Methyl Septanoside Hydrolysis as a Measure of Carbohydrate Ring Flexibility: Substrate Synthesis and Kinetics Analysis

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Methyl Septanoside Hydrolysis as a Measure of Carbohydrate Ring Flexibility: Substrate Synthesis and Kinetics Analysis

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Abstract

This thesis details the synthesis of β-D-glycero-D-guloseptanoside and the subsequent hydrolysis kinetics analysis of this compound and methyl-α-D-glycero-D-guloseptanoside, which was prepared independently of this work. The synthesis β-D-glycero-D-guloseptanoside used an established procedure. The sequence included common reactions, such as the Wittig reaction, and more advanced ones, such as ring-closing metathesis using Schrock Catalyst. Acid catalyzed hydrolysis kinetics analysis for both substrates was performed at 50°C. Reaction progress was monitored via $^1$H NMR spectroscopy. Using this data, rate constants for the reaction were calculated and determined to be $1.13 \times 10^{-4}$ s$^{-1}$ for and methyl-α-D-glycero-D-guloseptanoside and $4.82 \times 10^{-5}$ s$^{-1}$ for β-D-glycero-D-guloseptanoside. The pattern of the α anomer hydrolyzing faster than the β anomer is in reversal of what is observed for pyranose carbohydrates. With these rate constants being more than an order of magnitude faster than the rate constants of comparable, pyranose glycosides, it provides support to the argument that septanoses are more flexible than pyranoses.
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1. Introduction

1A. What are carbohydrates?

Carbohydrates are a large class of organic compounds that are crucial to a variety of biological processes, ranging from providing energy for living organisms to forming the structure of plant cell walls. Generally, a carbohydrate can be defined as a polyhydroxyl aldehyde or ketone. In other words, the compound contains a series of carbon atoms bonded to hydroxyl groups with a carbonyl group located somewhere along the carbon chain. This definition is slightly limiting, as carbohydrates can also contain heteroatoms, such as nitrogen and sulfur, and can also be connected to other moieties, such as nitrogenous bases and other carbohydrates. Because of the sheer number of functional groups present on a carbohydrate, they possess one of the highest densities of functional groups of any class of compounds and, as a result, carbohydrates can form structures ranging from the strong, linking backbone of DNA to easily broken polymers that provide easy energy.\(^1\)

Classification of carbohydrates is dependent on numerous factors, such as the number of carbons present in the molecule, the presence of an aldehyde or ketone, and the spatial orientation of the hydroxyl groups on the ring. The most basic carbohydrate is three-carbon glyceraldehyde (1), with common carbohydrates ranging from five-carbon ribose (2), found in DNA, to six-carbon glucose (3), a source of energy (Figure 1).

1, 2, and 3 are shown in Fischer projections as being a straight chain of carbons. This is true for small carbohydrates, but compounds containing five or more carbon atoms are able to enter a cyclic structure, changing 2 and 3 into 4 and 5, respectively (Figure 1).
Figure 1 – The structures of different carbohydrates in both Fischer and cyclic projections.

Figure 2 – The theoretical equilibrium of D-glucose as it is converted to and from the straight-chain form. Matching colors indicate which hydroxyl group must nucleophilically attack the aldehyde to produce the corresponding cyclic structure.
This process occurs via the reaction of one of the hydroxyl groups on the compound with the carbonyl group (Figure 3). Which hydroxyl group reacts with the carbonyl group is determined by thermodynamics. For example, three-carbon carbohydrates cannot enter cyclic structures because the steric strain is too great. Five-carbon carbohydrates are large enough to enter the cyclic structures. Six-carbon carbohydrates are able to enter both the five- (furanose) and six-membered (pyranose) cyclic structures through a straight-chain intermediate (Figure 2). However, the six-membered ring structure is, by far, the most prevalent due to its thermodynamic stability.

Figure 3 – The conversion of D-glucose in the straight-chain from into the pyranose cyclic structure via nucleophilic attack by the colored hydroxyl group on the aldehyde.

Theoretically, a carbohydrate containing six or seven carbon atoms should be able to form a seven-membered ring (septanose), in accordance to the pattern established furanose and pyranose structures. However, seven- and larger-membered rings have not been isolated in nature. This is attributed to the thermodynamic instability of such compounds, but has not yet been verified.

One of the most important features of a carbohydrate is the anomeric carbon (C1). As the only carbon atom bonded to two oxygen atoms, it is unique among all other carbons in the compound and possesses a number of properties that are dependent on its position in the ring. As shown in Figure 2, when a hydroxyl group attacks the aldehyde,
the resulting hydroxyl group at the anomeric carbon can be either axial or equatorial. In D-series sugars, these structures are labeled as α and β, respectively.

When the hydroxyl group on the anomeric carbon is replaced by another species, such as a halogen, alkoxy moiety or another sugar, the carbohydrate becomes a glycoside and the bond connecting the sugar to the functional group is called a glycosidic bond. Such bonds are very important in biological systems and whether a sugar is α or β has great influences on the properties of the compound that results when other moieties are bonded to the anomeric carbon. For example, glycogen 7 and cellulose 8 are very similar. Both compounds are polymers generated by 1,4-linking individual glucose 6 molecules (Figure 4).

![Figure 4](image-url) – Linking α-D-glucose in a 1,4 fashion produces glycogen (7). Linking β-D-glucose in the same manner produces cellulose (8). The two polymers have very different properties, even though they differ only in the manner of which one bond is oriented.

However, the simple fact that the two polymers differ only on whether the glycosidic bond is α or β has profound effects on the properties the polymers possess. Glycogen is a prominent method animal cells store energy in a form that can quickly be consumed when needed. In contrast, cellulose forms long, tough fibers that are a structural component of the cell walls of green plants and accounts for their sturdiness.
1B. Carbohydrates as Enzyme Inhibitors

As mentioned above, carbohydrates play crucial roles in a number of biological processes. One area in which carbohydrates have demonstrated therapeutic potential is in inhibition of enzymes, such as glycosidases. Many viruses that target mammals, including humans, do not carry genetic information necessary to force the infected cell to produce carbohydrates required to create the virus’ protein shell. Instead, the virus uses the cell’s natural hardware for glycosylation necessary for the virus to replicate. Inhibition of an infected cell’s glycosylation processes would slow and perhaps stop the virus from being able to replicate and infect new cells. Numerous sugar derivatives have been developed to test this concept and promising results have been found for diseases such as HIV and Hepatitis B. Another example of the effectiveness of carbohydrate-based pharmaceuticals is N-hydroxy-ethyldeoxynojirimycin, known by its trade name of Miglitol, an imino sugar. It treats non-insulin-dependent diabetes by limiting the effectiveness of enzymes in the intestinal tract that are designed to break down complex sugars, lowering the amount of glucose present after eating a meal.

