2013

Towards the synthesis of lipopolysaccharide fragments and development of new alkylating agents

Sinele Banelile Tsabedze
Iowa State University

Towards the synthesis of lipopolysaccharide fragments and development of new alkylating agents

by

Sinele Banelile Tsabedze

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Chemistry

Program of Study Committee:
Nicola L.B. Pohl, Major Professor
Jason Chen
Reuben Peters
Emily Smith
Yan Zhao

Iowa State University

Ames, Iowa

2013

TABLE OF CONTENTS

ABSTRACT

This document details the efforts towards the automated solution phase synthesis of fragments of the *Xcc* bacteria's lipopolysaccharide. The target fragment is a trisaccharide consisting of two glucosamine monomers with a 1-6 beta linkage connected to a 2-6 alpha link to 3-Deoxy-d-manno-Octulosonic Acid (KDO). First, the field of glycobiology is introduced and the challenges associated with the advancement of oligosaccharide synthesis highlighted. Then the synthesis of the glucosamine building block is described. Different approaches to the same general building block are investigated for the best possible route to the building block. Approaches that require less reaction steps, cheaper reagents, easier intermediates to handle, easier purification methods and safer reagents are sought and reported.

From the quest to produce easier intermediates, a different type of glycosyl donor, N-aryl trifluoroacetimidate donor is explored. Due to the change in stability observed with changing the substituent on the aryl group, stability studies of different methyl N-aryl trifluoroacetimidates are further studied. From the stability studies, the mechanism of acetimidate activation can be confirmed. Some acetimidates exhibit favorable characteristics and these are further studied for their role and ability as alkylating agents. Since some are crystalline at room temperature some acetimidates are reported for their appropriateness in commercialization.

The other part of the target LPS fragment is KDO and this rare and expensive sugar is synthesized through biosynthesis. Major problems with purification resulted in the chemical synthesis of KDO from oxaloacetic acid and this method also presents the same major problem. Future directions include better purification protocols for KDO and more runs of glucosamine building blocks on the automated solution phase workstation developed in the lab

ABBREVIATIONS

1D	Monodimensional
2D	Bidimensional
Ac_2O	Acetic anhydride
AcCl	Acetyl Chloride
AcOH	Acetic acid
AgOTf	Silver triflate
AllBr	Allyl bromide
Alloc	Allyloxy carbonyl
ASW	Automated Solution Phase Workstation
$BF_3.OEt_2$	Boron trifluoride dietherate
BnBr	Benzyl bromide
Bu_2SnO	Dibutyltin oxide
BzCl	Benzoyl chloride
CAN	Cerric ammonium nitrate
$CF_3C(NPh)CCl$	N-Phenyltrifluoroacetoimidoyl chloride
Cl_3CCN	Trichloroacetonitrile
COSY	Correlation Spectroscopy
CSA	(±)-Camphor-10-sulfonic acid (β)
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-(Dimethylamino)pyridine

DMF	*N*,*N*-Dimethylformamide
DMP	Dimethyl periodinane
DMSO	Dimethyl sulfoxide
DMTST	Dimethyl(methylthio)-sulfonium triflate
DNA	Deoxyribonucleic acid
ESI-MS	Electrospray Ionization Mass Spectroscopy
Et_2O	Diethyl ether
Et_3N	Triethyl amine
EtOH	Ethanol
EtSH	Ethanethiol
FSPE	Fluorous Solid Phase Extraction
F-tag	Fluorous tag
HMQC	Heteronuclear Multiple Quantum Correlation
HPLC	High Pressure Liquid Cromatography
HSQC	Heteronuclear Single Quantum Correlation
IDCP	Iodine dicollidine perchlorate
KDO	3-Deoxy-D-manno-Octulosonic Acid
LevOH	Levulinic acid
LPS	Lipopolysaccharide
$MeC(OMe)_3$	Trimethyl orthoformate
MeCN	Acetonitrile
MeI	Methyl iodide
MeOH	Methanol
MsCl	Mesyl chloride
NaOAc	Sodium acetate

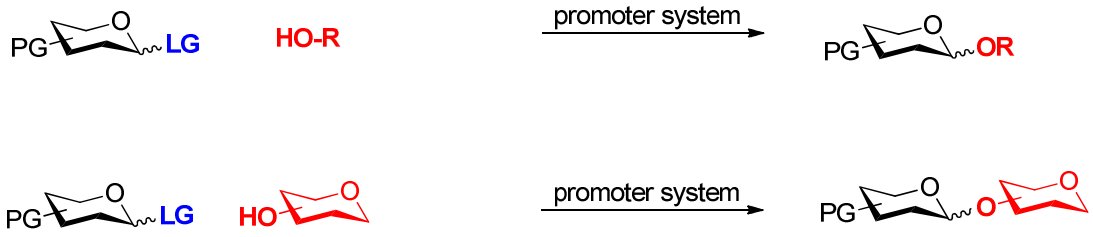
NaOMe	Sodium methoxide
NBS	*N*-Bromosuccinimide
NIS	*N*-Iodosuccinimide
NMR	Nuclear Magnetic Resonance
Pd/C	Palladium on carbon
Ph_2SO	Diphenyl sulfoxide
PhthN	Phthalimide
PMBCl	4-Methoxybenzyl chloride
Py	Pyridine
RNA	Ribonucleic acid
S_N2	Bimolecular Nucleophilic Substitution
SPPS	Solid Phase Peptide Synthesis
TBA	Thiobarbituric acid
TBAB	Tetrabutylammonium bromide
TBAI	Tetrabutylammonium iodide
TBDPS	t-Butyldiphenylsilane
t-BuOK	Potassium tert-butoxide
TCA	Trichloroacetyl
Tf_2O	Triflic anhydride
TfOH	Triflic acid
THF	Tetrahydrofuran
TIPS	Thin layer chromatography
TLC	Triisoproplysilane
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
Tr	Trityl

Troc	Trichloroethyl carbonyl
UV	Ultraviolet

CHAPTER 1. GLYCOSYLATION

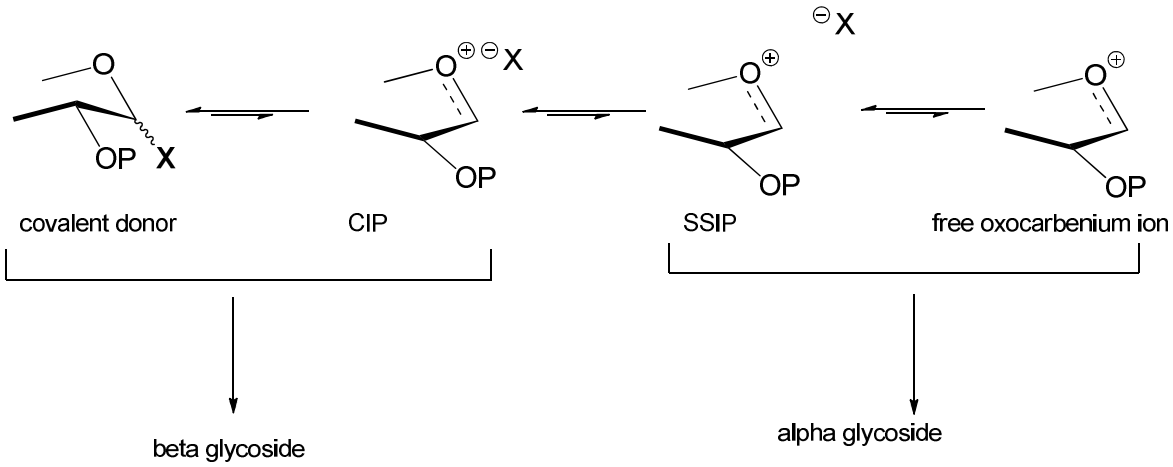
The Glycosidic Bond

The most important reaction in carbohydrate chemistry is glycosylation. It involves a nucleophilic displacement of a leaving group (LG) on the glycoside by another group, usually an alcohol (ROH). The glycoside with the leaving group is referred to as the glycosyl donor while the alcohol that displaces the leaving group is called the glycosyl acceptor. The glycosyl acceptor can be, but is not limited to glycosides as shown in the following figure: (Demchenko, 2008)



Scheme 1.1: General glycosylation reaction between a regular alcohol and a sugar alcohol

The promoter system is there to facilitate the departure of the leaving group. It consists of a solvent, an activating agent and sometimes, molecular sieves and acid scavengers may be used. The solvent can play a major role in determining the stereochemistry at the point of connection. At activation, a contact ion pair (CIP) or solvent separated ion pair (SSIP) can be formed, depending on the solvent used in the promoter system. For instance in non-polar solvents, the nucleophilic acceptor might attack on the tight ion pair in a S_N2-like pathway. In polar solvents the reaction proceeds mainly through the SSIP leading to the α-linked glycoside which is, actually, the main product coming from any oxacarbenium ion. This can be attributed to the anomeric effect which dictates that polar substituents such as halide, OR, or SR derivatives prefer an axial orientation.



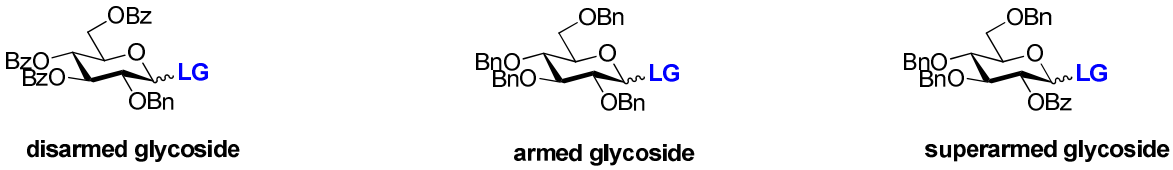
Scheme 1.2: The role of solvent in glycosylation.

The activating agent is usually a Lewis acid. They vary in strength and usefulness depending on the type of glycosyl donor used. Some activators are insoluble in reaction solvents and some are soluble. This makes a difference in whether the reaction is homogenous or heterogenous. All these consideration are taken into account when designing a synthetic plan for glycosylation. Some of the more commonly used activators are TMSOTf, $BF_3{\cdot}OEt_2$ (common in acetimidate donors), NBS, NIS/TfOH, IDCP, DMTST(common in thioglycoside donors) and silver metal based promoters (commonly used with glycosyl halides)

Acid scavengers can be necessary when there is a danger of accumulation of acid as the reaction progresses. Glycosidic bonds are not stable in acidic conditions and if too much acid accumulates, this can break the very glycosidic bonds being formed in the reaction. Some of the acid scavengers used in glycosylation include molecular sieves, 2,6-di-*tert*-butyl-4-methylpyridine(DTBMP)(Yan & Kahne, 1996) and tetramethylurea (Hanessian & Guindon, 1980).

Another important factor to keep in mind is the way or order of adding reagents. The promoter can be added over a mixture of the acceptor and the donor. This is appropriate for less reactive disarmed donors. For highly reactive armed donors, it is more appropriate to add the donor over a mixture of acceptor and promoter. This is done to limit the chances that a super reactive donor would decompose in the presence of the promoter before it has a chance to react with the acceptor.

In general, if a donor (or acceptor) is armed, it means that it is protected by mainly ether type, electron donating groups and if it is disarmed, it is mostly protected by ester type electron withdrawing groups. (Mydock & Demchenko, 2008). Differences in reactivity can be so pronounced that changing just one functional group can mean selectivity. This has brought about the popular advancement in oligosaccharide synthesis, namely, one pot sequential glycosylation. For instance in Scheme 1.3 below, changing the C-2 hydroxyl protection from Bn to Bz (super-arming) increased the reactivity that they could react a 2-O-benzoylated acceptor in the presence of the 2-O-benzylated acceptor. This is because even though ester-type protecting groups are deactivating, when they are on C-2, they can enhance reactivity by neighboring group participation. Other such selectivity can be achieved by reacting a mostly benzylated acceptor(armed) in the presence of a mostly acetylated one(disarmed) .

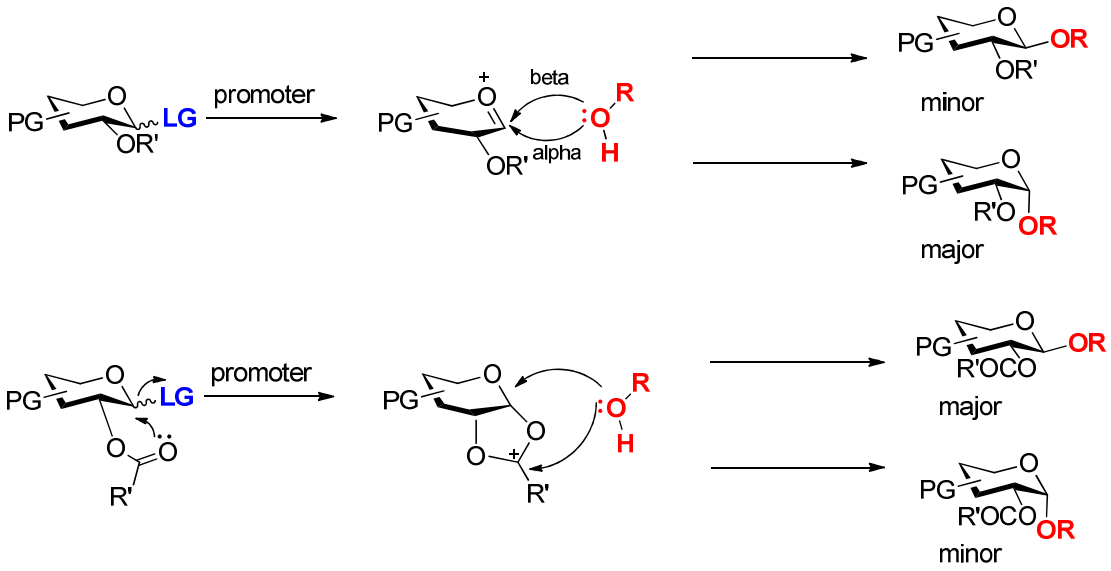


Scheme 1.3: Examples of disarmed donor, armed and super-armed

This concept has been observed to be effective even with similar protecting group. For instance, in the presence of phenylthio-glycoside, glycosylation will selectively occur with the ethanethio-glycoside as reported by concept Codee *et al* (Codee Jeroen, Litjens Remy, van den Bos Leendert, Overkleeft Herman, & van der Marel Gijsbert, 2005) . They report an increase in the reactivity of the carbamate protecting group Trichloroethyl carbamate (Troc), compared to the imide protecting group, Phthalimide(Phth.)

