compound **1c** is able to participate in the exo-anomeric effect. The

exo-anomeric effect occures when the lone pair of the nucleophile interacts with

the anti-bonding orbital of the anomeric carbon and the oxygen.[3] This causes the

oxygen carbon bond to lengthen. This reaction is run in an excess of the halide

ion so that compound **1c** becomes an abundant intermediate. When the acceptor

attacks the compound **1c** it forms the α-compound **1f**, and the halide is unable to

displace the acceptor because the compound **1c** is less stable. As the size of the

halide increases, the selectivity of the diastereomers increases as well (fluorine

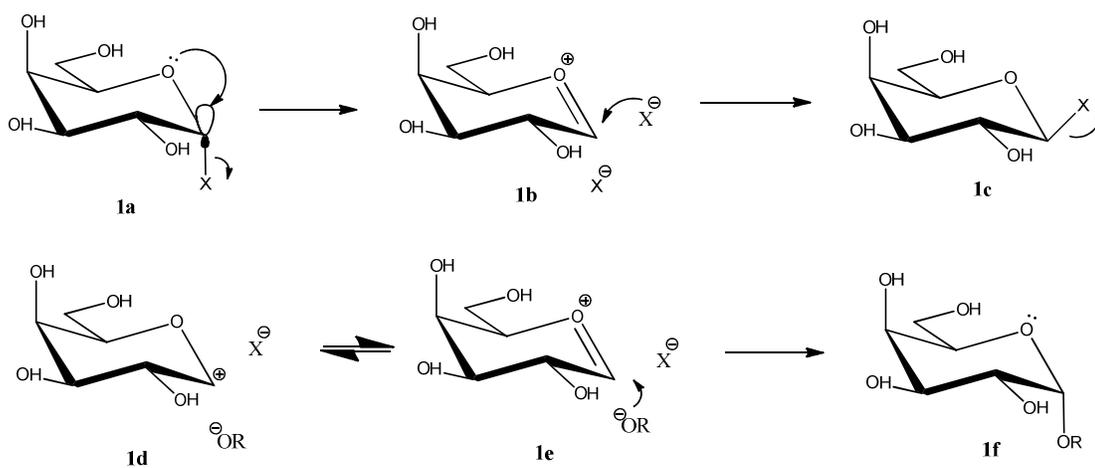mainly β, iodine only α).

Mechanism 1

Figure 5. Halide mechanism

The second class of functional groups can be generalized as reactions that

cause an S$_N$2 type reaction which causes an inversion of the stereocenter. This

class of leaving groups, promotes an S$_N$2 type reaction by causing an interaction

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with the leaving group which makes the leaving group unstable. The acceptor

displaces the leaving group, which allows the leaving group to stabilize while

also creating a more stable carbohydrate compound. For these types of

functional groups, it is imperative to insure the correct diastereomer was created

before the glycosylation step. If one uses a mixture of α and β donors, then one

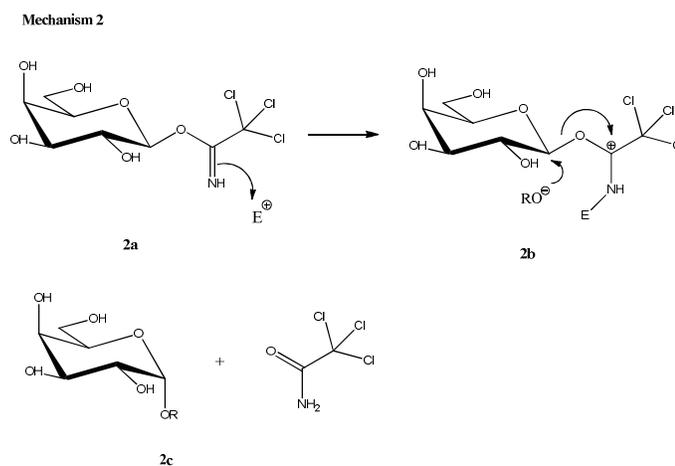will obtain a mixture of α- and β-linked disaccharides[4].