With furanose and pyranose carbohydrates being very common in nature, their possible medicinal properties have been probed extensively. However, unnatural septanose carbohydrates have not been studied nearly as completely. Evidence that septanoses may be able to bind to enzymes is present in studies where a class of compounds close to

![Figure 5 - Examples of polyhydroxyazepanes that have demonstrated inhibition against HIV proteases.](image-url)
carbohydrates, polyhydroxyazepanes (Figure 5), have demonstrated effectiveness in inhibiting enzymes involved in HIV and diabetes.\(^7\)

In order to probe the effectiveness of septanose carbohydrates in enzyme inhibition, the Peczuh group has focused on the synthesis of these carbohydrates and then performed biological assays on these substrates to test for enzyme binding. The concern of this thesis is to provide possible explanations for the activity of these carbohydrates. Should septanose carbohydrates exhibit enzyme inhibitory activity, the fact that they are more or less flexible than natural substrates will be an important factor in explaining why. In order to do this, an understanding of some of the peculiarities of carbohydrates must be gained.

1C. The Anomeric Effect

Among the unusual properties possessed by the anomeric carbon is one called the anomeric effect. When first coined, the anomeric effect was the observation that carbohydrates have a larger tendency over other ring systems, such as cyclohexanes, to have the hydroxyl group on the anomeric carbon axial relative to the ring.\(^7\) Non-carbohydrate cyclic structures with substituents on the ring prefer to have the substituents be equatorial relative to the ring in order to avoid 1,3-diaxial interactions (Figure 6). For example, a cyclohexane ring

\[\text{Figure 6} - \text{The equilibrium mixtures for methylcyclohexane and chloro-4-methyltetrahydrofuran. Note that conversion from 1 to 2 requires chair flipping while conversion from 3 to 4 is accomplished via the open-chain form, allowing for the methyl group to retain its orientation.}\]
monosubstituted with a methyl group produces a mixture where 95% of the methyl groups are equatorial (10) and 5% are axial (9). However, when looking at chloro-4-methyltetrahydropuran, there is a 97:3 preference for the chloride at the anomeric carbon to be in the axial conformation (11).\(^1\)

This preference for the axial conformation can be extended to non-carbohydrate systems and the term “anomeric effect” has been expanded to encompass all such similar effects. When limiting the discussion to only carbohydrates, defining this effect as the “Edward-Lemieux effect”, after the scientists who first observed it, may be more correct,\(^9\) but for the purposes of this paper, the term “anomeric effect” will be used. Furthermore, there are a number of other effects that are related to the anomeric effect, such as the exo-anomeric effect, gauche anomeric effect and reverse anomeric effect. These concepts are outside of the purview of this paper and will be discussed only briefly.

Explanations for the anomeric effect are varied and are debated even today, despite awareness of the effect since the late 1950’s. Early explanations involved favorable dipole-dipole cancellation when the glycosidic substituent is positioned axially (Figure 7).\(^10\) Positioning the glycosidic bond equatorially results in the glycosidic bond dipole being almost perpendicular to the oxygen-lone pair dipole, resulting in very little cancellation. An axially oriented substituent fixes dipoles in opposite directions. Another explanation called for a resonance structure where a lone pair from the...
cyclic oxygen was donated into the bond connecting it to the anomeric carbon, resulting in a “double bond, no bond” interaction (Figure 7). \(^\text{10}\)

Today, the most common explanation applies molecular orbital theory. When in the axial conformation, the \(\sigma^*\) antibonding orbital is positioned in such a way as to be parallel to the “axial” p-orbital lone pair of electrons on the cyclic oxygen atom (Figure 8). This results in a pseudo \(\pi\)-bond overlap between the p-orbital and the \(\sigma^*\) antibonding orbital, allowing the lone pair of electrons to donate themselves into the antibonding orbital. This delocalizes the charge of the entire molecule and is a stabilizing effect, resulting in a net lowering of the molecules energy. This comes at the cost of weakening the glycosidic bond and is an important part of the hydrolysis of such bonds, which will be discussed later. Conversely, glycosidic bonds in the equatorial conformation have \(\sigma^*\) antibonding orbitals that do not overlap well with the “axial” p-orbital lone pair and do not enjoy the same stabilization as the their axial counterparts. The end result is that axial hydroxyl and glycosidic bonds tend to be thermodynamically favored than equatorial structures. This theory is called the antiperiplanar lone-pair hypothesis (ALPH).

![Figure 8](image)

**Figure 8** – Donation of p-orbital lone pair on cyclic oxygen into \(\sigma^*\) orbital of glycosidic bond.
1D. Acid-catalyzed Hydrolysis of Glycosides

The cleavage of glycosidic bonds is an important biologic process that is regulated by enzymes in the body. For example, glycogen is broken down into individual glucose molecules via the enzyme glycogen phosphorylase by cleavage of the 1,4-glycosidic bond. Exactly how this is done has been studied using acid-catalyzed hydrolysis. One of the earliest and most debated questions of acid-catalyzed glycoside hydrolysis was the mechanism by which the glycosidic bond is cleaved. Early work believed that bond cleavage occurred via hexose-oxygen bond fission with an intermediate carbonium ion.\textsuperscript{11} There are two possible mechanisms by which these characteristics can be accounted for (Figure 9).

![Figure 9](image)

**Figure 9** – Two mechanisms for the acid-catalyzed hydrolysis of β-methyl-D-glucoside in water.

Mechanism A protonates on the exocyclic oxygen, which then results in the glycosidic bond cleaving in an $S_N$1 fashion with nucleophilic attack by water on the intermediate carbonium ion to produce the product. Mechanism B protonates on the cyclic oxygen to open the ring, water attacks the carbonium ion to form a tetrahedral intermediate, with attack by the free alcohol to close the ring and form the product. Experiments involving the amount of inversion at the anomeric carbon and the lack of a kinetic isotope effect of the cyclic oxygen on the hexose-oxygen bond rupture indicated that Mechanism A was the most likely candidate for acid-catalyzed hydrolysis.\textsuperscript{12} The conclusion that exocyclic bond
Fission occurs was reinforced and refined via kinetic isotope studies on the acid-catalyzed hydrolysis of α and β-methyl glucopyranosides. Replacing the exocyclic oxygen with $^{18}$O resulted in kinetic isotope effects on the order of 1.026 and 1.024 (α and β), which are consistent with what would be expected for exocyclic bond fission. Furthermore, it was determined to be a primary kinetic effect, demonstrating that the protonation of the exocyclic oxygen is a key step. Subsequent studies altered the mechanism slightly to include the formation of a cyclic oxacarbenium intermediate instead of a carbonium ion (Figure 10).

The influence of the anomeric effect on acid-catalyzed hydrolysis of glycosides is a relatively recent development that, even today, is not complete. Generally, it is necessary for ALPH conditions to be present in order for glycosidic bond cleavage to proceed due to the weakening of the glycosidic bond that occurs upon lone-pair electron donation into the $\sigma^*$ orbital.