Glycosylation mechanism

The stereochemistry of the product gives a hint on the mechanism of the reaction. We can glean at the type of intermediates involved when we know the stereochemistry of the product. But before we get the product, the stereochemistry can be predicted based on the glycosyl donor, particularly, the protecting group on carbon 2. This is because of the phenomenon of anchimeric assistance. Also known as neighboring group participation, anchimeric assistance is the participation by a neighboring group in the rate-determining step of a reaction; most often encountered in reactions of carbocation intermediates.



Scheme 1.5: Depiction of the role played by anchimeric assistance in the determination of stereochemistry at the glycosylation site.

If the neighboring group is an ether-type protecting group, there is no participation, meaning the leaving group leaves to form the oxocarbenium ion, the most likely/major product is the more stable α glycoside and the β glycoside is only a minor product. On the other hand if the group on carbon 2 is an ester-type protecting group, due to the lone pairs, the carbonyl oxygen can attack the anomeric center forming a 5 membered ring. This largely blocks attack from the alpha position, leaving only the top face of the ring open for attack as shown in Scheme 1.3. This is the reason why, even though the alpha anomer is more stable, in the case where anchimeric assistance is involved, the beta anomer is usually the major product.

Types of donors

The number of types of glycosyl donors is daunting and yet more and more are still being discovered. One cannot know which donor is ultimately best for certain glycosylation, but an educated guess can be made. For instance, highly reactive donors are perfect since they usually proceed very quickly and reduce the chance of acceptor degradation, but on the other hand, they are themselves, very prone to degradation. When designing a donor, one has to keep this in mind since a careful manipulation of protecting groups can arm or disarm a donor as discussed earlier. The other important consideration is the type of leaving group on the donor.

The leaving group (LG) in a donor is very important. Leaving groups can vary from very stable options such as those in thioglycoside to very reactive ones such as those in trichloroacetimidate. The leaving group activation should be in conditions suitable for other protecting groups on both the acceptor and donor, and usually milder conditions are preferable to harsh conditions.

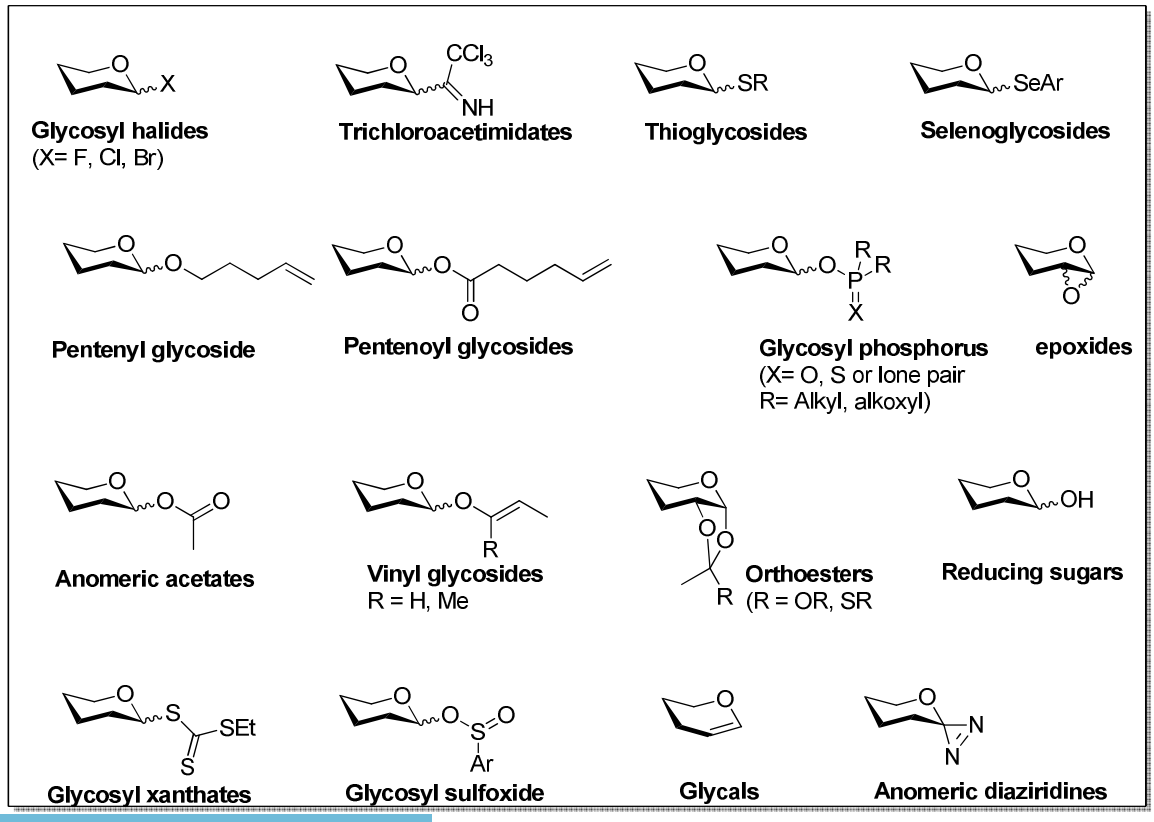


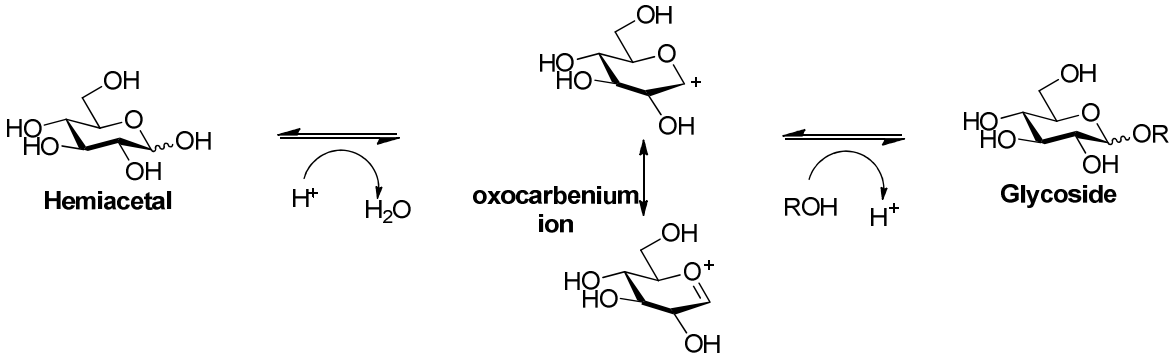
Figure 1.1: Glycosyl donors

A Sample of glycosylation methods

There are many glycosylation methods available and at different times in history, different methods were popular. Some methods, though not widely used today, can be the most suitable ones for certain glycosylation needs. Whether its Fischer glycosylation or the more recent and popular trichloroacetimidate method, glycosylation methods' have different levels of usefulness depending on specific cases.

Fischer Glycosylation

Developed at the end of the nineteenth century, this method uses acid and an alcohol to achieve glycosylation. It produces a mixture of alpha/beta furanosides and pyranosides. Furanosides are formed first and as time goes on, the equilibrium shifts to the pyranosides. This can increase the chances of another anomer being formed. Today, Fischer glycosylation can still be used in changing the anomeric configuration of certain glycans. Say there is a beta anomer and yet the alpha anomer is desired; one can treat the mixture with an acid and this will result in the formation of the alpha anomer. This also works the alpha anomer is the more stable of the two anomers (anomeric effect)



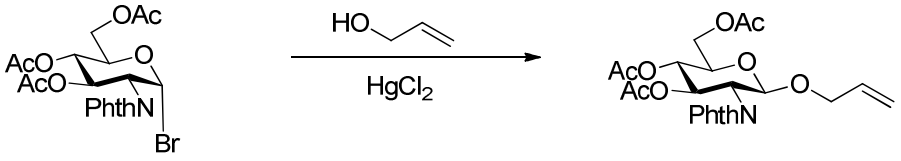
Scheme 1.6: Portrayal of Fischer glycosylation.

Today the Fischer method or Fischer like method is being used with some modifications, such as using it in the microwave for better yields or using ultrasound for more green chemistry. The main advantage with this method is still the fact that it can be used to selectively glycosylate unprotected sugars

(Artner, Stanetty, Mereiter, Zamyatina, & Kosma; Fischer, 1895; Guchhait & Misra; Shaikh, Russo, Cipolla, & Nicotra). Regio and stereoselectivity are still the major downfalls.

Koenigs-Knorr and (later) Mukaiyama

Koenigs-Knorr method has been in use since the beginning of the 20th century and there are some cases today in which this method is the best option available. Koenigs-Knorr method uses glycosyl halides (chlorides and bromides, later Mukaiyama developed glycosyl fluorides) as donors in the presence of heavy metal promoters. At its inception, this method used insoluble silver metal promoters such as Ag_2O and Ag_2CO_3, but later more soluble mercury based promoters have been used.



Scheme 1.7: Allylation of glucosamine in the presence of mercury chloride.

Today it is more common to use Koenigs-Knorr method as a way of installing protecting groups on the anomeric center rather than installing another glycoside. The obvious downfalls with this method are the use of heavy metal promoters. They are both very harzadous materials and they present a major problem for waste disposal. The other problem is with the donor. Gylcosyl halides(chlorides and bromides) are not very stable, requiring in situ preparations in environmentally unfriendly conditions.(Claffey, Casey, & Finan, 2004; Garegg, 2004; Gouliaras, Lee, Chan, & Taylor; Pazynina et al.; Saman, Wimmerova, & Wimmer, 2006; Steinmann, Thimm, & Thiem, 2007)

Mukaiyama developed the glycosyl fluoride methodology because it produces the more stable glycosyl fluorides. Glycosyl fluorides are easier to prepare(generally from glycosyl acetates with HF-pyridine, though other methods are available if the desired glycosyl fluoride is the beta anomer) and their activation does not require heavy metal promoters. The chemistry is the same as that of other halides and there is no evidence that fluorides are superior/inferior to their bromide and chloride counterparts.(Harada

& Mukaiyama, 1981; Mukaiyama, 1982, 2004; Mukaiyama, Hashimoto, & Shoda, 1983; Mukaiyama, Murai, & Shoda, 1981; Sakurai, Ikegai, & Mukaiyama, 2007)

There has to be a careful balance between the types of donor and promoter used. Since some promoters are harsh and some mild, one has to think about what type of promoter to use with what donor for both reactivity's and stereochemistry's sake. Solvents are are also an important consideration. With all the advances and studies into the process of glycosylation, there still remain major problems in generalizing reactions. The issue of reproducibility is always a problem and the need for highly skilled carbohydrate chemists to carry out these reactions is another. The use of an automated platform that can be operated by low skill level individuals is being explored in our lab. This method also has the added advantage of ensuring reproducibility.

Automated Oligosaccharide Synthesis

Carbohydrates/polysaccharides are one of only four major classes of biomolecules. Others include polypeptides(proteins) , nucleic acids(DNA,RNA) and lipids. Even though carbohydrates of most, if not all degrees of polymerization are extremely important, it is still a huge task to chemically synthesize even some disaccharides, not to mention longer polymers/oligomers. In an attempt to fill the gap between the advances made in polypeptide /nucleic acid synthesis and that made in carbohydrate synthesis, it was important to follow the leaders and develop an automated method for the synthesis of oligosaccharides. Earlier work in oligosaccharide automation synthesis focused on mimicking the Merrifield's Solid Phase Peptide Synthesis(SPPS.)

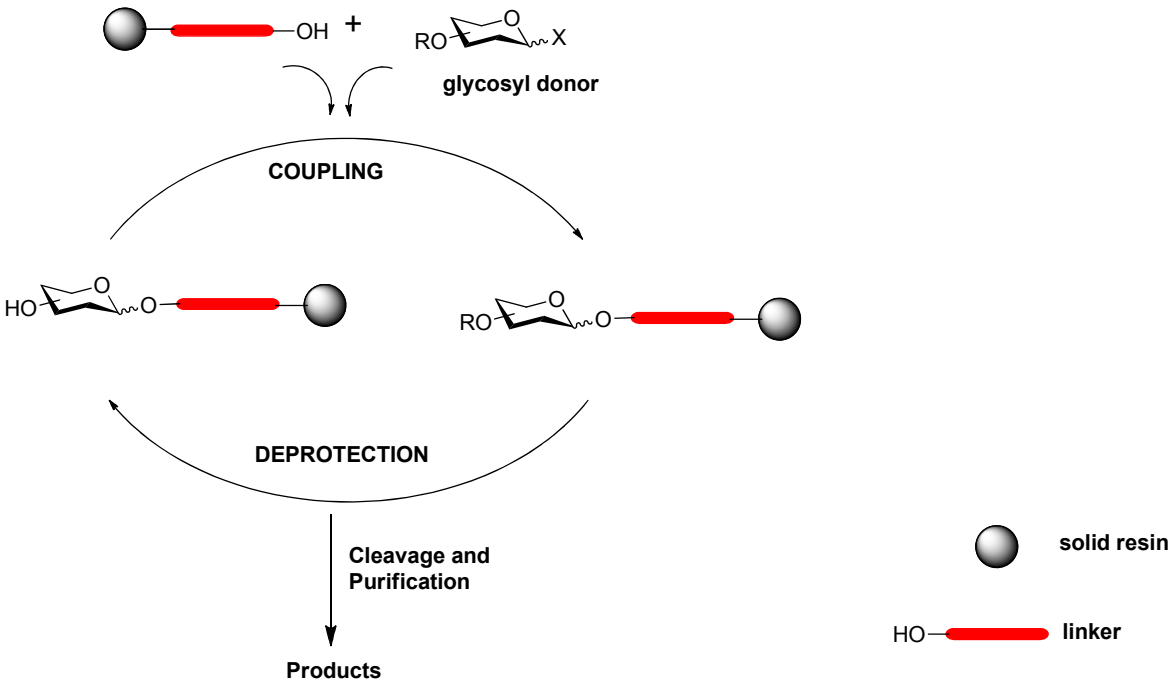
The success of automated oligosaccharide synthesis has been sluggish given the nature of the building block. Monosaccharides are far more complicated than amino acids. They contain multiple hydroxyl groups with multiple stereocenters. Fine tuning a raw monosaccharide to the stage where it can be applied to an automated platform takes a lot of time and in most cases, can only be done by experts.