Figure 6. Mechanism of inverting the stereocenter

The interaction of the protecting groups with the anomeric carbon can

negate the design of the donor. If an ester protecting group is used to protect the

C-2 oxygen, then the carbonyl oxygen is able to add to the anomeric carbon

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blocking the addition of a nucleophile to the cis conformation relative to the C-2

oxygen. Then the trans product will be dominant. The interaction with the

neighboring group is faster than the interaction with the nucleophile. The

nucleophile is limited to traveling no faster than the rate of diffusion, while the

reaction rate of the neighboring group is determined by the rate of rotation. Since

galactose has an equatorial hydroxyl group at C-2, neighboring-group

participation will cause the trans- product to form which would be an equatorial

attachment at the anomeric carbon.
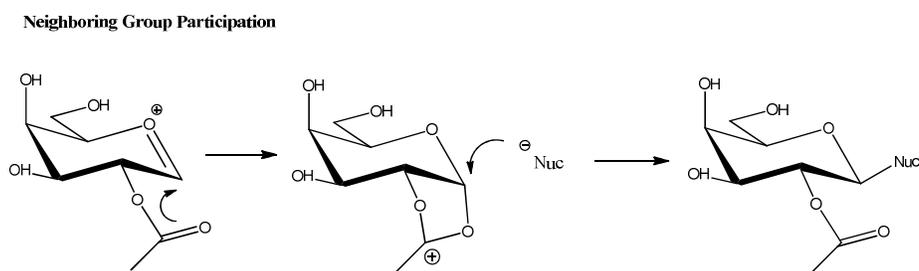
Neighboring Group Participation

Figure 7. Neighboring group participation

Another commonly used approach for protecting the alcohol functional

group is using a benzyl ether protecting group. The benzyl ether protecting

group does not take part in neighboring-group participation, so it is frequently

used for reactions in which the α-compound is desired. The problem that we a

into with this form of protecting group is that the usual way to remove a benzyl

group is hydrogenolysis. Unfortunately in this case hydrogenation will react

with our other functional groups, which will destroy our product. Another common technique to remove the benzyl ether protecting group is a Birch reduction. This is performed in ammonia which may cause problems since it could interfere with our acetate protecting groups during the evaporation of ammonia.

A protecting group that is also used to protect alcohols, including the hydroxyl sugars is the trisubstituted silyl moiety. After several litature searches I was able to find several papers on synthesis that used glycosyl iodide donors together with silyl protecting groups. Trimethylsilyl (TMS) will not interact with the neighboring anomeric carbon, and it can be removed with an acidic ion-exchange resin. Another benefit of the glycosyl iodine donor being protected by TMS seems to be a decrease in the reactivity of the glycosyl donor. The other form of glycosyl iodide donor is one in which the benzyl-protecting group is used. Only one other research group has reported using the benzyl protected glycosyl iodide donor in glycosylation reactions. The glycosyl iodide donor protected by TMS is that which we chose to use for our reactions.
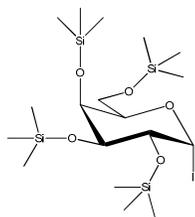
Figure 8. TMS-protected glycosyl iodide donor

Design of the Acceptor

One major obstacle faced when designing the acceptor for this research

project was the available time to adequately research the problem. Since I would

only have a few months to work on this project, the four galactose acceptors

would need to be synthesized preferably by a convergent synthesis, and the

reaction processes should be high yielding and as regiospecfic as possible.

Synthesis of a C-6 free hydroxy compound
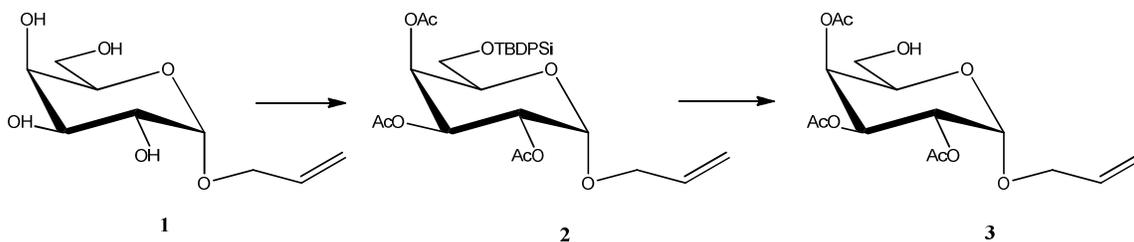
Scheme 1. Synthesis of allyl – *O* - 2,3,4- tri – *O* - acetyl-α-D-galactopyranoside

Compound **1** reacts with *tert*-butylchlorodiphenylsilane (TBDPSCl)

through an S_N2 type reaction to produce a compound that has its C-6 alcohol

protected.   This compound is then reacted with acetic anhydride to yield a fully

protected galactose compound **2**. Compound **2** is then reacted with a fluoride

source to remove the TBDPS protecting group leaving us with the desired

galactose triacetate compound **3**with the C-6 alcohol unprotected.