However, the presence of hydroxyl groups on normal glycosides affects the hydrolysis of glycosidic bonds to some degree and makes them poor choices for study (*vida infra*). To avoid these complications, rigid bicyclic acetals 13 and 14 have been chosen for anomeric effect studies due to the lack of hydroxyl groups. *Ab initio* studies of the acid-catalyzed hydrolysis of these substrates focusing on the energy of structural changes gleaned many insights. A reaction coordinate diagram of the hydrolysis of 13 and 14 is seen in Figure 11.
Figure 11 – Reaction coordinate diagram and conformational changes in the acid-catalyzed hydrolysis of alpha and beta-methoxy tetrahydropyrans. Credit to Deslongchamps, P. *The Anomeric Effect and Associated Stereoelectronic Effects*; Thatcher, G.R., Ed.; ACS Symposium Series 539; American Chemical Society; Washington, DC, 1993.
As expected, the ground state unprotonated axial anomer is more stable than the equivalent ground state equatorial anomer by an amount of .45kcal/mol. Upon protonation, both the α and β anomers increase in energy. The α glycoside is then able to change into a twist-boat conformation 79 that is stabilized by the anomeric effect, while the β-glycoside 83 is already stabilized by this effect. However, neither of these conformations is in the correct orientation to undergo hydrolysis according to the ALPH model. The α-glycoside must return to the chair conformation 80 while the β-glycoside must convert to a twist-boat conformation 84, with the β-twist boat higher in energy than the α-chair. The subsequent transition states 81 and 85, respectively, have a .7kcal/mol energy difference, with the β-glycoside higher in energy. Both glycosides then convert to the oxacarbenium ion 86 upon cleavage of the glycosidic bond. The differences in energy between all intermediates and transition states should result in a rough difference in energy of .25kcal/mol between the two reactions, corresponding to a rate ratio of α:β of 1.5:1. Empirical studies showing rate constants of $6.2 \times 10^{-5}$ s$^{-1}$ and $3.8 \times 10^{-5}$ s$^{-1}$ for α and β, respectively, at 35.2°C and 2.5x$10^{-3}$M HCl support this conclusion, lending credence to both the ALPH theory and the mechanism hydrolysis undergoes.16

1E. Kinetics Analysis of Acid-Catalyzed Hydrolysis of Glycosides as a Study of Ring Flexibility

The flexibility of a compound is an important factor when considering binding effectiveness with enzymes. In order to bind, the substrate must be able to not only enter the active site, but also submit to the correct steric and electronic interactions that are necessary for the enzyme to perform its function. A flexible substrate should more easily be able to fit into the active site. If inhibition of an enzyme is desired, a compound that is
more flexible than the natural substrate may be able to out compete the natural substrate. The Peczuh group believes that septanose carbohydrates may be more flexible than smaller pyranose carbohydrates by virtue of the addition of a carbon to the ring. Septanose based inhibitors of glycosidases may, therefore, act as effective competitors for pyranose processing enzymes. In the case of carbohydrates, ring flexibility can be assessed through kinetics analysis of acid-catalyzed hydrolysis.

The necessity for the carbohydrate ring to position the glycosidic bond antiperiplanar to the lone-pair of electrons on the cyclic oxygen to allow hydrolysis to occur is clear. In the case of D-series carbohydrates, it is the β-anomers that must contort to reach this required orientation. It stands to reason that a flexible ring would more easily able to reach this conformation and hydrolyze faster than a less flexible ring. By comparing the hydrolysis rate constants of septanose carbohydrates to pyranose carbohydrates, a larger rate constant for the septanose carbohydrate may indicate a more flexible ring.

Hydrolysis kinetics experiments of glycosidic bonds have been performed for decades, but are by no means exhaustive. In order to make a comparison, a substrate that has a comparable pyranose structure with available hydrolysis data must be chosen. We chose the septanose equivalent of β-D-methylglucoside, β-D-glycero-D-guloseptanose. The substrates are identical, except for the addition of a single carbon and hydroxyl group to the ring. The substrate was chosen because glucose is a common carbohydrate and has been studied extensively, providing a solid foundation from which we can begin to compare the properties of pyranose and septanose structures.
2. Synthesis of methyl β-D-glycero-D-guloseptanoside via Ring-Closing Methathesis

2A. Synthesis of Oxepine Intermediate

The synthesis of β-D-glycero-D-guloseptanoside involves the generation of a key oxepine intermediate, using an established synthesis that is shown in Scheme 1.\textsuperscript{17}

The synthesis begins by protecting the free hydroxyls commercially available α-D-methylglucoside (15) as their benzyl ethers (16). The reaction is affected by the addition of benzyl chloride and potassium hydroxide to 15 with stirring overnight. Yield was 69% on average for this reaction. Because of the high boiling point of benzyl chloride, the removal of most of the benzyl chloride had to be done using column chromatography. However, due to the sheer size of the reaction, complete separation of
the entire product from the benzyl chloride proved very difficult. As a result, the yield of pure product was reduced by the large amount of mixed fractions that resulted, even after multiple purifications. A clear, colorless oil resulted. Characterization was performed using $^1$H and $^{13}$C NMR spectroscopy.

Following benzyl protection, hydrolysis of the methyl glycoside in 16 was performed using acetic acid and catalytic methane sulfonic acid with heating to 80°C to produce lactol 17. Careful monitoring of temperature was required, as temperatures below 70°C resulted in a sluggish reaction and temperatures above 90°C resulted in product mixtures that also contained large amounts of decomposed material. The length of reaction also had to be considered, as even reactions that ran at 80°C overnight produced some decomposed material. Purification of the product using column chromatography also proved troublesome, as the product tended to crystallize on the column in the low-polarity solvent systems required to remove nonpolar impurities. Percent yields ranged between 50%-75% to produce a fluffy, white solid. Characterization was performed using $^1$H and $^{13}$C NMR spectroscopy.

The lactol ring was methylenated to produce 18 using a Wittig reaction. Flame-drying the reaction vessels was required to prevent quenching of the n-butyllithium with residual water. A reaction temperature of −20°C had to be maintained at all times in order to prevent reaction of the n-butyllithium with the tetrahydrofuran solvent. The ylide solution was prepared separately and then added to the lactol substrate. Various amounts of the ylide always remained unreacted and left behind after the addition, due to a slightly less-than-stoichiometric amount of n-butyllithium used to create the solution. Purification of the reaction mixture using column chromatography produced a clear,
colorless oil. Percent yields ranged from 34% to 66%. Yields on the lower end were common on large-scale reactions, likely due to non-stoichiometric amounts of \( n \)-butyllithium being used because of loss of potency of the stock solution over time, which was present during smaller batches, but not as noticeable. Characterization of the hepten-1-itol was performed using \(^1\)H and \(^{13}\)C NMR spectroscopy.