Sometimes various protecting groups are required within one building block and one has to make sure that conditions used later on in the protection sequence, do not tamper with protecting groups already installed.

Coupling can also introduce problems of its own. This is because coupling a glycoside to something else can result in one of two stereogenic centers, alpha or beta. Care has to be taken when formulating the building block to make sure that the odds are in favor of the desired stereochemistry. Sometimes ensuring the product's stereochemistry can be near impossible. This problem is not encountered in protein synthesis, and neither do amino acids contains numerous reaction centers.

To that end, a solid support systems were tested for their usefulness in automated oligosaccharide synthesis by Dr. Seeberger and his group(Bindschadler, Dialer, & Seeberger, 2009; Love & Seeberger, 2005; Melean, Love, & Seeberger, 2002; Seeberger, 2008). They settled on chloromethylated styrene resin which is commercially available. The use of solid support has the same advantages as in Merrifield's original design, ease of purification at the end of the automation. By simply filtering out the liquid part of the reaction flask, one is left with the oligomer attached to a linker and solid support. A linker is necessary to attach the sugar moiety to the solid phase resin. This helps in the cleavage of the sugar from the solid support at the end of automation. The linker of choice was based on the pentenyl glycoside as shown in Scheme 1.4.

The process of automation begins with a glycoside donor being attached to the solid resin. This is then filtered to remove untagged glycosyl donors and prevent them from entering the next stage of automation. This is followed by the deprotection of one hydroxyl at the desired point of attachment. To quantify the efficiency of deprotection within the system, one can use a UV sensitive temporary protecting group, as this will inform of the amount of cleaved UV sensitive material. This deprotected hydroxyl reacts with another glycosyl donor to form a disaccharide connected to the solid support. Any untagged material is easily washed off and the process continues.



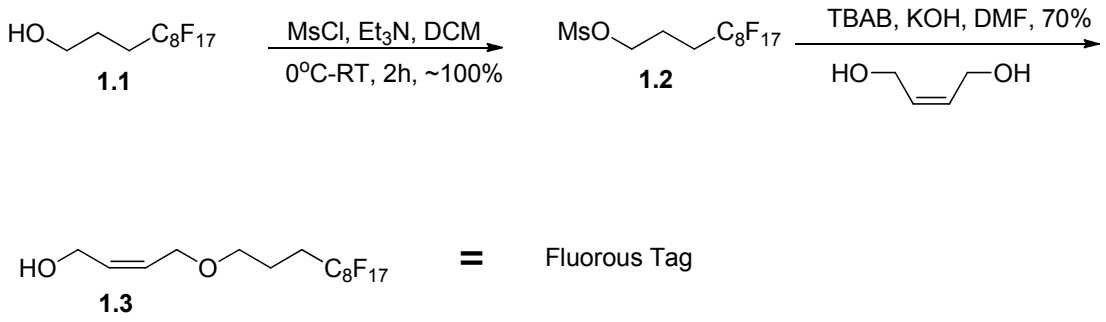
Scheme 1.8: Coupling cycle of automated oligosaccharide synthesis.

As genius as this idea is, it has a lot of shortcomings. The first major shortcoming is the fact that it does not allow room to rectify any errors in the glycosylation. For instance, if there is an error in the second glycosylation, there is no way of knowing this and the user can continue building up a long chain oligosaccharide that is wrong, without knowing. The other major shortcoming is the amount of glycosyl donors used in the glycosylation. Most bench top glycosylations require 1-3 equivalents of the donor. Using two or more equivalents of donor already feels like a waste of material, especially because it can be very difficult to synthesize these glycosyl donors. In Seeberger's solid phase oligosaccharide synthesis, at least 5 equivalents, but usually 10 or more are used for decent glycosylation efficiencies. Bearing in mind that in oligosaccharide synthesis, the tricky part is not the glycosylation itself, but the building block synthesis, this method seems to be a good idea, but not a great solution to the problem of lagging advances to oligosaccharide synthesis compared to their oligonucleotide and oligopeptide counterparts. To solve the problem of the major shortcoming, i.e. the use of huge excess of donors and failure to monitor the success of automation at every step, our group has come up with the solution phase automated oligosaccharide synthesis.

Solution Phase Oligosaccharide Synthesis

As the name suggests, this new method relies on the use of a support that is soluble but is still easy to purify. This system, Chemspeed Automated Synthesis Workstation (ASW) takes advantage of fluorous chemistry and the separation is carried out using Fluorous Solid Phase Extraction (FSPE) and the use of fluorophobic and fluorophilic solvents. The basic idea is similar to solid phase oligosaccharide synthesis with the option for the inclusion of a Thin Layer Chromatography(TLC) step and the option to pause the automated run and make sure the run was succesful. The other great advantage of this method is the use of an average of about 2 equivalents.

The fluorous tag that is key to the design and success of this system shown below as **1.3**. It was first reported by Mamidyala *et al.* in 2006. The linker is a *cis* but-2-ene-1,4-diol. Other functional group linkers have been discovered and shown to be just as efficient as this linker and some even more stable. The presence of a double bond makes this linker unsuitable for use with reducing promoter systems because the alkene functionality can be reduced to an alkane presenting a huge problem for cleavage of the linker. In cases where the cleavage of the linker is not necessary, such as in microarray experiments, this linker proceeds without a problem.



Scheme 1.9: Synthesis of allyl-based fluorous tag(Allyl-F-tag)

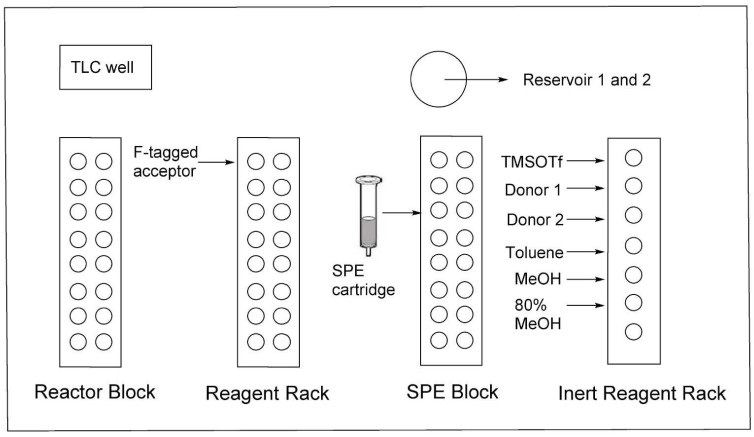


Figure 1.2: Chemspeed Automated Synthesis Workstation (ASW) design

The design of the system includes a two reagent racks, one sealed and connected to an inert gas source for those reagents that are air and moisture sensitive, and the other, a regular reagent block for the more stable reagents. The SPE block is the purification station. FSPE cartridges are places in the block and two different washes carried out. The first wash is with a fluorophobic solvent and this washes away all the untagged oligosaccharides, then the fluorophilic solvent unhinges the tagged sugar from the FSPE column making sure that only the tagged sugars are able to continue with the cycle. 80% methanol in water is the fluorophobic solvent used in this work and 100% methanol is the fluorophilic solvent used.

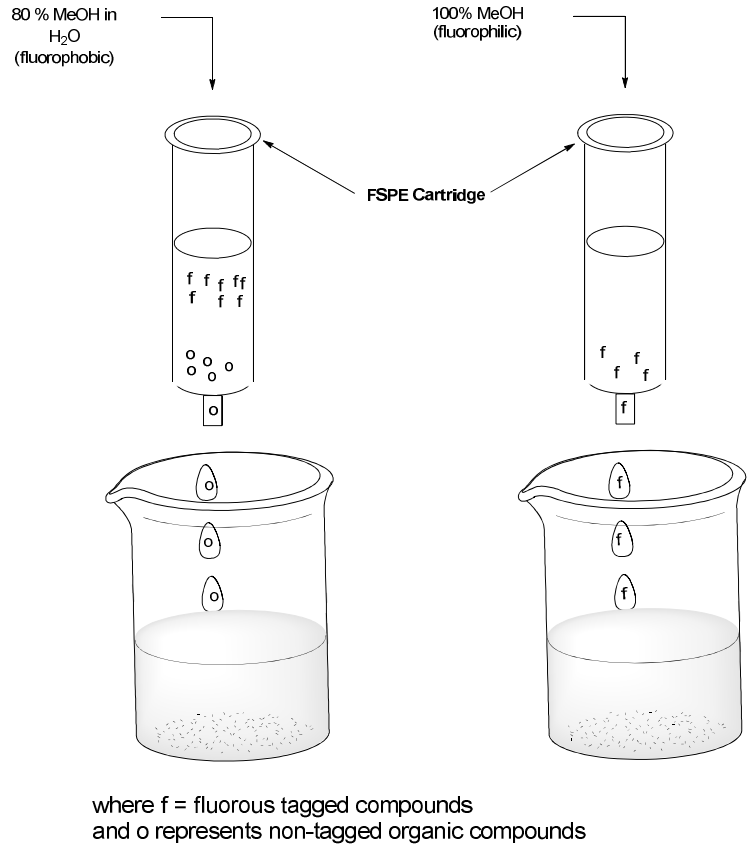


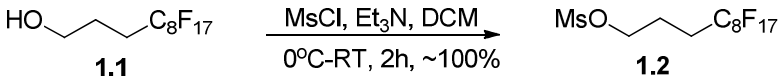
Figure 1.3: Demonstration of separation of fluorous versus non-fluorous compounds using Fluorous Solid Phase Extraction (FSPE.)

The reagent block is the place where glycosylation happen. The system is equipped with the ability to shake at different speeds and currently has the capacity to carry out reactions at temperatures as low as -20^{o}C and as high as 70^{o}C. The reservoirs are used to store solvents, and more solvents can be stored on the reagent racks, inert or not.

Even though still lagging, carbohydrate chemistry is slowly becoming more and more easily accessible. The main problem that most carbohydrate chemist are working on is making building blocks more readily available. Improvements such as limiting the amount of waste by using alternative purification methods such as crystallization are being explored. Like in any other field, there is an interest to find the shortest and cheapest route to most building blocks.

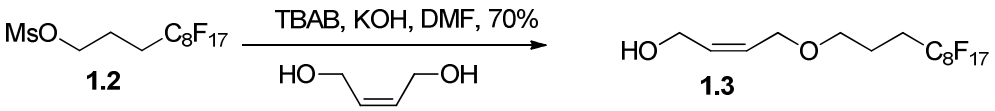
EXPERIMENTAL SECTION (CHAPTER 1)

***Synthesis of 3-(perfluorooctyl)propanyl methyl sulfonate* (1.2)**



3-(perfluorooctyl)propanol **1.1** (1.0 g, 2.1 mmol) was mixed with 10 mL methylene chloride. Triethylamine (0.6 mL, 4.18 mmol) was added and cooled to 0°C. Mesyl chloride (0.32 mL, 4.2 mmol) was added over a period of 5 min and the reaction mixture was allowed to warm to ambient temperature over 2 h. The resulting mixture was washed with water , 2N HCl , and brine and dried over MgSO4. The solvent was removed under reduced pressure to provide 2 (1.10 g, quantitative yield) as a solid. ^{1}H NMR (400 MHz, CDCl3): d 2.05–2.13 (m, 2H), 2.20–2.36 (m, 2H), 3.04 (s, 3H), 4.31 (t, 2H) as reported.

***Synthesis of 4-[3-(perfluorooctyl)propyloxy]-cis-2-butenyl alcohol* (1.3)**

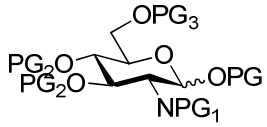


To a solution of cis-1,4-butenediol (0.24 g, 2.7 mmol), 3-(perfluorooctyl)propanyl methyl sulfonate (1.10 g, 2.08 mmol), and tetrabutylammonium bromide(TBAB) (0.15 g, 0.44 mmol) in DMF was added freshly ground KOH pellets (0.235 g, 4.16 mmol). The reaction mixture was heated at 70 °C for 1 h quenched by the addition of water. The aqueous layer was extracted with ethyl acetate and the organic layer was washed with water and brine and dried over MgSO4. The solvent was removed under reduced pressure. Flash chromatography of the product at 30% ethyl acetate in hexane gave the product as a yellow oil(0.75g, 70%) ^{1}H NMR (400 MHz, CDCl3): d 1.78–1.88 (m, 2H), 2.04– 2.19 (m, 2H), 2.99 (s, 1H), 3.45 (t, , 2H), 4.0 (d, 2H), 4.13 (d, 2H), 5.57–5.65 (m, 1H), 5.70–5.78 (m, 1H) as reported

CHAPTER 2. SYNTHESIS OF A GLUCOSAMINE BUILDING BLOCK

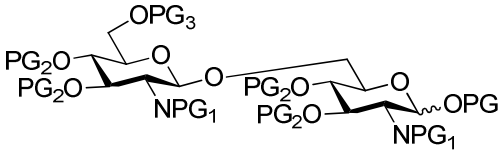
Introduction

The synthesis of glucosamine building block has been studied intensively. Glucosamine is a deoxy sugar with an amine on the second carbon. The presence of the amine on the C2 presents a challenge that cannot be overlooked. For this project, I present various methods to the synthesis of the following building block:

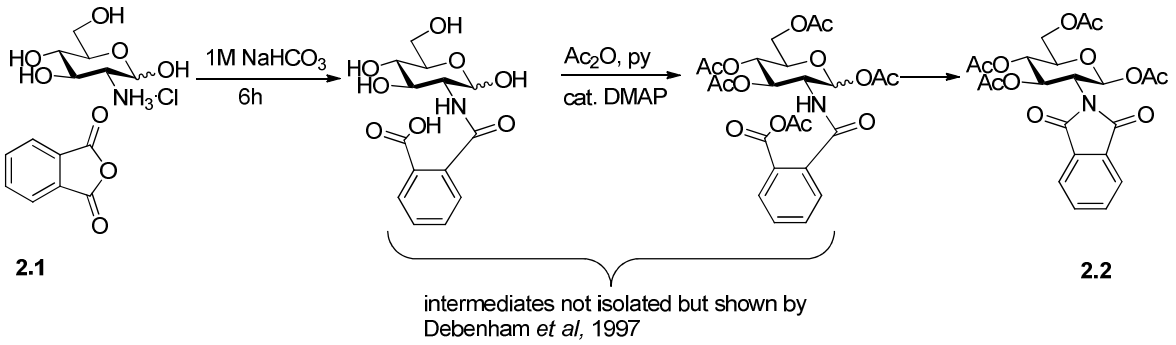


PG= **P**rotecting **G**roup

Various protecting group combinations were tried in order to formulate a building block for the synthesis of a 1-6 linked glucosamine disaccharide that can still be connected to another sugar, KDO on the 6 position. The aim of this process is to find a building block with a combination of protecting groups that are less time consuming, very cost effective and very reactive.