Following our initial reference from Murata et al. we were able to produce

a galactose compound with its C-6 OH protected by TBDPS.[5] The reaction was

performed in *N,N*-dimethylformamide (DMF) with a stocichimetric amount of

imidazole as the base. The reaction was run for 5 hours, and the workup was the

addition of water followed by a separation using ethyl acetate.   However, the

residual DMF caused problems when running the chromatography column

because of poor separation. From an NMR spectrum of the major product, I was

able to interpret the spectra and determine that we had our desired product,

which TLC had shown to be dominant product. The yield, though, for this

experiment was unexpectedly low in the 40% range. The problem with this

reaction was the solvent: DMF. For the next experiment I used the same

procedure except I used dichloromethane (DCM) instead of DMF. In the workup

I used DCM to wash the reaction mixture during the separation. From this

reaction our product was obtained in a 60% yield.   This yield was an increase,

but since our initial reagent is very insoluble in DCM, the reaction could not be

run to completion. The residue left over in the flask was our initial reagents.

From another reference by Miura et al. the reaction was run in pyridine, and the

second step was the addition of acetic anhydride to give the fully protected

compound in a one-pot reaction for a 90% yield.[6] This produced a fully protected

galactose triacetate compound. I tried this reaction again by running the reaction

longer before the addition of acetic anhydride, but the desired product was not

obtained. The current reaction scheme being used can be found in the

experimental section.

The silyl protecting group is removed by fluoride, and for this reaction we

needed to find a suitable fluoride source. The initial fluoride source that we used

was from $BF_3 \bullet Et_2O$ which was recommended by the Murata et al. group. This

reaction produced the desired compound at a 30% yield, but allowed for the

collection of our starting material. Since this did not produce the desired

compound in a large enough yield, we continued to look at two different sources

of the fluoride. The most common way to remove a silyl protecting group is

tetrabutylammonium fluoride (TBAF). The problem with this reaction is it is run

under basic conditions where an acetate shift occurs which leads to a more stable

triacetate compound. From the reference Baker et al. the addition of formic acid

to the reaction will help quenched anion formation which will prevent the

acetate shift. The small scale that I was running my reaction at required

microliter amounts of formic acid, which caused some problems. The percent

yield from this reaction was 70%, but the yield would have no doubt been higher

if an excess of formic acid had not been used. For this reason I choose to use

HF•pyridine which gave us a higher percent yield of our desired product.

Synthesis of C-4 and C-2 free hydroxy compound
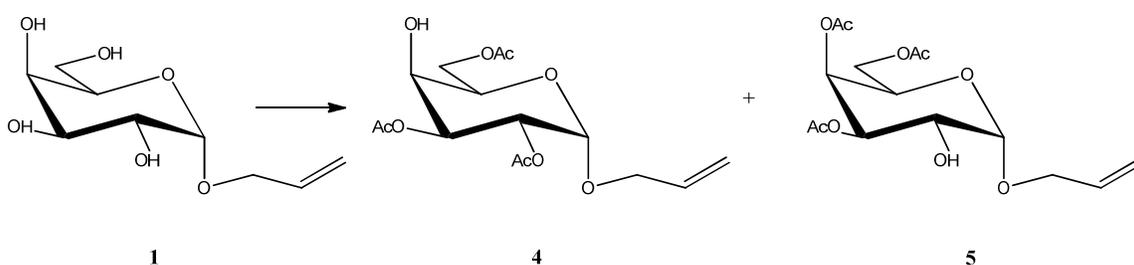
Scheme 2: Synthesis of allyl – *O* - 2,3,6-tri - O - acetyl- α-D-galactopyranoside and

allyl – O - 3,4,6 – tri – O- acetyl-α-D-galactopyranoside

Through several litature searches I was able to find a paper from Zhang et al.

that described the reaction of dibutyltin oxide with vicininal hydroxyl-type

compounds that leads to a selective acetylation.[7] This regiospecific acetylation

occurs because the carbohydrate forms a complex with the dibutyltin oxide. The

way the complex works is that the dibutyltin forms a transient five-membered

ring with two oxygen atoms (a tin acetal). When acetic anhydride is added, the

alcohol functional groups interact with acetic anhydride to from an acetate

protecting group. Depending on the temperature at which the reaction is carried out, we can form two different compounds in which the dibutyltinacetal remains intact. After the workup, one of the hydroxyl groups that had the tin compound attached is now unprotected.