Formation of diene \( 19 \) was performed using two methods. The first method used a palladium(II) acetate as a heterogeneous catalyst in the presence of catalytic 1,10-phenanthroline to add ethyl vinyl ether to the hepten-1-itol. The reaction was slow, requiring refluxing from three days to a week to produce yields ranging from 14% to 39%. The reaction mixture was purified directly via silica gel chromatography to produce a clear, colorless oil. The second method used Ir(cod)Cl\(_2\) catalyst to add a vinyl group to the hepten-1-itol to produce \( 19 \). A reaction mixture of the substrate, vinyl acetate and iridium catalyst was heated to near reflux conditions overnight. The mixture was then purified directly via silica gel chromatography to give the product in yields ranging from 14% to 64%, with an average percent yield of 32%. However, instead of producing a colorless oil, the product had a yellow color, possibly due to residual iridium. The newly added olefin is particularly labile and \( 19 \) can readily decompose back to \( 18 \) over time. Characterization was performed using \(^1\)H and \(^{13}\)C NMR spectroscopy.

Oxepine \( 20 \) was subsequently prepared via ring-closing metathesis (RCM) using Schrock catalyst. Due to the air-sensitive nature of the catalyst, the reaction had to be carried out in a glovebox under an inert atmosphere. Upon addition of the catalyst, the reaction was allowed to stir overnight at room temperature. Silica gel chromatography of the product mixture produced percent yields ranging from 9% to 91%, giving a clear,
colorless oil. Characterization was performed using $^1$H and $^{13}$C NMR spectroscopy. The wide inconsistency in the effectiveness of the reaction is difficult to explain. In cases where the iridium catalyst was used to produce the diene, yields were generally lower than those where the substrate was generated using the palladium catalyst, but some reactions still produced high yields. Furthermore, sometimes no reaction would occur and the substrate would decompose to 18. To ensure conditions were as optimal as possible, the toluene still was cleaned and replaced to ensure no water was getting into the reaction, the glovebox was purged and regenerated and the substrate was azeotroped to ensure dryness, but the reaction still failed to work. The last variable was the catalyst itself. Investigations into the age of the catalyst revealed that it is produced only once every year or so, with the catalyst used being the tail end of the last batch. However, Strem Chemicals Inc., the manufacturer, insisted that the catalyst still passed the activity assays used to assess its potency. New catalyst from a subsequent batch resulted in product formation again, so while it cannot be said for certain that the catalyst from the previous batch was bad, evidence would indicate that was the case.

2B. Synthesis of methyl $\beta$-D-glycero-D-guloseptanoside from Oxepine

The synthesis of $\beta$-D-glycero-D-guloseptanoside from oxepine 20 was done using an established synthesis and is shown in Scheme 2.$^{18}$
Intermediate oxepine 20 was treated with freshly prepared dimethyl dioxriane (DMDO) to produce a mixture of 1,2-anhydroseptanose diastereomers 21 and 22. To avoid complications with purification of this reactive epoxide, the reaction mixture was simply concentrated, resulting in a clear, colorless oil, and taken directly onto the next step as a mixture. 100% conversion was assumed.

The mixture of 21 and 22 was dissolved in methanol and treated with sodium methoxide to open the epoxide ring to produce a diastereomers 23 and 24. Purification of the mixture using silica gel chromatography proved very difficult, due to the very similar polarities of the two diastereomers. Separation eventually produced percent yields of 10% for 24 and 33% for 23, with significant amounts of both diastereomers remaining in mixed fractions. 23 was a white solid 24 was a clear, colorless oil. Characterization was performed using $^{1}$H and $^{13}$C NMR spectroscopy.

Methyl septanoside 24 was then deprotected to produce final product 25 using palladium(II) hydroxide on carbon and hydrogen gas. Purification was performed by...
filtering the reaction mixture through Celite and then removing the solvent and toluene byproduct under reduced pressure, giving quantitative conversion. The final product was a clear, colorless oil.

3. Hydrolysis Kinetics Analysis of methyl-\(\beta\)-D-glycero-D-guloseptanoside and methyl-\(\alpha\)-D-glycero-D-guloseptanoside

3A. Conditions for Acid-Catalyzed Hydrolysis of methyl-\(\alpha\)- and \(\beta\)-D-glycero-D-guloseptanoside

The acid-catalyzed hydrolysis of glycosidic bonds is a first-order reaction with respect to the acid. The rate of the reaction is dependent on the temperature and the concentration of the acid used, but not on the concentration of carbohydrate. However, to obtain a data set with enough data points in a smooth curve to acceptable for curve fitting, enough carbohydrate must be used to allow the reaction to proceed for a long enough time to allow for several dozen data points to be obtained. At the same time, acid concentration and temperature must be carefully selected to ensure that the amount of carbohydrate selected for the reaction hydrolyzes fast enough such that a plot of concentration can be obtained in a reasonable amount of time while not being so fast such that a very steep, and therefore difficult to fit, curve is produced.

To monitor this reaction, \(^1\)H Nuclear Magnetic Resonance (NMR) spectroscopy was used. This method was chosen because of the presence of characteristic \(^1\)H peaks of the substrate that could be used to find the concentration of the carbohydrate over time. Furthermore, it allowed for a specific temperature to be held during the reaction as well as being convenient and easy to use. The acquisition program used in this experiment was designed to take 32 scans for each \(^1\)H spectra taken, with individual acquisitions
taking place at intervals of 15 minutes over a time period of approximately 12.5 hours, resulting in a total of 50 \(^1\)H spectra taken.

Once the method for monitoring the reaction was chosen, the use of an internal standard was found necessary. Due to the noise inherent in any analytical instrument, the variable being measured, the integration value of the peak, will fluctuate over time. As a result, a correction factor must be applied so that the integration value of any given peak at any given time can be normalized to account for this fluctuation. An internal standard is an artificially added compound that is inert under the reaction conditions being used. For this experiment, dimethylsulfoxide (DMSO) was chosen because it is inert under acidic aqueous conditions, is miscible in water, the solvent, and has a \(^1\)H chemical shift far upfield of the protons of the substrate, meaning it will not overlap with any other signals.

The choice of acid used in this experiment is important. A strong, monoprotic acid is desirable because the exact amount of free protons in solution is known. A diprotic strong acid’s conjugate base will release protons according to an equilibrium, and determination of the exact concentration of protons in solution, while possible, can be bypassed by using a monoprotic acid. Hydrochloric acid (HCl) fits the criteria for being a strong, monoprotic acid. However, the presence of free hydrogen ions in the solution would result in exchange with the hydroxyl functional groups on the substrate, possibly allowing the hydroxyl protons to appear on the NMR and obscuring necessary peaks. To avoid this problem, dueterated hydrochloric acid (DCl) was chosen.

Because the reactivity of the substrate was unknown at the beginning, initial experiments were chosen to have a carbohydrate concentration of 5 mM, DCl
concentration of .5M and DMSO concentration of 1mM with a total reaction volume of 600µL of dueterated water and a temperature of 50°C using a 400Mz NMR spectrometer. Prior to running the experiment, the NMR probe was raised to temperature and tuned to $^1$H using a reference sample composed of a 600µL solution composed of carbohydrate and DMSO concentrations equivalent to the reaction conditions, but without acid.

Taking a known amount of DCl solution and adding it to a known amount of carbohydrate solution gave a total volume of 600µL for the actual experiment. This mixture was quickly mixed using a shaker and then placed into an NMR tube. The sample was then placed into the spectrometer and the acquisition program was activated. Simultaneously, the operator manually modified the shim in an attempt to produce the best resolution possible. Once the temperature of the reaction mixture stabilized, the shimming was further refined and the program was allowed to run its course.