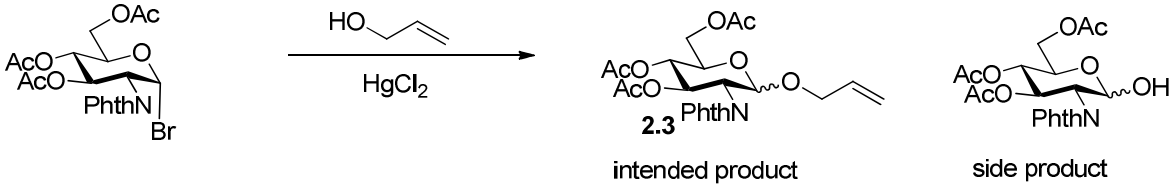


In amine sugars, the first functional group to be protected is the amine since the amines are generally more reactive than their hydroxyl counterparts. In this case the phthalimide protecting group was used initially. It is a relatively straight forward and one step protecting process and favors the formation of a mainly beta intermediate products. The installation of a phthalimide group on the amine requires that it be followed by an acetylation reaction since this allows for the closing of the imidic ring.(Debenham, Rodebaugh, & Fraser-Reid, 1997) This is because closing the imidic ring without the acetyl group on the carboxylic acid would be impossible.



Scheme 2.1: The importance of acetylation reaction, following the protection of the amine functionality with phthalic anhydride.

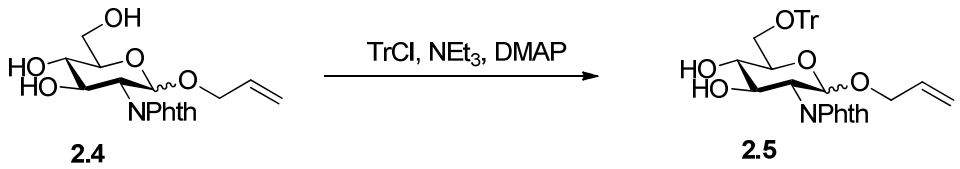
On top of helping close the ring, the presence of the acetyl group at the anomeric center helps with the next reaction. The anomeric protecting group needs to be one that is stable under most protecting group conditions because the group will be carried through until the final step before formation of the glycosyl donor. As a result, the allylic group was chosen as it is both acid and base stable and it can be selectively removed by the use of iridium catalyst. Initially the attempt to allylate was carried through the bromination followed by the Koenigs-Knorr reaction in the presence of $HgCl_2$.



Scheme 2.2: Allylation via the Koenigs-Knorr reaction

This procedure proved to be inefficient; it is a two-step reaction that uses highly sensitive (HBr/AcOH) and dangerous reagents ($HgCl_2$). The formation of the bromoglycoside is very unstable that it was hydrolyzed to the deprotected anomeric OH. There was NMR evidence for the formation of the highly unstable beta halide. A more efficient allylation procedure was the allylation of **2.2** using boron trifluoride dietherate in dichloromethane at zero degrees. Allylation provided an efficient method for the anomeric protection but it does require heavy metal catalyst for deallylation and thus alternatives were explored.

2.3 was deacetylated followed by the installation of a protecting group that is selective to primary alcohols and does not react with secondary alcohols. The Zemplen deacetylation with sodium methoxide in the presence of phthalimido group can be challenging and needed to be monitored closely. Since the other objective of the project was to decrease the cost of the building block, the silyl-protecting groups were not considered at this time. The trityl (Tr) group seemed a very attractive option since it is very cheap and very selective for the primary alcohol. It gives very good yields and can easily be extracted out as a solid in most solvents.



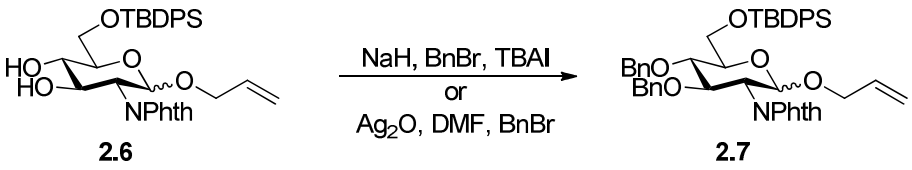
2.4 **2.5**

Scheme 2.3: Masking of the primary hydroxyl of glucosamine with trityl group (Tr)

The tritylated glycoside seemed to be perfect but subsequent reactions could not happen because there were challenges with solubility. The other problem was the fact that the OTr is a very stable protecting group that requires harsh deprotection conditions. Detritylation is achieved by using extremely acidic conditions, 80% acetic acid in water under reflux. In these conditions glycosidic bonds would be destroyed, so this building block is not ideal. The sequence of glycosylation and deprotection as depicted by Scheme 2-3 below, starts with the glycosylation of the donor and acceptor (F-tag). This is followed by the demasking of C-6 protecting group. If trityl ether protection is used, the extreme deprotection protocol would cleave the glycosidic bond. At this point, the use of bulky silyl protecting groups though expensive, seemed necessary because they have had better success in masking primary alcohols while producing intermediates that are useful in the path of building block synthesis. The triisopropylsilyl(TIPS) chloride was chosen as the reagent of choice because it had never been used on the automated solution phase platform while others bulky reagents such as tert-butyldiphenylsilyl(TBDPS) chloride had been used. This reaction has been reported and continued without any problems. Problems arose in the deprotection step of

the automated platform. It was a very slow process requiring upwards of 24hours to finish the deprotection protocol. This is not only unfavorable because of the amount of time but also because the longer you allow your reagents to sit in the automated synthesizer the higher the chances they will degrade. It was for this reason that at the end, TBDPS was used.

After that the protection of C3 and C4 hydroxyls was necessary and benzyl was the masking agent of choice. This presented quite a lot of problems. Using neutral conditions of Ag_2O resulted in incomplete reactions. The need to freshly prepare silver oxide and the use of heavy metal reagents made this option not very attractive. Benzylation in basic conditions (NaH) presented problems for the phthalimido protecting group on C2 and a strategy which involved tweaking the amount of NaH and time allowed for a reaction resulted in an acceptable result.



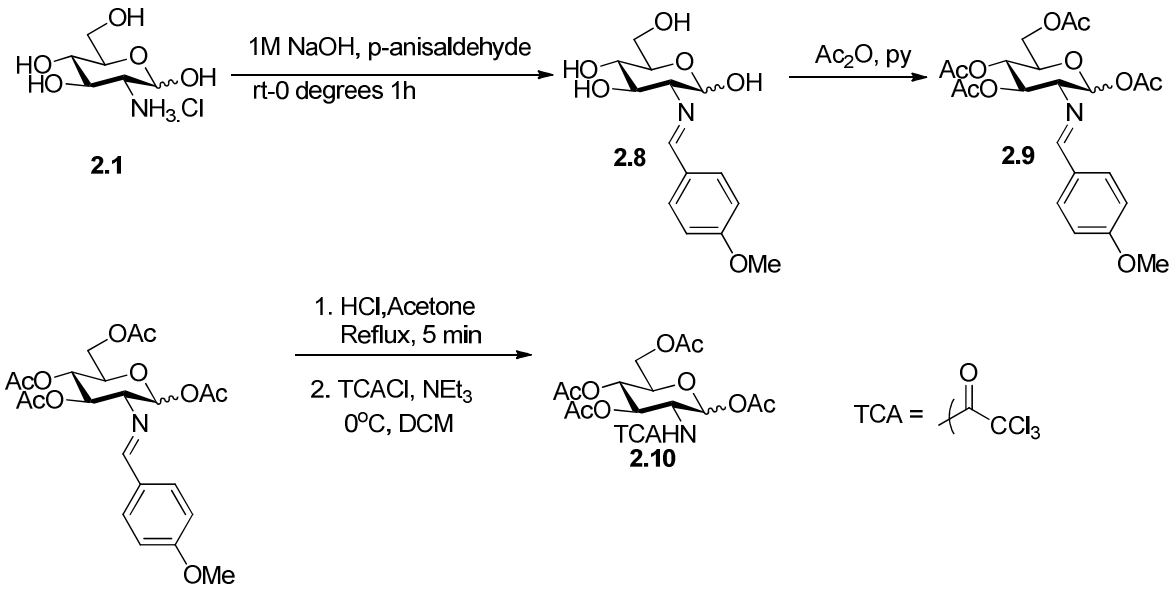
Scheme 2.4: Different benzylation reactions on glucosamine building block

Removing the allyl group at the anomeric position using iridium catalyst easily affords the predonor intermediate.

The quest to find the most efficient and cost effective route to the glucosamine donor led to a consideration of a lot of other building blocks. This meant revising every protecting group including the amine protection. Since different protecting group experimental procedures work differently with various other protecting groups, it was important to work unilaterally with donors from different N protection. some reactions and procedures, for instance, allylation of the anomeric center may be a recurring theme.

Other Amine Protecting Groups

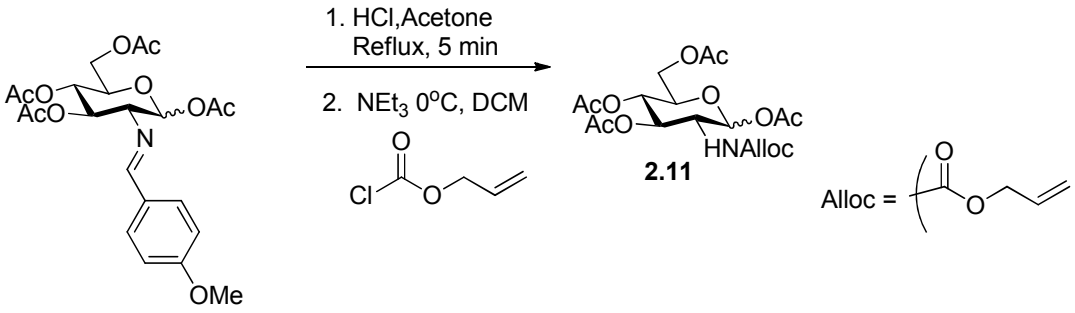
The phthalimide protecting group is very easy to install and not very difficult to remove. The main problem associated with it is the lack of stability in basic conditions. Since a lot of the conditions in the building block synthesis are basic, this presents a problem especially because the amine protection is installed first and does not get removed until after the glycosylation. For this reason, the trichloroacetimidate protecting group was also explored. The disadvantage of the trichloroacetimidate protection is the fact that installation involves a multi step process and this takes more time and money, but a viable method nonetheless if it has the possibility of avoiding problems that would be encountered by the more labile phthalimide donor. Being so close to the anomeric position, it would be interesting to find out how changing the protecting group, from a more rigid and somewhat bulky phthalimide group to a more flexible and very bulky trichloroacetimidate group would affect building block synthesis and glycosylations.



Scheme 2.5: Steps necessary for the protection of amine group with the N- trichloroacetyl (NTCA) group.

The formation of the N-Alloc protecting group follows the same procedure as the one above, except instead of using trichloroacetyl chloride; allylchloroformate was used as shown by the formation of **2.11** below. Even though the carbamate is not as stable as the acetamide protecting group, both building block

approaches were carried on. The carbamate building block provides the comparison for a non-rigid but slightly unhindered 2 position, and its effect on glycosylation would be important to record. Note that all the protecting groups provide neighboring group participation, and this is a deliberate choice since all the intended glycosylation are expected to be beta.



Scheme 2.6: N-Alloc protecting group procedure

With 3-4 candidates for the protection of the amine, attention can be focused in the anomeric protecting groups that can be used in the building block synthesis. The allyl group has already been discussed briefly. Initial problems with it involved the use of mercuric reagent and hydrobromide in acetic acid solution. Both reagents are toxic and the reaction is not user friendly. This problem was solved by the use of a less expensive, 1 step process using boron trifluoride as the promoter from the glycosyl acetate. The procedure for the general allylation reaction can be found in the experimental section at the end of the chapter. The deallylation procedure also requires the use of yet another expensive heavy metal based catalyst. The cost and type of reagents involved with the allyl protection necessitated the search of yet another anomeric protecting group.

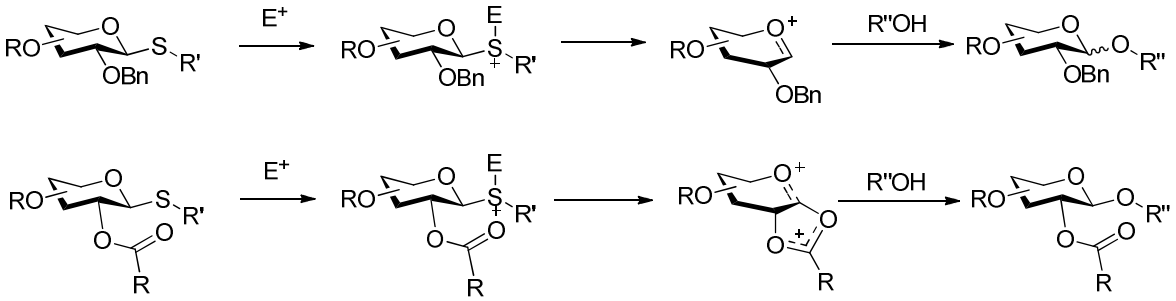
Another anomeric protecting group that has been widely explored is the use of alkylthiols. These are interesting because thioglycosides can be directly activated to allow for a shorter journey to glycosylation and thereby reducing time and costs associated with building block synthesis. It is important to explore the usefulness of this reagent in the automated solution phase platform.