The initial reference paper reacted a compound with dibutyltin oxide which was dissolved in toluene. The reaction was refluxed for several hours, and the water produced from the complexing of the dibutyltin with the carbohydrate was collected using a Newman take-off condenser. Benzoyl chloride was then added to the reaction mixture, and the reaction was allowed to run at the desired temperature. The reaction was then quenched, the solution was filtered through Celite, and the solvent was evaporated. The resulting crude product was then purified on a silica gel column. The main problems experienced with this reaction were that the final product was very difficult to isolate and the percent yield was dismal. To increase the percent yield we focused on the removal of water from the reaction flask. To do this we added molecular sieves as well as adding insulation to prevent the water from condensing before it had reached Newman take-off condenser. After several failed reactions and continued difficulty with the separation, I looked at different references to find a better procedure with which to run this reaction. The

reference that I came upon by Dong et al. was able to produce the desired

compound by using acetate instead of benzoyl protecting groups.[8] The authors

dissolved the compound and dibutyltin oxide in methanol. This was then

refluxed, the solvent was removed, and the reaction was dried on the high

vacuum rotavap. After this, the mixture was dissolved in toluene, and the

reaction was performed at a specific temperature to get the desired compound.

This new reference gave us a reasonable means of removing the water from the

reaction, and the acetylated product was much easier to purify.

Synthesis of the C-3 and C-4 free hydroxy compound
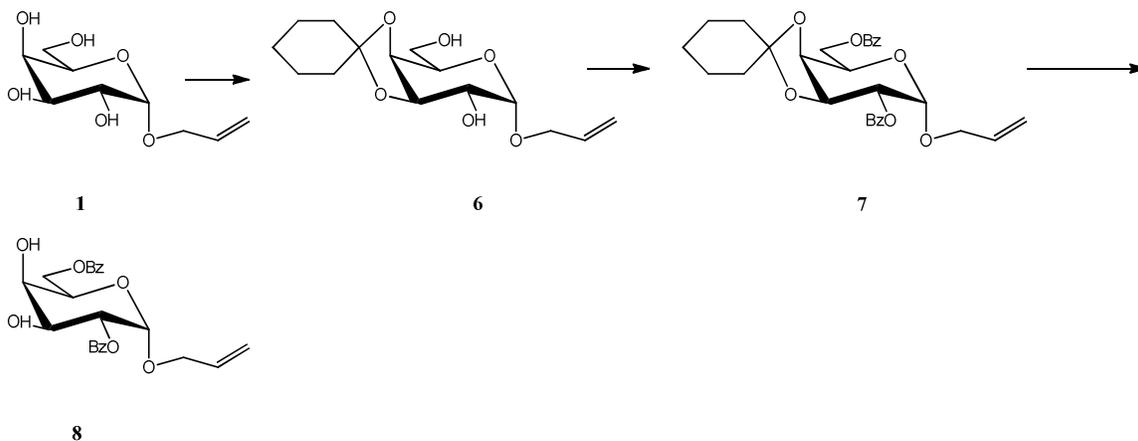
Scheme 3. C-3 and C-4 free-hydroxy compound

In this synthetic scheme we start with compound **1** and reacted this compound

with cyclohexanone with a catalytic amound of "dry" acid. According to the

Baldwin's rules, the ketone functional group prefers to form a five-membered

ring with two diols in the cis conformation. This will give the C-3 OH and C-4

OH protected compound **6** rather than its C-2 OH/C-3 OH analog since the C-2

and C-3 OHs are trans to one another. This solution is then reacted with benzoyl

chloride in a pyridine solution to give compound **7**. This fully protected

compound is then reacted with a slightly acidic solution in methanol to remove
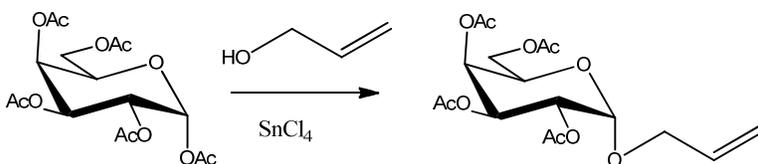
the cyclohexanone protecting group to give compound **8.**[9]