3B. Kinetics Analysis of methyl-β-D-glycero-D-guloseptanoside

Three experimental runs under identical conditions, as described above, were performed. In all runs, integration values from the first spectrum were discarded due to poor resolution of the peaks that had resulted from poor shimming as the sample was brought up to temperature. This produced 49 usable data sets for each run.

The raw data for the reaction were analyzed using the program Topspin, by Brüker. For each run, the individual $^1$H spectra were calibrated to bring the DMSO peak to 2.71ppm. As time elapsed, the integration of the methyl peak clearly becomes smaller (see Figure 12 - arrows).
Figure 12 – $^1$H spectra of methyl-β-D-glycerod-guloseptanoside as time elapses. The purple arrows point to the methyl peak being monitored. As time elapses, the peak clearly becomes smaller.

Figure 13 – An overlay of three separate runs of the acid-catalyzed hydrolysis of methyl-β-D-glycerod-guloseptanoside plotting carbohydrate concentration versus time.
The range for the methyl peak was to be integrated over was determined by eye based on the spectral resolution. Each methyl peak in the 49 spectra was integrated over this area. Using the integration value of the DMSO internal standard, the integration of the methyl peak was converted to concentration of carbohydrate present in the solution. The amount of time elapsed between each spectral acquisition was known. The concentration of carbohydrate was plotted versus time for each experimental run (Figure 13).

The plots appear to follow a first-order exponential decay function. There is some discrepancy between the individual runs in terms of the time the plot levels out and the concentration at which the plot levels off. For example, the concentration of carbohydrate near time zero is much higher for smm-ii-053 than for the other two runs and smm-ii-055 levels off at a much lower concentration value than the other runs. There are a few factors that may explain these differences.

600µL of water is a large enough volume to give the NMR spectrometer enough sample to view completely. However, due to the high surface tension of water and the small diameter of the NMR tube, transfer of the mixed solution from the Eppendorf vial into the NMR tube prior to placement in the spectrometer always resulted in portions of the solution stuck to the sides of the NMR tube instead of being with the rest of the solution at the bottom of the tube. Neither long pipets nor shaking could cause the entirety of the solution to reach the bottom of the tube. As a result, while the concentration of sugar and acid was constant between all runs, the raw amount of each reagent may have been slightly different. Any solutions that had water stuck to the sides of the NMR tube would have had less carbohydrate being monitored by the spectrometer.
and so would have leveled off faster. Fortunately, this does not affect the rate at which the reaction occurs and so is not a problem.

Different concentrations each experiment leveled off at can also explained. The resolution of experiment smm-ii-055, seen in Figure 12, was high enough so that a second peak adjacent to the methyl peak was completely distinguishable and the integration of the methyl peak did not include this adjacent peak. In the other two runs, the resolution was not as high and so the two peaks could not be distinguished, so the integration of the methyl included this second peak. As a result, the leveling-off concentrations for smm-ii-051 and smm-ii-051 are roughly equivalent while the leveling-off concentration for smm-ii-055 is much lower, because it the integration of the second peak was excluded. If the integration value of the second peak does not fluctuate over time, the only effect of the inclusion of the integration of the second peak will be a change in the vertical shift of the graph. The rate of reaction should be unchanged. However, should the integration of the second peak change over time, the observed rate of the reaction for smm-ii-051 and smm-ii-053 will be different than for smm-ii-055. Fortunately, analysis of the spectra for smm-ii-055 does not indicate that the integration for the second peak changes over time, so the observed rate of reaction should not be affected.

Using Kaleidagraph, these plots were fitted using an exponential decay function. A first order exponential decay function is of the form $Y = m_1 + m_2 e^{(-m_3 t)}$, where $Y$ is the concentration of carbohydrate at time $t$, $m_1$ is the vertical shift of the function, $m_2$ is the original concentration of carbohydrate, $m_3$ is the observed rate constant ($k_{obs}$) and $t$ is time elapsed. A representative fit for smm-ii-055 is seen in Figure 14. For this particular
fit, the value of $k_{obs}$ (m$^3$) is $5.26 \times 10^{-5}$ s$^{-1}$. Similar fits for smm-ii-051 and smm-ii-053 gave $k_{obs}$ values of $4.36 \times 10^{-5}$ s$^{-1}$ and $4.87 \times 10^{-5}$ s$^{-1}$, respectively. Averaging all three $k_{obs}$ values gives a final $k_{obs}$ of $4.82 \times 10^{-5}$ s$^{-1}$.

As mentioned earlier, there were several differences between the three runs that gave different looking plots, but should not have significantly affected the rate constant. All of the individual rate constants are within 10% of the average value. While not ideal, when taken in the context of the small concentrations being dealt with, where even a small amount of error resulting from something such as the pipet used to measure these volumes could result in a fairly large deviations from true values, within ten percent is reasonable accuracy.

3C. Acid-Catalyzed Hydrolysis of methyl-$\alpha$-D-glycero-D-guloseptanoside

Generation of the methyl-$\alpha$-D-glycero-D-guloseptanoside substrate was performed by Dr. Shankar Markad. The experimental conditions used in the analysis of methyl-$\alpha$-D-glycero-D-guloseptanoside were the same as described in the section 3A. As with the $\beta$ anomer, the raw data for the reaction was analyzed using the program Topspin, by
Brüker, and the data was calibrated so that the DMSO peak came out at 2.71ppm. As with the β anomer, the methyl peak clearly decreases in integration value as time elapses (Fig. 15). The range for the methyl peak was to be integrated over was determined by eye based on the spectral resolution. Each methyl peak in the 49 spectra was integrated over this area. Using the integration value of the DMSO internal standard, the integration of the methyl peak was converted to concentration of carbohydrate present in the solution. The amount of time elapsed between each spectral acquisition was known. The concentration of carbohydrate was plotted versus time for each experimental run (Fig. 16).

Fig. 16 – $^1$H spectra of methyl-α-D-glycero-D-guloseptanoside as time elapses. The purple arrows point to the methyl peak being monitored. As time elapses, the peak clearly becomes smaller.
The plots appear to follow a first-order exponential decay function. Here there is excellent agreement between all three experiments. All runs begin to level off at the same time and level off at the same carbohydrate concentration. The problems encountered with the β anomer experiments centered on the overlap of the methyl peak with other peaks, inflating the concentration of carbohydrate slightly. While there is similar overlap with the α, as seen in Figure 16, there is sufficient resolution in all three runs to integrate only the methyl peak. As a result, any affects that might have resulted from including the integration of the overlapping peak are bypassed.