Thioglycosides in automated solution phase synthesis

Thioglycosides are a major class of glycosyl donors. They are an attractive donor because of their stability which allows for early introduction of the thio group and that in turn limits the number of steps in building block synthesis. Though not as reactive as other glycosyl donors, the thioglycoside is activated by an electrophile to produce an intermediate sulfonium ions, which then gives rise to carbenium intermediate which when reacted with an acceptor, gives the glycosylation product.

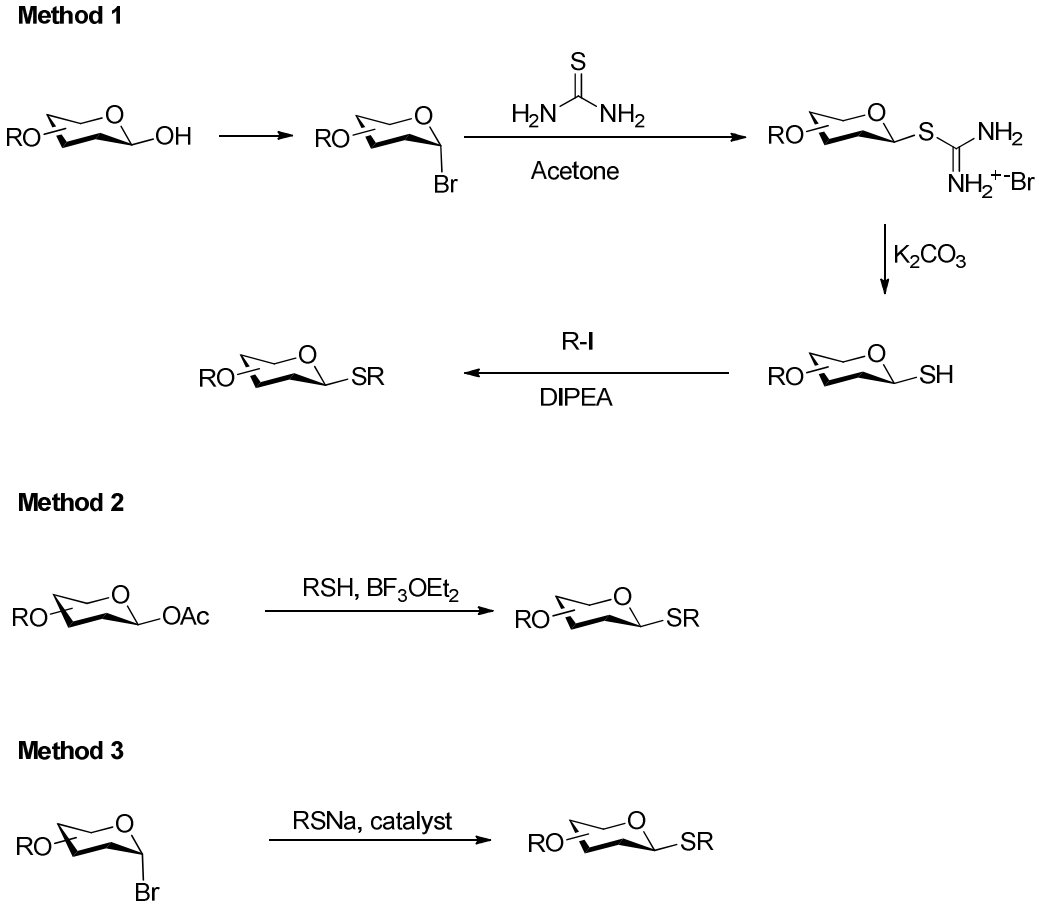
Thioglycosides can be activated using any number of reaction conditions and the appropriateness depends on the conditions available, the donor and acceptors reactivity, consideration for the safety. The most common method was first introduced in 1980 (Hanessian & Guindon, 1980) and uses a halonium ion as an electrophile. NBS and NIS are commonly used to supply the brominium and iodinium ions respectively. It is always necessary to use an additive for instance, a triflate source to increase the yields of these glycosylation reactions.

For the promoter system to be applicable to solution phase automated synthesis, it has to be in liquid form or soluble in some solvent. This is to facilitate the transfer of the promoter system to the reaction vial in the automated solution phase platform. Most of the promoter systems for thioglycosides are not suitable for our purposes because they are in solid form and do not dissolve in most solvents. This simplifies the candidates for glycosylation trials. Of particular interest, is the application of a new promoter system reported by Tatai *et al* which involves the use of the cheap and readily available dimethyl disulfide and triflic anhydride.



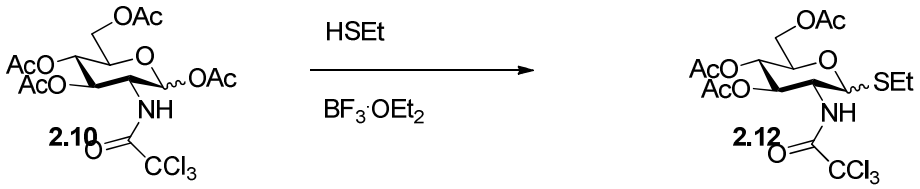
Scheme 2.7: Thioglycoside activation for glycosides with and without neighboring group participation.

There are several ways of synthesizing thioglycosides. Horton D, *et al* described a method that goes from an anomeric OH to an anomeric SH and then alkylation/arylation of the thioglycoside as shown in Method 1 of Scheme 2.7. Even though this method involves very high yielding reaction steps and utilizes very cost effective reagents, it is a long process. Another method for making thioglycosides is described by Ferrier R. *et al* in 1980 (Method 2). In their work, they show an acid displacement at the anomeric center in the presence of a Lewis acid. The base displacement at the anomeric center was reported by Tropper *et al* in 1991



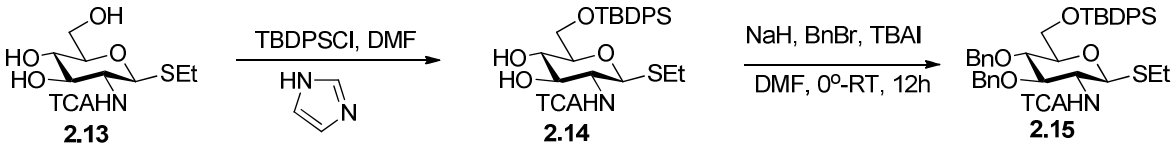
Scheme 2.8: Synthesis of thioglycosides

Synthesis of the thioglycoside with both phthalimide protection and trichloroacetamide protection was carried out. Most of the reactions were the same after the initial differentiation between thio and O-allyl protection.



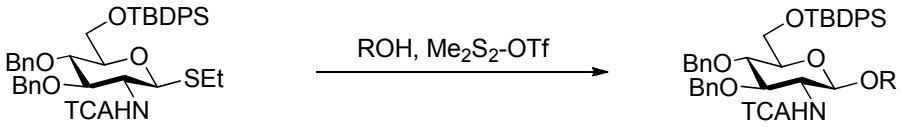
Scheme 2.9: Ethane-thiolation of glycosyl acetate in the presence of a Lewis Acid, $BF_3{\cdot}OEt_2$

After Zemplen deacetylation, a bulky silylating agent was used to mask the primary alcohol. Triisopropylsilyl(TIPS) group was first used and then tert-butyldiphenylsilyl(TBDPS) group because it is easier to remove this group on the automated solution phase synthesizer. This was followed by the usual benzylation reaction using NaH and benzyl bromide. It is important to note that the same reactions were carried out with allyl protection and similar reactions with the phthalimide protecting group at C2.



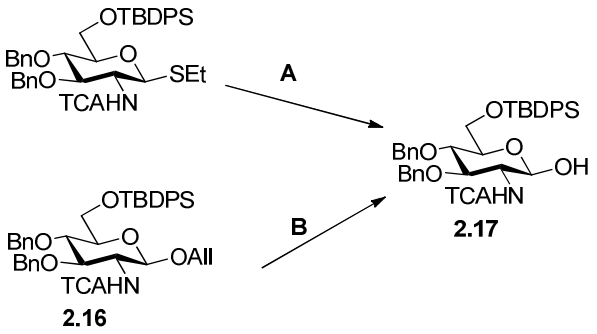
Scheme 2.10: Silylation followed by benzylation of a thioglycoside.

As a thioglycosyl donor, building block **2.15** is ready for glycosylation. This reaction was tried on the bench top to see whether it would be viable for the automated solution phase synthesizer. The dimethyl disulfide triflate promoter system was used and was moderately reactive, giving percent yields of 70%. The main problem with the reagent is that the smell of dimethyl disulfide makes it difficult to work with and would present odor problems on the automated synthesizer.



Scheme 2.11: Glycosylation using a thioglycoside donor

Another option is to remove the anomeric protection group and transform it into an imidate donor. Clearly, it is advantageous to use the thioglycoside as a donor because one can avoid two expensive steps. Since thioglycoside are not as reactive as their imidate counterparts, it is sometimes necessary to go dethiolate and this could be followed by donor synthesis. Scheme 2.11 below shows the

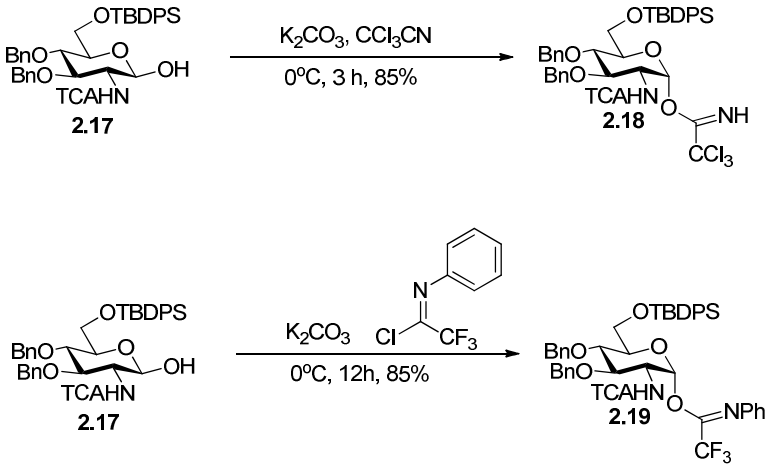


Scheme 2.12: Predonor synthesis with subsequent donor.

A: NBS in 10% water in acetone and **B** is a two-step reaction going through the hydrogenation/isomerization of the double bond using an iridium catalyst which is followed by the cleavage using I_2 and THF/water as a solvent. Both reactions are fairly reactive with approximately 80% yield.

Trichloroacetimidate and trifluoroacetimidate donors

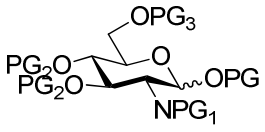
After deprotection of the anomeric hydroxyl, two options are available. The more common trichloroacetimidate donor could be formed using trichloroacetonitrile(CCl_3CN) in the presence of K_2CO_3. The other option was any one of the N-aryl trifluoroacetimidate donors. The trichloroacetimidate was expected to be very unstable in most conditions and thus the use of the backup method with the more stable N-phenyl trifluoroacetimidate.



Scheme 2.13: Synthesis of glucosamine trifluoroacetimidate and trichloroacetimidate donors.

Butane Diacetal Sugars

Since the building block of interest involves the protection of 3,4 hydroxyls with permanent protecting groups, the butane diacetal protecting group seems like the perfect choice. While the selective protection of primary and anomeric hydroxyl groups is very easy to achieve, selective protection of secondary alcohols is usually not so easy. The butane diacetal protecting group is very selective to trans-diequatorial-1,2-diols such as found in glucosamine. According to a review by Lence *et al* (Lence, Castedo, & Gonzalez-Bello, 2008) the selectivity for trans-diequatorial-1,2 diols is due to the formation of a relatively sterically favorable trans-ring junction.

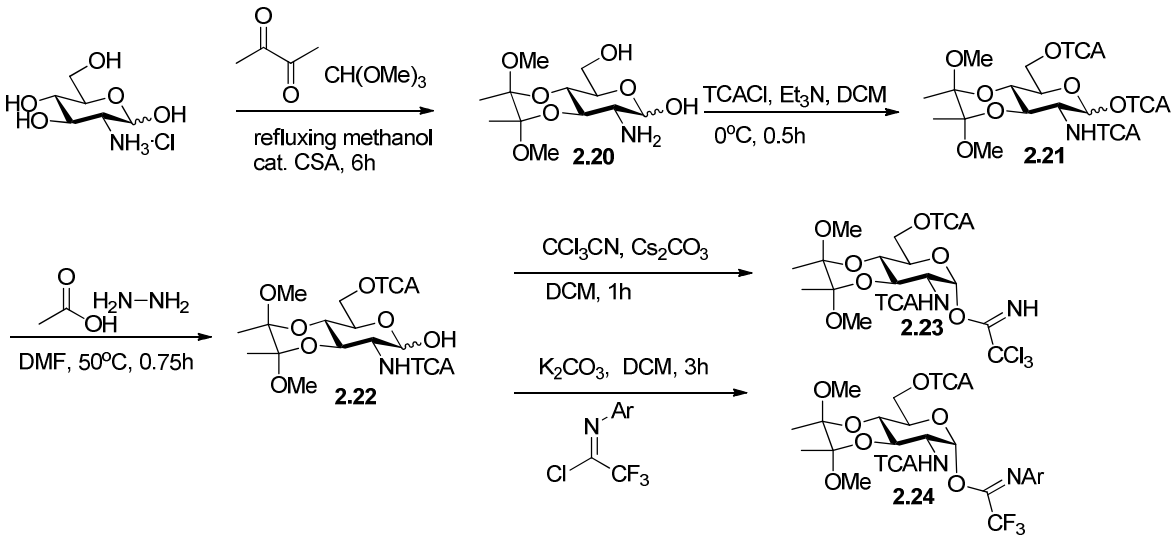


Using the butane diacetal could help with two problems; first, one can potentially work in a straightforward manner to get to the desired building block. Theoretically, the protection can start with amine protection, followed by butane diacetal formation and then anomeric and primary hydroxyl protection. Because of the manner in which the protection happens, it is possible to avoid the use of the expensive silyl groups. This will provide a very cheap, building block made with fewer all high yielding

steps. Possible synthetic route explored to this end is shown below. The other option is to install the diacetal straight from the glucosamine salt. This significantly decreases the length, in number of steps and amount of time. It also increases the yield and decreases the cost to an efficient building block. Forming another ring on the sugar moiety is usually frowned upon since it has been shown that it increases rigidity and therefore decreases the reactivity of the building block (Baeschlin et al., 2000). During glycosylation, the formation of a carbenium intermediate results in the bending of the chair structure into more of a boat conformation. Having a ring on the structure confines the flexibility and prevents the ring from easily changing its conformation to accommodate the formation of an oxazoline ring.