Experimental

Penta-*O*-acetyl-D-galactopyranose
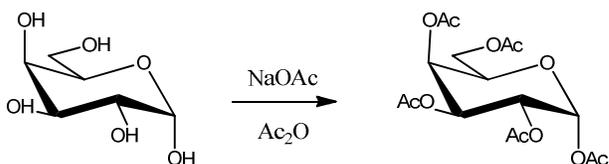
Sodium acetate (5 g, .0601 mole) was dissolved in a stirring solution of acetic anhydride (30 mL, .3005) and heated to 70°C. Dried galactose (10 g, .055 mole) was added to the solution and then it was heated to 95°C. The reaction was stirred for 18 hrs. and was quenched with the addition of a saturated solution of sodium bicarbonate. The reaction was washed with DCM and separated. The organic layer was collected and the solvent was removed under reduced pressure. Galactose pentaacetate was obtained in 98% yield (19.86 g, .0539 mole).
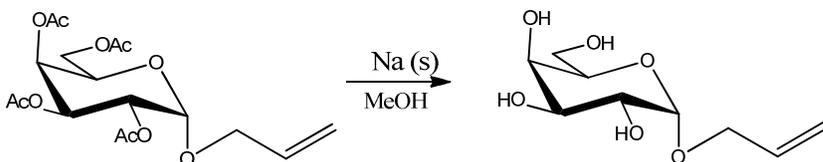
Allyl -tetra-*O*-acetyl-α-D-galactopyranoside

Dried galactose pentaaceate (5 g, .0139 mole) was dissolved in DCM (50 mL) and refluxed. Stoichiometric amounts of stannic chloride (1.63 mL, .0139 mole) and allyl alcohol (.945 mL, .0139 mole) were added to the stirring solution. The reaction was run for 20 hrs. at refluxing temperature. The reaction was quenched over ice by the addition of sodium bicarbonate. The solution was washed and separated with DCM. The organic layer was collected and the solvent was removed under reduced pressure. The desired α-D-galactopyranoside (2.7 g, 75

mmole) was obtained in a 54% yield with trace amounts of the β-D-galactopyranoside.

Allyl -α-D-galactopyranoside

Dried allyl-tetra-*O*-acetyl-α-D-galactopyranoside (2.7 g, 7.5 mmole) was dissolved in methanol (50 mL) and sodium methoxide (50 ml) was added to the stirring solution. Sodium methoxide was prepared in a separate flask by dissolving sodium metal (.5 g, 270 mmole) in methanol (50 mL). The reaction was stirred for 2 hrs. at room temperature. The reaction was quenched by the addition of prepared hydrogen ion-exchange resin. Hydrogen ion-exchange resin (1 g) was allowed to react with the solution until the pH of the solution dropped between 5 and 6. The reaction was filtered, the filtrate was collected, and the solvent was removed under reduced pressure. The desired allyl -α-D-galactopyranoside (1.6 g, 7.2 mmole) was obtained in 97% yield.

Allyl -2,3,4-tri-O-acetyl-6-*O-tert*-Butyldiphenylsilyl-α-D- galctosepyranoside
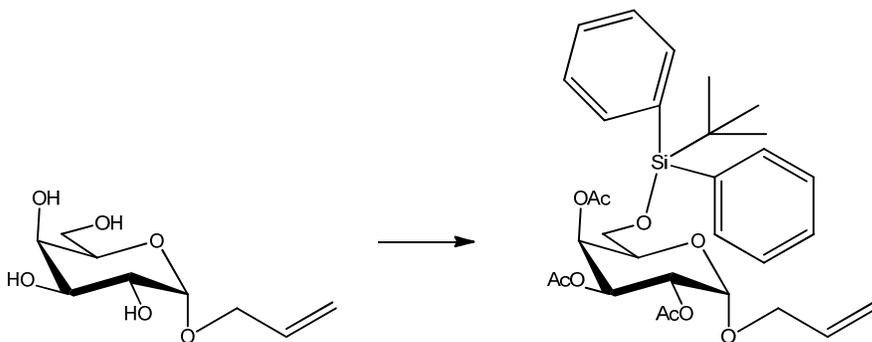
Allyl -α-D-galactopyranoside (1.6 g, 7.2 mmole) was dissolved in DMF (15 ml) at

room temperature, and stoichiometric amounts of *tert*-butylchlorodiphenylsilane

(1.89 mL, 7.2 mmole) and imidazole (.5 g, 7.2 mmole) were added to the stirring

solution. The reaction was stirred for 24 hrs and was quenched with the addition

of water. The solution was extracted with ethyl acetate, and the organic layer was

collected. The solvent was removed under reduced pressure. The dried mixture

was then dissolved in pyridine (10 mL) and acetic anhydride (2.05 mL, 21.8

mmole) was added to the stirring solution. The reaction was quenched by the

addition of methanol. The solvent was evaporated under reduced pressure,

water was added, and the mixture was extracted with DCM. The organic layer

was collected and dried, and a chromatography column was run with a solvent

system of (hexane- N-ethyl acetate 3:1).

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