Using Kaleidagraph, these plots were fitted using an exponential decay function. A representative fit for experiment smm-i-186a is seen in Figure 16. For this particular fit, the value of \( k_{\text{obs}} \) (m3) is \( 1.04 \times 10^{-4} \text{s}^{-1} \). Similar fits for smm-ii-186a and smm-ii-193a gave \( k_{\text{obs}} \) values of \( 1.13 \times 10^{-4} \text{s}^{-1} \) and \( 1.22 \times 10^{-4} \text{s}^{-1} \), respectively. Averaging all three \( k_{\text{obs}} \)
values gives a final $k_{obs}$ of $1.13 \times 10^{-4} \text{s}^{-1}$. All values are within 8% of the average value. Given the very close correlation between all three runs, this value is deemed accurate.

3D. Comparison of $\alpha$ and $\beta$ Septanose Hydrolysis Rate Constants To Each Other

To summarize the results of the determination of the acid catalyzed hydrolysis of methyl-$\alpha$- and $\beta$-$D$-glycero-$D$-guloseptanoside, the $k_{obs}$ for the $\alpha$ anomer was found to be $1.13 \times 10^{-4} \text{s}^{-1}$ and $4.82 \times 10^{-5} \text{s}^{-1}$ for the $\beta$ anomer. From this data, it is clear that the $\alpha$ anomer hydrolyzed faster than the $\beta$. Furthermore, the increase in rate is significant, with $k_{obs}$ for the $\alpha$ anomer being more than twice that of the $\beta$ anomer. This result is intriguing, because $\beta$ pyranose carbohydrates have a higher rate constant than their $\alpha$ anomers. The origin of this reversal of reactivity cannot be explained without a thorough analysis of the structure of the compound as it undergoes the reaction, which has not been done, but some comments can be made.

In pyranose ring structures, the $\beta$ anomer hydrolyzes faster than the $\alpha$ anomer, despite needing to contort the ring in order to reach the necessary lone-pair anti-periplanar orientation. The exact reasoning behind this phenomenon has not yet been explained, but it is known that the electron withdrawing power of substituents on the ring
can have a great effect on the rate of hydrolysis.\textsuperscript{19} Subsequent studies have shown that
electronic, not steric, effects are the primary contributors to the hydrolysis of
glycosides.\textsuperscript{20} In particular, the stabilization or destabilization of the oxonium ion
intermediate by the substituents on the ring is crucial to the rate of reaction. From this, it
may be inferred that the cost of rotating the $\beta$ anomer to the correct orientation is offset
by lessening the destabilization of the oxonium ion intermediate by altering the positions
of the other substituents on the ring. The $\alpha$ anomer does not have to twist itself, but the
destabilization of the oxonium ion by the substituents may be so high as to cause the $\alpha$
anomer to react slower than the $\beta$ anomer.

Translating this reasoning to septanose rings is difficult, because the strictly axial
and equatorial designations of pyranose structures are replaced by pseudo-axial and
pseudo-equatorial designations, but some assumptions can be made. The apparent
destabilization of the oxonium ion present in the pyranose structure is likely mitigated by
the loss of strictly equatorial and axial orientations in the septanose structure. As a result,
the advantage of being close the required lone-pair anti-periplanar orientation for the $\alpha$
anomer is larger than the energy required to twist the $\beta$ anomer into the correct
orientation. Further studies that probe the effects of changing the substituents on the ring
so as to have different electronegativities will provide insight into which anomer is more
sensitive to destabilizing effects and may be of aid in elucidating why the $\alpha$ septanose
anomer hydrolyzes faster than the $\beta$ septanose anomer, in a reversal of the patterns found
for pyranose carbohydrates. In addition to empirical studies, \textit{ab initio} studies of the
conformational changes the protonated substrates undergo would provide insight into
how each structure must change to reach the required anti-periplanar orientation.
3E. Comparison of α and β Septanose Hydrolysis Rate Constants to Hydrolysis Rate Constants of Comparable Pyranose Carbohydrates

Table 1 provides a summary of the acid-catalyzed hydrolysis of glycosides rate constants and conditions used to obtain them for methyl-α- and β-D-glycero-D-guloseptanose and α- and β-D-methylglucoside.

<table>
<thead>
<tr>
<th>Ring Type</th>
<th>Anomer</th>
<th>HCl Concentration (M)</th>
<th>Temperature (°C)</th>
<th>Average Rate Constant (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyranose</td>
<td>α</td>
<td>1.0</td>
<td>75</td>
<td>1.06x10⁻⁶</td>
</tr>
<tr>
<td>Pyranose</td>
<td>β</td>
<td>1.0</td>
<td>75</td>
<td>1.91x10⁻⁶</td>
</tr>
<tr>
<td>Septanose</td>
<td>α</td>
<td>0.5</td>
<td>50</td>
<td>1.13x10⁻⁴</td>
</tr>
<tr>
<td>Septanose</td>
<td>β</td>
<td>0.5</td>
<td>50</td>
<td>4.82x10⁻⁵</td>
</tr>
</tbody>
</table>


Before comparing methyl-α-D-glycero-D-guloseptanose to α-D-methylglucoside, the conditions the rate constants were determined under must be examined. The pyranose carbohydrate was hydrolyzed at an acid concentration of 1.0M and a temperature of 75°C. Both of these conditions are harsher than those used for the septanose carbohydrate, which used an acid concentration of .5M and temperature of 50°C. If both carbohydrates were of equal reactivity, the calculated rate constant for the pyranose structure would be higher than the septanose structure due to an accelerated reaction from the extra acid and energy. However, the data clearly shows that, even though the pyranose structure has a clear advantage in terms of conditions, the septanose carbohydrate still reacts more than two orders of magnitude faster. While a direct comparison of rate constants at the same reaction conditions would be ideal, it is clear
from this data that the α-septanose carbohydrate is much more reactive than the equivalent α-pyranose carbohydrate.

A similar analysis of methyl-β-D-glycero-D-guloseptanoside to β-D-methylglucoside shows that same differences in reaction conditions are present. Again, in spite of the more robust reaction conditions for hydrolysis of the pyranoses, the septanose carbohydrate reacts at higher rate than the pyranose carbohydrate, although only by a single order of magnitude. Again, a direct comparison of rate constants under identical reactions would be preferable, it is evident from the data that the β-septanose carbohydrate is more reactive than the β-pyranose carbohydrate.