In a recent review (Zhu & Schmidt, 2009) Schmidt *et al* explain the mechanism of glucosamine donors with neighboring group participation. This mechanism is said to go through a very stable oxazoline intermediate (Demchenko, 2008), which even under very harsh conditions exhibit limited glycosyl donor properties. Having a structure that is prohibitive to the formation of a very stable intermediate may just work best for glucosamine.

The butane diacetal protecting group would not be suited long chain oligosaccharide synthesis because of the harsh conditions required to remove them. Usually, more than 80% trifluoroacetic acid in water is required for extended periods of time (~12 hours) and this would break the glycosidic bond. Even though this is true, it is still a viable method to use to decrease both the cost and number of steps associated with this particular building block.

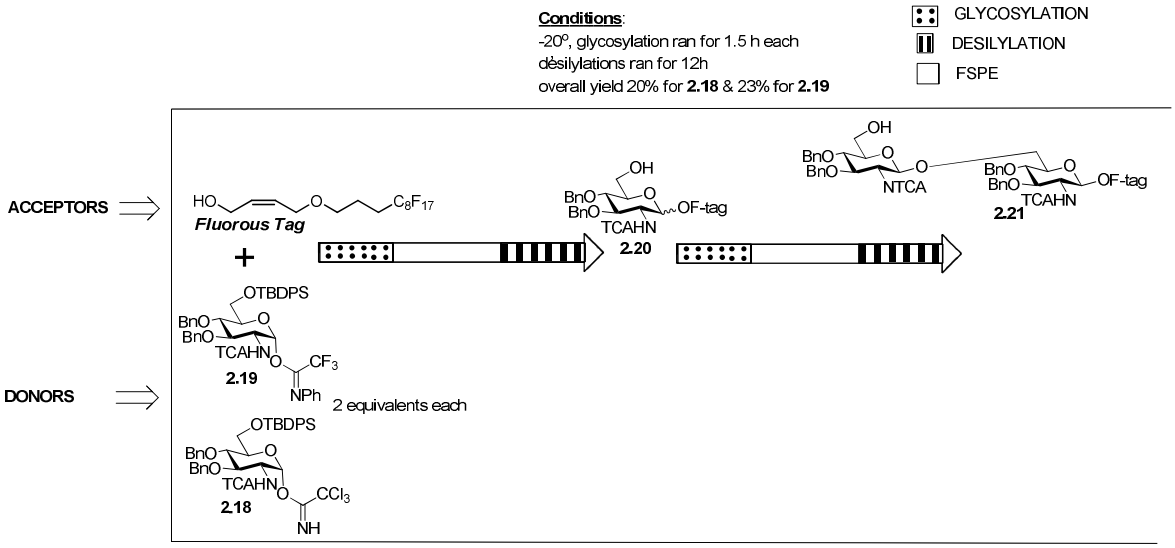


Scheme 2.15: Formation of a glucosamine donor through protection with 1,2 butane diacetal.

Many different methods have been described to get to the glycosyl donor. What is important ultimately, is the glycosylation. What type of linkage will occur and what stereochemistry it will result in.

Synthesis of a glucosamine disaccharide

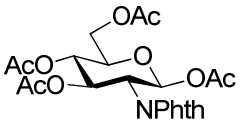
Glycosylation was carried out in the presence of catalytic amounts of TMSOTf for both the trichloroacetimidate and trifluoroacetimidate donors. There was no significant differences in the yield from the two different donors. It did however take more time to complete the reaction with trifluoroacetimidate (allowed to run for 3 hours as opposed to ~1 hour for the trichloroacetimidate reaction. The initial tagging with the fluorous marker resulted in a high yielding reaction, ~85% yield. This is because of the simpleness of the alcohol, it is allylic and primary. That compared to adding any hydroxyl groups from a sugar would be easier, even if the sugar hydroxyl is also primary. The reaction ran on the automated platform gave similar results.



Scheme 2.16: Glycosylation.

EXPERIMENTAL SECTION (CHAPTER 2)

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-D-glucose (**2.2**)

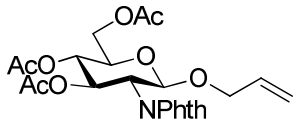


2.2

Glucosamine hydrochloride (15g, 70mmol) was dissolved in 50mL of 1M $NaHCO_3$ nd phthalic anhydride (10.5g, 71mmol) was added, reaction stirred at room temperature until all traces of the phthalic anhydride had disappeared(about 6 hours.) Reaction mixture evaporated under reduced pressure and co-evaporated with toluene (3 x 20mL) several times and allowed to dry under vaccum. This intermediate was used without purification directly with 75mL of pyridine (870mmol) and 83mL of acetic anhydride (870mmol.) This reaction was stirred at room temperature for another 6 hours. The resultant mixture was worked up with water, saturated sodium bicarbonate and dried over $MgSO_4$. Flash chromatography of the concentrated product with 30% ethyl acetate in hexane gave the foamy product (22g, 46 mmol) in 66% yield in about 2:3 alpha beta ratio. ^{1}HNMR (300MHz) δ 1.87, 2.03-2.11, 12H Me; 4.01 ,m, H5; 4.15 ,m,

H6; 4.4, m, H6; 4.48, m, H2; 5.22, m, H4; 5.89, m, H3; 6.5, d, and 6.55, t, H1 in 2:3 α/β; 7.72 and 7.83, m, 4H aromatic protons as reported.

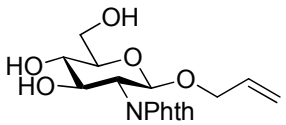
Allyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**2.3**)



2.3

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-D-glucose, (5g, 10.5mmol) was dissolved in dry methylene chloride and allyl alcohol (1.82g, 31.5 mmol) was added at 0^{o}C. Boron trifluoride diethetate (2.23g, 15.75mmol) was added and reaction mixture stirred for 5 hours and allowed to warm up to room temperature. The reaction was quenched with triethylamine and worked up with saturated sodium bicarbonate, water and dried over $MgSO_4$. The organic layer was evaporated under reduced pressure and Flash Chromatography 40% ethyl acetate in hexane afforded a foamy product (4.3g, 9.0mmol) 86% in the pure beta form. δ 7.8-7.7 (m, 4 H, aromatic protons), 5.80 (dd, 1 H, H3), 5.67 (m, 1 H, -CH=), 5.42 (d, 1 H, J 8.5 Hz, Hl), 5.19 (dd, 1 H, H4), 5.14-5.08 (qAB, 2 H, =CH,), 4.36 (dd, 1 H, J 10.7 and 8.5 Hz, H2), 4.02-4.18 (m, 2 H, -OCH,CH=), 3.84 (m, 1 H, H5), 2.12, 2.02, and 1.86 (3 s, 9 H, 3Ac) as reported by Kito and Anderson **1985**.

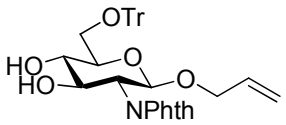
1-O-allyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**2.4**)



2.4

2.3 was dissolved in dry methanol at room temperature. A chunk of sodium metal was added and the reaction allowed to stir for 0.5 h and monitored by TLC using 100% Ethyl Acetate solvent. When only one spot(blob) around Rf 0.4 was observed, the reaction was neutralized with acidic Dowex resin and filtered and concentrated to afford compound **2.4** which was used in the next step without characterization.

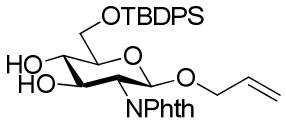
1-O-allyl-2-deoxy-2-phthalimido-6-trityl-β-D-glucopyranoside (**2.5**)



2.5

2.4 was dissolved in DMF and 1.2 equivalents of imidazole added. Mixture stirred at 80^{o} C and 1.2 equivalents of trityl chloride added and allowed to run overnight. Mixture cooled to room temperature and washed with $NaHCO_{3,}$ water and dried with $MgSO_4$. Concentration and Flash Chromatography produced a white solid as the product. (85% yield) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86- 7.83 (d, 2H, Phth), 7.73-7.70(d, 2H, Phth)\, 7.49-7.46(d, Ph_3), 7.33-7.26(m, $Ph_{3)}$, 5.75- 5.70(m, 1H, CH=), 5.28- 5.25(d, 1H, H-1), 5.17-5.04(m, 2H), 4.30- 4.07(m, 4H), 3.62-, 3.45(m,4H), 2.91-2.90(d, 1H), 2.42- 2.41(d,1H)

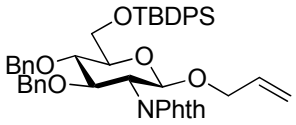
Allyl 6- tert-butyldiphenylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (2.6)



2.6

2.4 was dissolved in DMF and 1.2 equivalents of imidazole added. Mixture stirred at 0^{o} C and 1.2 equivalents of TBDPSCl added and allowed to run overnight. Mixture cooled to room temperature and washed with $NaHCO_{3,}$ water and dried with $MgSO_4$. Concentration and Flash Chromatography resulted in the product. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75-7.64(m), 7.42-7.39(m), 7.05- 6.91(m), 5.67- 5.61(m, 1H, CH=) , 5.23-5.20 (d, 1H, H-1), 5.09-4.96 ((m,2H), 4.81-4.77 (d, 1H), 4.58- 4.54 (d, 1H), 4.29 - 3.98 (m, 3H), 3.62- 3.59 (m, 1H, H-5), 3.19, 3.18, 2.00, 1.09(s,9H, tBu).

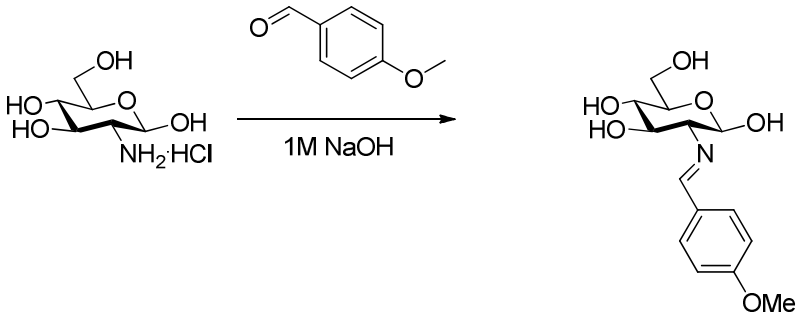
Allyl,3,4-benzyl-,6-tert-butyldiphenylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside(2.7)



2.7

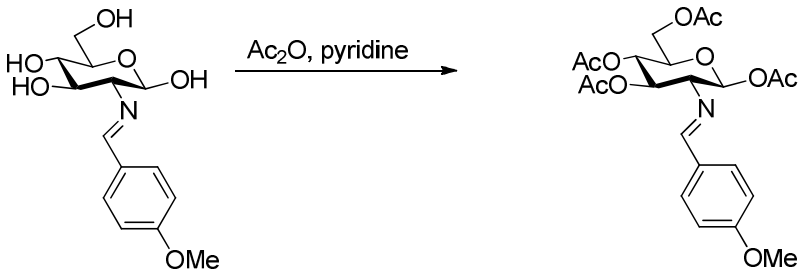
Starting material was dissolved in DMF and BnBr + TBAI added. NaH was added in small amounts in a space of an hour. The reaction was stirred for 8-12 hours and quenched by addition of ice pellets. Washed with brine and dried over $MgSO_4$. Rough spectra available in the appendix.

2-deoxy-2-[p-methoxybenzylidene(amino)-β-D-glucopyranose(2.8)



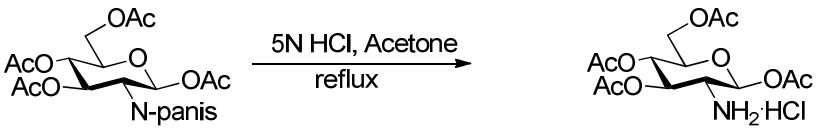
D-glucosamine hydrochloride (25 g, 0.12 mol) was added to an aqueous solution of 1 M NaOH (120 mL) and under vigorous stirring, p-anisaldehyde (17 mL, 0.14 mol) in an ice water bath, p-anisaldehyde was added. After a short time the mixture solidified and was allowed to stay in ice water bath for another hour. The product was then filtered off and washed with cold water, and followed by a mixture of 1:1 MeOH–Et_2O to give 2-deoxy-2-[p-methoxybenzylidene(amino)]- β -D-glucopyranose. This mixture was either coevaporated with toluene under high vacuum for an hour and used in the next step, or it was left to dry overnight then used directly for the next step.

1,3,4,6-tetra-Oacetyl- 2-deoxy - 2 - [p - methoxybenzylidene(amino)]-β-Dglucopyranose(2.9)



This intermediate product (25g, 0.084 mol) was added successively to a cooled (ice water) mixture of pyridine (135 mL) and Ac_2O (75 mL). The mixture was stirred in ice-bath 1 h and then at room temperature overnight. The yellow solution was poured into 500 mL of ice-water. The precipitated white product was filtered, washed with cold water and dried. This product was identified as 1,3,4,6-tetra-*O*acetyl- 2-deoxy - 2 - [*p* - methoxybenzylidene(amino)]- β –D-glucopyranose. This intermediate was coevaporated with toluene for an hour and the product used directly in the next step. ^{1}H NMR (400 MHz, CDCl3): δ = 8.16 (s, 1H), 7.66 (d, 2H), 6.92 (d, 2H), 5.95 (d, 1H,), 5.44 (t, 1H,), 5.15 (t, 1H), 4.39 (dd, 1H), 4.13 (dd, 1H,), 3.98(ddd, 1H), 3.84 (s, 3H), 3.45 (dd, 1H), 2.10 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.89 (s, 3H); ^{13}C NMR : δ= 170.9, 170.1, 169.8, 169.0, 164.5, 162.5, 130.4, 128.4, 114.2, 93.3, 73.4, 73.1, 72.9, 68.2,62.0, 55.6, 21.0, 20.9, 20.8, 20.7.

1,3,4,6-tetra-O-acetyl- β-D-glucosamine hydrochloride (2.9a)

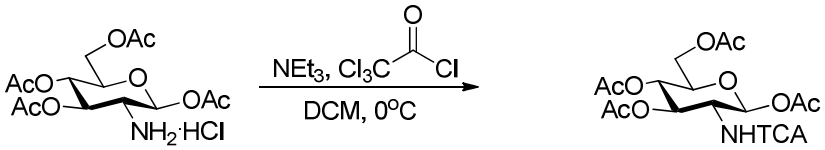


Product was dissolved in refluxing acetone and stirred. Drops of 5N HCl were added until a solid formed. This was then cooled and the product filtered out and washed with cold acetone and dried overnight. This product was used directly in the next step.

^{1}HNMR (250 MHz, DMSO-d6): δ = 8.93 (s, br, 3H, -NH3+), 5.93 (d, 1H, H1), 5.37(dd, 1H), 4.98 (dd, 1H), 4.19 (dd, 1H, H6a), 4.08–3.94 (m, 2H, H6b), 3.55 (t, 1H, H2), 2.17 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.97

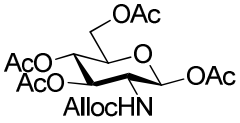
(s, 3H); 13C NMR ($DMSOd_6$) : δ = 170.0, 169.8, 169.3, 168.7, 90.1, 71.6, 70.3, 67.8, 61.3, 52.1, 21.0, 20.9, 20.5, 20.4.

Acetyl 3,4,6-Tri-O-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside(2.10)



Acetyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-amino- β -D-glucopyranoside (10 g, 23.5 mmol) was dissolved in CH_2Cl_2 (100 mL) and cooled to 0 °C. triethylamine (6.6 mL, 47.0 mmol) was added and then trichloroacetyl chloride (3.4 mL, 30.6 mmol). After 30 min, the solution was diluted with CH_2Cl_2 (150 mL) and washed with water (100 mL) and saturated aqueous $NaHCO_3$ (2 _ 150 mL). The organic extracts were dried over $MgSO_4$, and purified with 40% EtOAc in Petroleum Ether(PE) 25% EtOAc/hexanes to yield 10.9 g ^{1}H NMR (400 MHz,CDCl3): δ = 7.27–7.17 (m, 1H, NH), 5.76 (d, 1H, H1), 5.36 (dd, 1H, H3), 4.98 (t, H4), 4.24 (dd, 1H, H6a), 4.28 (ddd, 1H, H2), 4.11 (dd, 1H, H6b), 3.86 (ddd, 1H, H5), 2.07 (s, 3H,CH3CO), 2.06 (s, 3H, H3CO), 2.03 (s, 3H, CH3CO), 2.01 (s, 3H, CH3CO); 13C NMR (62.9 MHz, CDCl3): δ = 171.7, 170.8, 169.5, 169.4, 162.4, 92.3, 92.1, 73.2, 72.0, 68.0, 61.8, 54.5,20.8 (2C), 20.7, 20.6.

1,3,4,6-Tetra-O-acetyl-2-(allyloxycarbonylamino)-β-D-glucopyranoside (2.11)

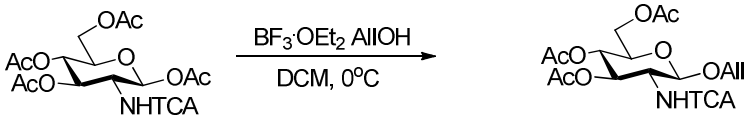


2.11

Acetyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-amino- β -D-glucopyranoside (10 g, 23.5 mmol) was dissolved in CH_2Cl_2 (100 mL) and cooled to 0 °C. triethylamine (6.6 mL, 47.0 mmol) was added and then allyl chloroformate (30.6 mmol). After 1h, the solution was diluted with CH_2Cl_2 (150 mL) and washed with

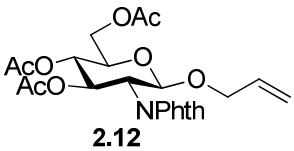
water (100 mL) and saturated aqueous $NaHCO_3$ (2 X 150 mL). The organic extracts were dried over $MgSO_4$, and purified with 40% EtOAc in Hexanes 25% EtOAc/hexanes to yield.

β-D-Glucopyranoside, 2-propen-1-yl 2-deoxy-2-[(2,2,2-trichloroacetyl)amino]-,3,4,6-triacetate (2.12a)



Acetyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-amino- β -D-glucopyranoside was dissolved in dry CH_2Cl_2 and 3 equivalents of allyl alcohol were added under stirring in an ice bath. Boron trifluoride etherate(1 equivalent) was added and the solution allowed to stir till room temperature for 5 hours. Solution quenched by aqueous workup of saturated sodium carbonate solution and water, $MgSO_4$ and filtered and dried. Flash chromatography with 30% EtOAc in PE afforded the product in 80% yield. 1H NMR (400.1 MHz, CDCl3): δ =6.77 (d, 1H, 3J = 8.8 Hz, NH), 5.77 (dddd, 1H, CH=CH2), 5.29 (dd, 1H, H3), 5.21 (dq, 1H, $CH=CH_{cis}H_{trans}$), 5.14 (dq, 1H $CH=CH_{cis}H_{trans}$), 5.06 (t, 1H, H4), 4.66 (d, 1H,H1), 4.30 (tdd, 1H, CHaHbCH=CH2), 4.22 (dd, 1H, H6a), 4.10 (dd, 1H, H6b), 4.03 (tdd, 1H CHaHbCH=CH2), 3.93 (td, 1H, H2), 3.67 (ddd,1H, H5), 2.04 (s, 3H, CH3CO), 1.97 (s, 3H, H3CO), 1.97 (s, 3H, CH3CO); 13C NMR (100.6 MHz, CDCl3): δ = 170.9, 170.7, 169.3, 161.9, 133.1, 118.2, 99.3, 92.3, 72.0, 71.5, 70.3, 68.4, 62.0, 56.0, 20.8, 20.6, 20.6

Ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-l-thio-β-D-glucopyranoside (2.12)



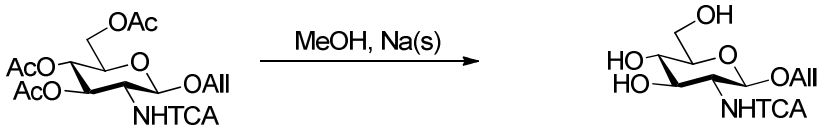
2.12

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-D-glucose, (5g, 10.5mmol) was dissolved in dry methylene chloride and ethanethiol (1.95g, 31.5 mmol) was added at 0°C. Boron trifluoride diethetate (2.23g, 15.75mmol) was added and reaction mixture stirred for 5 hours and allowed to warm up to room

temperature. The reaction was quenched with triethylamine and worked up with saturated sodium bicarbonate, water and dried over $MgSO_4$. The organic layer was evaporated under reduced pressure and Flash Chromatography 50% ethyl acetate in hexane afforded a foamy product (4.5g, 9.0mmol) 90% in form. 1H NMR (200 MHz, CDCl3): δ = 1.22 (m, 3 H, CH3), 1.87 (s, 3 H, CH3), 2.04 (s, 3 H, CH3), 2.11 (s, 3 H, CH3), 2.62–

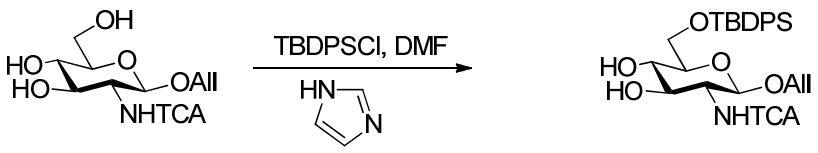
2.73 (m, 2 H, SCH2), 3.87–3.91 (m, 1 H, H2), 4.14–4.36 (m, 2 H, H6), 4.40 (t, 1 H, H5), 5.18 (t, 1 H, H3), 5.49 (d and t, 1 H, H1, 1:2 α:β), 5.84 (t, 1 H, H4), 7.70–7.90 (m, 4 H, Phth) as reported by Sun *et al.* **2006**

Allyl 2-deoxy-2-trichloracetamido-β-D-glucopyranoside (2.13a)



The starting material was dissolved and in methanol and a chunk of Na(s) was added. The reaction was allowed to stir for 3 hours and monitored through TLC. After 100% EtOAc showed one spot at around 0.25 R_f the reaction was quenched with acidic DOWEX and evaporated to give a powdery substance. This was dried and used without purification in the next step.

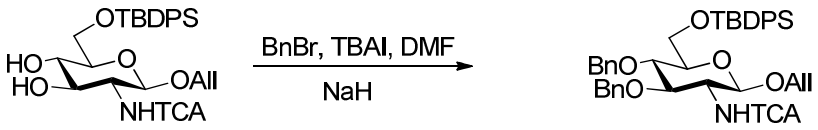
Allyl 6- tert-butyldiphenylsilyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside(2.14a)



The starting material was dissolved in DMF and imidazole was added at 0°C. TBDPSCl was added and the mixture warmed to room temperature and allowed to stir overnight. After completion, the reaction

was washed with $NaHCO_3$ and dried over $MgSO_4$. Product was used directly in the next step without purification.

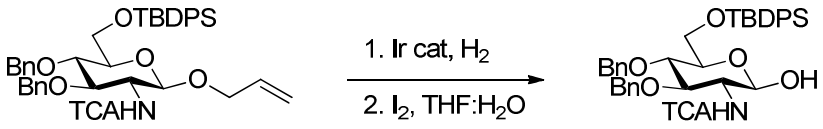
Allyl,3,4-benzyl-,6-tert-butyldiphenylsilyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside(2.16)



Starting material was dissolved in DMF and BnBr + TBAI added. NaH was added in small amounts in a space of an hour. The reaction was stirred for 8-12 hours and quenched by addition of ice pellets. Washed with brine and dried over $MgSO_4$. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.74-7.67 2X(d, 2H Phth), 7.44-7.21(m, 20H, 2X Bn + 2X Ph), 7.00-6.98 (d, 1H, NH), 5.90- 5.85(m, 1H, CH=), 5.30- 5.25 , 5.19-5.16, 4.88- 4.81, 4.75- 4.71, 4.35-4.31, 4.16- 4.13, 4.09-4.05, 3.99- 3.93, 3.86- 3.83, 3.66- 3.62, 3.52- 3.49 (m, 1H, H-5), 1.06, 1.06(s, 9H, tBu)

^{13}C NMR (151 MHz, $CDCl_3$) δ 161.78, 137.91, 137.80, 135.91, 135.56, 133.70, 133.61, 133.02, 129.69, 128.56, 128.51, 128.05, 127.96, 127.88, 127.81, 127.73, 127.60, 117.72, 98.05, 92.55, 79.45, 78.13, 77.25, 77.03, 76.82, 75.78, 74.99, 74.69, 69.77, 62.64, 58.02, 53.44, 26.81, 19.31

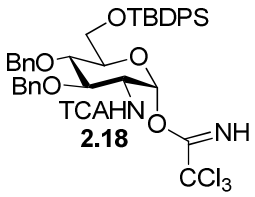
3,4-benzyl-,6-tert-butyldiphenylsilyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside(2.17)



2.11 was dried and flask evacuated. Ir catalyst added followed by evacuation of the flask. THF was added and stirred at room temperature. A balloon of H_2 was attached and this reaction was monitored for 4 minutes (or until the red color of the solution turned to pale yellow.) H_2 balloon removed and the flask flushed with Ar and stirred for another 6 hours. Solution concentrated and dissolved in 5 mL 80% THF in

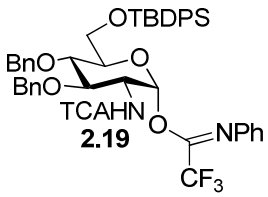
water and I_2 added. Stirred at room temperature for an hour and quenched by addition of saturated sodium thiosulfate solution and washed with sodium bicarbonate, brine and water. Concentrated and evaporated then separated by Flash Chromatography to afford **2.17** in 70% yield over 2 steps.

3,4-benzyl-,6-tert-butyldiphenylsilyl-2-deoxy-2-trichloroacetamido-α-D-glucopyranosyl trichloroacetimidate (2.18)



2.17 was dissolved in dry toluene and Cs_2CO_3 was added, followed by trichloroacetonitrile. Reaction stirred under argon at 0^o degrees Celsius for 3 hours under argon. Reaction filtered and purified using Flash Chromatography. Product foamy and 87% yield.

3,4-benzyl-,6-tert-butyldiphenylsilyl-2-deoxy-2-trichloroacetamido-α-D-glucopyranosyl, N-phenyl-trifluoroacetimidate (2.18)



2.17 was dissolved in dry toluene and K_2CO_3 was added, followed by N-phenyl-trifluoroacetimoyl chloride. Reaction stirred under argon at room temperature for 3 hours under argon. Reaction filtered and purified using Flash Chromatography. Product foamy and 85% yield.

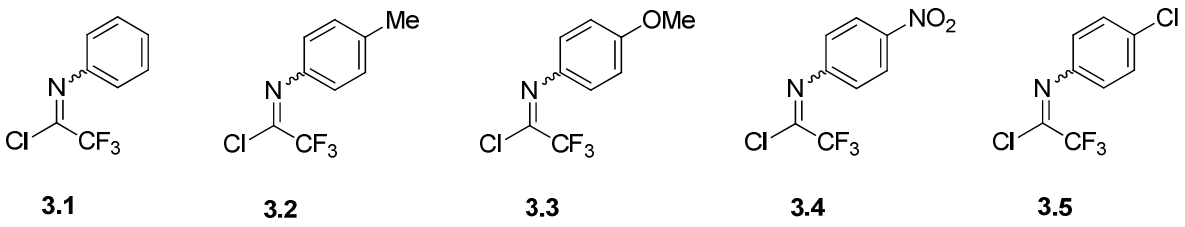
CHAPTER 3. N-ARYL TRIFLUOROACETIMIDATES

Introduction to N-Aryl trifluoroacetimidates

The three components involved in glycosylation are the glycosyl donor, the promoter system and the glycosyl acceptor. In improving glycosylation one or more of these components is changed/improved. In this project the main component that is changed is the glycosyl donor. Glycosyl donors are very varied and there are advantages and disadvantages associated with using different types of donors. So far, no one glycosyl donor is applicable to all glycosylation conditions.