The significant increase in reactivity of the septanose series of carbohydrates over the pyranose series of carbohydrates is promising support for the hypothesis that seven-membered ring carbohydrates are more flexible than six-membered ring carbohydrates. However, the presence of other possible influences must be considered. As mentioned in the previous section, the magnitude and orientation of electronegative groups on the ring has a profound effect on the rate of glycoside hydrolysis. It is possible that increasing the ring size has caused the substituents on the ring to be positioned in such a manner as to mitigate the destabilizing effects of the substituents such that the increase in rate is due to electronic instead of flexibility reasons. In order to probe whether electronic or flexibility effects are the root causes of the demonstrated increase in reactivity, further experiments must be performed, as described in Section 3D. Again, ab initio studies of the conformational changes the protonated substrates undergo would provide insight into how each structure must change to reach the required anti-periplanar orientation.
4. Comments and Conclusions

In summary, the synthesis of β-D-glycero-D-guloseptanoside was performed using an established procedure with moderate success. Acid-catalyzed hydrolysis kinetics analysis of this substrate and the independently prepared methyl-α-D-glycero-D-guloseptanoside was performed at 50°C and .5M DCl. Monitoring of the reaction was done via time-elapsed $^1$H NMR spectroscopy. Using this data, rate constants for the reaction were calculated and determined to be $1.10 \times 10^{-4} \text{s}^{-1}$ for and methyl-α-D-glycero-D-guloseptanoside and $5.35 \times 10^{-5} \text{s}^{-1}$ for β-D-glycero-D-guloseptanoside. These rate constants indicate that α septanose glycosides hydrolyze twice as fast than their β anomers, which is a reversal of the reactivity shown by pyranose carbohydrates. Furthermore, with these rate constants being more than an order of magnitude faster than the rate constants of comparable, pyranose glycosides, support is given to the argument that septanoses are more flexible than pyranoses. However, the effect of electron-withdrawing substituents on the ring must be examined to determine their contribution to the rate of hydrolysis. Future work to probe this problem may include further kinetics analysis using differently substituted substrates to alter the electronics of the compound and ab initio calculations to determine the conformational changes septanoses undergo during hydrolysis.
5. Detailed Reaction Protocols

2,3,4,6-tetra-O-benzyl-D-methylglucoside (16). A solution of α-D-methylglucoside was generated by dissolving the substrate (8.0 g, 41.20 mmol) in 60mL dioxane in a three-neck roundbottom flask. Ground KOH (39.68 g, 708.64 mmol) was added to the solution. A condensor was attached to the left neck of the flask and a mechanical stirrer was placed into the flask through the center neck. The stirrer was activated and BnCl (48.37 mL, 420.24 mmol) was added to the mixture through the right neck. After the addition was completed, the right neck was sealed with a glass stopper and the mixture was refluxed at 105°C using an oilbath overnight. The reaction was cooled to room temperature and the solvent and excess BnCl were removed under high vacuum. The resulting oil was purified by column chromatography (silica, 15 mm×100 mm) eluting with 20% EtOAc/Hex to give a clear, colorless oil.

2,3,4,6-tetra-O-benzyl-D-glucose (17). Into a flask containing 16 (42.658 g, 77.0 mmol) dissolved in 300mL glacial acetic acid was slowly added an aqueous solution of methane sulfonic acid (10.14 mL, 2M). Reaction mixture was heated to 80°C using an oilbath and allowed to stir overnight. The mixture was allowed to cool to room temperature and the acetic acid was removed under hi-vacuum. The residue was dissolved in DCM and washed with sodium bicarbonate three times. The aqueous layers were combined and extracted with DCM three times. All organic layers were combined, dried using Na₂SO₄ and concentrated under reduced pressure. The resulting solid was purified by column chromatography (silica, 15 mm×100 mm) eluting with 30% EtOAc/Hex to give a white solid.
**3,4,5,7-tetra-O-benzyl-1,2-dideoxy-D-gulo-hept-1-ene (19).** Starting material 18 (5.21 g, 9.65 mmol) was placed in a flame-dried roundbottom (RB#1), placed under N<sub>2</sub> atmosphere and dissolved in 30mL dry THF added via syringe and needle. In a second flame-dried roundbottom (RB#2), Wittig Salt (12.06 g, 33.77 mmol) was added and placed under N<sub>2</sub> atmosphere. 40mL of dry THF was added to RB#2. Both roundbottoms were cooled to -20°C using dry ice/acetone baths. n-BuLi (26.93 mL, .394 M) was added dropwise to RB#1 via syringe and needle, resulting in a pale yellow solution color. n-BuLi (88.15 mL, .394M) was added dropwise to RB#2 via syringe and needle, giving a cloudy, yellow solution as the salt dissolved. Both roundbottoms stirred at -20°C for thirty minutes. The contents of RB#2 were transferred to RB#1 via canula. Mixture allowed to stir overnight warming to RT. Reaction was quenched using ammonium chloride and the mixture was extracted using DCM three times. The organic layers were combined, dried using Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The resulting solid was purified by column chromatography (silica, 15 mm×100 mm) eluting with 10% EtOAc/Hex to give a clear, colorless oil.

**3,4,5,7-tetra-O-benzyl-1,2-dideoxy-6-O-vinyl-D-gulo-hept-1-ene (Pd(OAc)) (19).** Into a roundbottom flask containing 18 (1.74 g, 3.24 mmol) in dry DCM (22.5 mL) under N<sub>2</sub> atmosphere were sequentially added ethyl vinyl ether (90mL), 1,10-phenanthroline (0.132 g, 0.732 mmol) and Pd(OAc)<sub>2</sub> (0.164g, 0.732 mmol). The flask was fitted with a reflux condensor, maintained under N<sub>2</sub> atmosphere and heated to reflux for 72 h. The reaction mixture slowly turned black, indicating formation of palladium black. The reaction was cooled to room temperature and the solvent removed under reduced
pressure. The resulting oil was purified by column chromatography (silica, 15 mm×100 mm) eluting with 2.5% EtOAc/Hex to give a clear colorless oil: $R_f$ (25% EtOAc/pet. ether)=0.92

$^1$H NMR 400 MHz (CDCl$_3$) $\delta$ 7.31–7.23 (m, 20H), 6.23 (dd, $J$=14.0, 6.5 Hz, 1H), 5.86 (ddd, $J$=17.5, 10.3, 7.4 Hz, 1H), 5.28 (dd, $J$=10.3, 1.2 Hz, 1H), 5.20 (d, $J$=17.5, 1H), 4.78 (d, $J$=11.2 Hz, 1H), 4.70–4.58 (m, 3H), 4.48 (dd, 2H), 4.36 (ddd, $J$=14.3, 1.6 Hz, 1H), 4.15 (ddd, $J$=5.3, 5.3, 3.4 Hz, 1H) 4.10 (dd, $J$=6.8, 6.8 Hz, 1H), 3.99 (dd, $J$=6.5, 1.6 Hz, 1H), 3.97 (dd, $J$=5.0, 5.0 Hz, 1H), 3.84 (dd, $J$=10.7, 3.3 Hz, 1H), 3.69 (dd, $J$=6.3, 5.0 Hz, 1H), 3.66 (dd, $J$=10.7, 5.8 Hz, 1H); $^{13}$C NMR 100 MHz (CDCl$_3$) $\delta$ 151.3, 138.9, 138.4, 138.3, 135.5, 128.5 (2), 128.4 (2), 128.3, 128.2, 127.9 (2), 127.7, 127.6 (2), 119.4, 89.4, 81.7, 81.5, 79.3, 79.0, 76.9, 75.3, 74.3, 73.5, 70.8, 69.1. FAB MS $m/z$ (M$^+$+H) calcd 565.2954, found 565.2944.

3,4,5,7-tetra-O-benzyl-1,2-dideoxy-6-O-vinyl-D-gulo-hept-1-ene (Ir(cod)Cl$_2$) (19).

Into a roundbottom flask containing 18 (0.623 g, 1.160 mmol) in dry toluene (8 mL) under N$_2$ atmosphere was added sodium carbonate (0.074 g, 0.696 mmol), Ir(cod)Cl$_2$ (0.078 g, 0.116 mmol) and vinyl acetate (2.04 mL, 23.200 mmol). The flask was fitted with a reflux condensor, maintained under N$_2$ atmosphere and heated to 90°C and allowed to stir overnight. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The resulting oil was purified by column chromatography (silica, 15 mm×100 mm) eluting with 2.5% EtOAc/Hex to give a clear, brown oil.
1,6-Anhydro-3,4,5,7-tetra-O-benzyl-2-deoxy-D-gulo-sept-1-enitol. (20). (In a glovebox) Diene 19 (0.319 g, 0.565 mmol) was dried via azeotropic distillation from toluene (3 × 5 mL) under reduced pressure. To a solution of 19 in 140 mL dry toluene was added Schrock Catalyst (0.080 g, 0.105 mmol). The reaction was sealed in an airfree screw-top roundbottom and removed from the glovebox and stirred at RT overnight. The solvent was removed under reduced pressure and the resulting dark brown oil was purified by column chromatography (silica, 15 mm×85 mm) using 5% EtOAc/Hex as eluent to give a clear colorless oil: \( R_f \) (25% EtOAc/pet. ether)=0.63; \([\alpha]_D^\text{c}=+68.8^\circ \) (c 1.7, CHCl\(_3\)); \(^1\)H NMR 400 MHz (CDCl\(_3\)) \( \delta \) 7.56–7.06 (m, 20H), 6.51 (d, \( J=6.9 \) Hz, 1H), 4.80 (dd, \( J=6.8, 6.8 \) Hz, 1H), 4.67–4.55 (m, 8H), 4.50 (d, \( J=11.2 \) Hz, 1H), 4.09 (dd, \( J=5.9, 5.9 \) Hz, 1H), 3.96 (dd, \( J=4.5, 4.5 \) Hz, 1 Hz), 3.87 (dd, \( J=9.4, 3.8 \) Hz, 1H), 3.78 (d, \( J=3.2 \) Hz, 2H), \(^{13}\)C NMR 100 MHz (CDCl\(_3\)) \( \delta \) 149.4, 138.6, 138.4(2), 128.6, 128.5, 128.0(2), 127.9(2), 127.8, 127.7(2), 123.0, 103.8, 82.8, 81.2, 80.3, 73.6(2), 73.5, 72.8, 70.9, 70.4; FAB MS \( m/z \) (M\(^+\)−H) calcd 535.2484, found 535.2455.

1,2-Anhydro-3,4,5,7-tetra-O-benzyl-\(\alpha/\beta\)-D-glycero-D-ido/guloseptanose (21,22). Oxepine 20 (0.123 g, 0.229 mmol) was dried via azeotropic distillation from toluene (3 × 5 mL) under reduced pressure and dissolved in dry DCM (2 mL). The solution was cooled in an ice bath to 0 °C and a DMDO (1.91 mL, 0.18 M) solution was added dropwise. The mixture was stirred at 0 °C for 30 min and the solvent was removed under reduced pressure. TLC indicated complete conversion and the product was taken on directly to next step.
**Methyl-3,4,5,7-tetra-\(\text{-}\)benzyl-\(\beta\)-\(\text{-}\)glycero-\(\text{-}\)d-guloseptanoside (24).** Mixture of epoxides 21 and 22 was generated was dissolved with some difficulty in CH\(_3\)OH (4 mL) and cooled in an ice bath to 0 °C. To the mixture was added NaOCH\(_3\) (0.014 g) and the mixture was stirred overnight (12 h). The reaction was quenched with water (2 mL), and the solvent was removed under reduced pressure. The residue was dissolved in CH\(_2\)Cl\(_2\) (15 mL), washed with water (2 × 15 mL), and dried (Na\(_2\)SO\(_4\)), and the solvent was removed under reduced pressure. The residue was purified by column chromatography (3:1 hexanes/EtOAc) to give two products, of which one was 24 and gave a clear, colorless oil: Rf 0.24 (3:1 hexanes/EtOAc); [\(\alpha\)]D \(+23.9\) (c 0.45, CHCl\(_3\)); 1H NMR 400 MHz (CDCl\(_3\)) \(\delta\) 7.33–7.27 (m, 18H), 7.18–7.17 (m, 2H), 4.72 (d, J = 11.5 Hz, 1H), 4.60–4.54 (m, 4H), 4.51–4.46 (m, 3H), 4.31 (d, J = 11.3 Hz, 1H) 4.04–4.03 (m, 2H), 3.96–3.95 (m, 2H), 3.69 (d, J = 9.1 Hz, 1H) 3.61–3.57 (m, 2H), 3.54 (s, 3H); 13C NMR 100 MHz (CDCl\(_3\)) \(\delta\) 138.6, 138.3, 138.1, 128.6(2), 128.5, 128.2(2), 128.1, 128.0, 127.9(2), 127.7, 106.9, 81.3, 79.2, 77.8, 77.6, 73.9, 73.5, 72.9, 72.8(2), 71.7, 56.5; FAB-MS m/z (M + H)+ calcd 585.2852, found 585.2883.

**Methyl-\(\beta\)-\(\text{-}\)glycero-\(\text{-}\)d-guloseptanoside (25).** To a solution of 24 (0.039 g, 0.0.066 mmol) in CH\(_3\)OH (2 mL) was added Pd(OH)$_2$ (0.042 g, .397mmol). The mixture was purged with N\(_2\) for twenty minutes. The reaction vessel was then purged with H\(_2\) using a balloon and then the reaction was placed under an H\(_2\) atmosphere via a balloon, and the mixture was stirred for overnight at RT. The balloon was removed from the flask and the mixture was filtered through a short pad of Celite. The Celite was washed with additional CH3OH (4 × 5 mL). The solvent was removed from the combined filtrates by rotary
evaporation under reduced pressure to give a clear, colorless oil: [α]D +7.8 (c 1.01, CH3OH); 1H NMR 600 MHz (CD3OD) δ 4.33 (d, J = 5.4 Hz, 1H), 3.83 (dd, J = 11.8, 2.6 Hz, 1H), 3.80 (dd, J = 5.4, 4.0 Hz, 1H) 3.70 (dd, J = 9.1, 7.3 Hz, 1H), 3.67 (dd, J = 9.1, 4.0 Hz, 1H), 3.57 (dd, J = 11.6, 6.7 Hz, 1H), 3.48 (ddd, J = 8.3, 6.7, 2.6 Hz, 1H), 3.43 (s, 3H), 3.29 (dd, J = 8.3, 7.3 Hz, 1H); 13C NMR 150 MHz (CD3OD) δ 110.0 82.0, 75.7, 75.6, 73.9, 72.0, 64.5, 56.7; FAB-MS m/z (M + H)+ calcd 225.0974, found 225.0992.
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