One of the most popular glycoside donors is the trichloroacetimidate donor. This donor boasts of short reaction time, and generally associated with high percent yield. Even with such a good record, there are examples of glycosylation in which the trichloroacetimidate donor is superseded by the simple thioglycoside(Yu & Tao, 2001). The biggest problem with most trichloroacetimidate donors (especially glucosamine donors) is their sensitivity to normal reaction conditions and their lack of stability. Handling highly sensitive reactants can be a problem especially if one needs to do further studies. Their lability limits their accessibility to non-experts. The introduction of the aryl-trifluoroacetimidate donor aims to increase the stability of the donor while retaining a lot of the reactivity. Trifluoroacetimidate donors are less reactive than the corresponding trichloroacetimidate donors, and this is hypothesized to be as a result of the lower basicity of the nitrogen atom, the presence of a substituent on the nitrogen atom, and the smaller conformational changes caused by the trifluoromethyl group(Zhu & Schmidt, 2009)

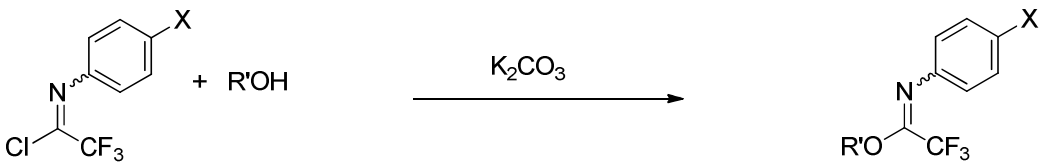
In 1993 Tamura *et al.* reported a method for one pot synthesis of several aryl trifluoroimidoyl chlorides. These can all be reagents for use in glycosyl donor synthesis and our interest in this project is with the following reagents:



3.1 **3.2** **3.3** **3.4** **3.5**

Scheme 3.1: N-aryl-trifluoroacetimidoyl chlorides

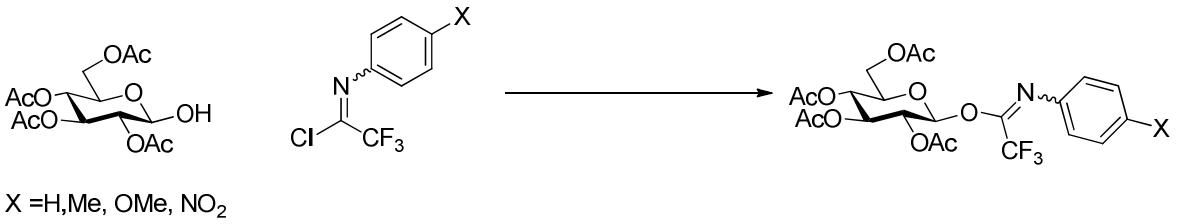
These reagents can react with any alcohol in the presence of base to give an acetimidate, which when reacted with the anomeric alcohol in a sugar would give a glycosyl donor:



Scheme 3.2: General reaction for the formation of N-aryl-trifluoroacetimidates

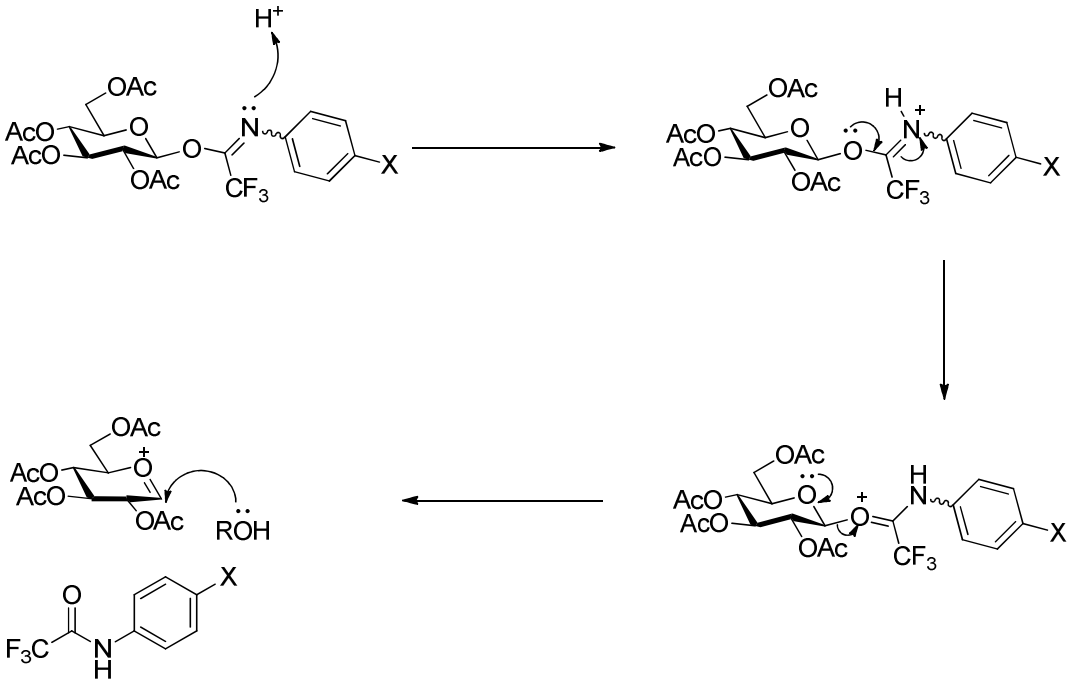
Glycosyl donors made with different substituents on the ring (X) are expected to have varying reactivity. This is because at their position, the substituent can either donate electrons or withdraw them from the reactive center. In some cases using a reagent that is mildly reactive is the better option to using a highly reactive reagent because the latter may not be able to survive in workable conditions in the laboratory setting. It is therefore vital to obtain as much information about a reagent as possible in order to tailor the most appropriate donor possible.

Stability studies for the donors are a way to figure out how appropriate they are in laboratory settings. Glycosylations happen in acidic conditions and this study will observe the stability of various trifrluoroacetimidates in acidic conditions. These reactivities will confirm the proposed mechanism of glycosylation reaction. To this end, a simple donor was synthesized and observed for stability in certain conditions. At first, a glucose donor was synthesiszed and monitored for degradation.



Scheme 3.3: Formation of a glucose N-aryl-trifluoroacetimidate donor.

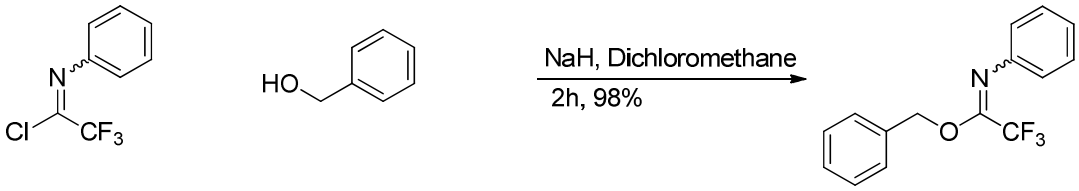
In acidic conditions, the N is protonated and a movement of electrons shown below in Scheme 3.4 results in the displacement of the imidate by the alcohol. The imidate is reaaranged into the corresponding amide.



Scheme 3.4: Proposed mechanism of the activation of trifluoroacetimidate donors in the presence of acid.

There are many disadvantages associated with using the glucose donor. First donor synthesis requires multiple steps, acetylation, selective deacetylation with hydrazine acetate and then donor formation. This means increased costs and time for getting the data. Also, after monitoring the degradation, the results have to be measured in a reliable fashion. In our case, ^{1}HNMR was used. The ^{1}HNMR of

protected sugars has too many peaks and the spectra can be difficult to read. In a situation where we are looking for even the smallest changes in spectra, this is not ideal. Since the sugar part of the donor remains the same and does not affect the study, an easier way would be to replace the sugar with a simple alcohol. This way, a readily available starting material can be used and this will increase the ease with which we decipher the ^{1}HNMR data. In this case a few alcohol candidates were tested. Benzyl alcohol and allyl alcohol were among the favorites. This is because the reagents thus synthesized can have use as allylating and benzylating reagents respectively. For the benzylating agent, the case where R is H, the reagent has been reported by Okada *et al* in 2007. An even simpler alcohol, methanol was chosen for the ^{1}HNMR kinetic studies. Methanol is both cheap and much more readily available.

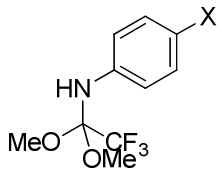


Scheme 3.5: Okada *et al* in 2007 reported a new benzylating agent using N phenyl trifluoroacetimidoyl Chloride

To achieve this task, the trifluoroacetimidoyl chlorides were synthesized as they were not all commercially available, and those that are, are much expensive to buy. The same method used by Tamura *et al.*was employed and improved by performing the reaction in the microwave. This greatly reduced the time for the reaction from 3 hours to 1 hour.. The yield which was already 90+% with the original method was not affected by using the microwave and the shortened time.

Synthesis of methyl 2, 2, 2-trifluoro-N-arylacetimidates

Methyl alcohol with potassium carbonate was stirred at room temperature. Aryl trifluoroacetimidoyl chloride added and the reaction stirred at room temperature overnight under inert gas. The solution filtered out and the filtrate concentrated under high vacuum. Flash chromatography at 5% ethyl acetate in hexanes was performed. Leaving the reaction overnight produced a diacetal side product.



Attempts to make the acetimidate donor were unsuccessful. Different conditions were tried, including drying the solvents, using an excess of imidoyl chloride but all these were futile, not yielding the desired product. Yu *et al.* 2001 reported using solvent that is not completely dry in order to increase the basicity of the solution by solubilizing the potassium carbonate would be more beneficial. With this development, the reaction was allowed to stay and closely monitored. After 1 hour, the product obtained was a mixture of the desired compound and the undesired diacetal product. This means that the desired product, when allowed to stay in basic conditions further reacts with methanol to form the undesired diacetal product. Decreasing the reaction time compromised the reaction since there was a lot of starting material left. The ideal situation would be one in which the starting material **A** in Figure 3.1 is totally consumed but before **C** starts to accumulate.

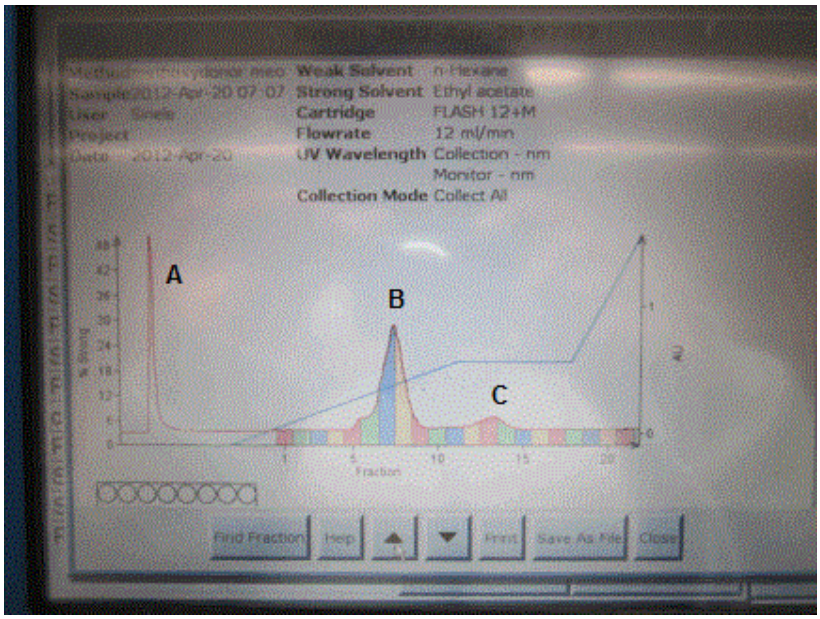


Figure 3.1: Separation of the reaction mixture after 1 hour. **A** is the starting material **B** is the desired product and **C** is the undesirable diacetal product. The x axis shows the sensitivity to UV light by each component. The y-axis shows the amount of solvent containing each compound.

A balance between the reagents and amounts used and the time of reaction has to be established in order to make sure that the reaction mixture shows product **B** and insignificant amounts of reagent **A** and **C.** The trial reactions were carried out with the N-(p-Nitrophenyl)-2,2,2-trifluoroacetimidoyl chloride since it is the easiest to work with, high yielding and forms nice crystalline product.

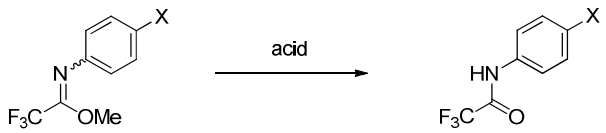
The easiest way to get rid of the problem of diacetal formation would be to limit the amount of methanol applied to the reaction. When methanol was used as a limiting reagent, the reaction did not show any displacement of Cl by OMe. Rather, the reaction still had the starting material. Even after the reaction was allowed to stir overnight, there was no imidate formation. It must be noted that using methanol as a limiting reagent meant using miniscule amounts of methanol.

When tried with an excess of methanol, the undesired diacetal forms concurrently with the desired product. Since it is fair to assume that the undesired product formation goes through the formation of the desired product, attempts to disable the reaction from crossing the activation barrier for the next step, low

temperatures were used immediately after the imidoyl chloride was added. This did not stall the formation of the diacetal especially at extended periods of time (2 hours.) At the end, separating these 3 compounds was the only way to ensure that the desired product was obtained for the kinetic studies.

Stability studies (Kinetics)

A sample of 40µmol of imidate was treated to 2% trifluoroacetic acid in DMSO d_6 and allowed to react over at least 120 minutes. The recording were taken by ^{1}HNMR every 5 minutes and recorded for the determination of the rate of transformation of the imidate to the corresponding amide.



Scheme 3.6: Imidate to amide transformation.

The rate of transformation was quantified as the percent acetimidate aromatic peak divided by the total amount of aromatic peaks (starting material and product aromatic peaks). The difference between the aromatic peaks of the acetimidate and the degraded product is very distinct, making it very easy to quantify the rate of transformation.

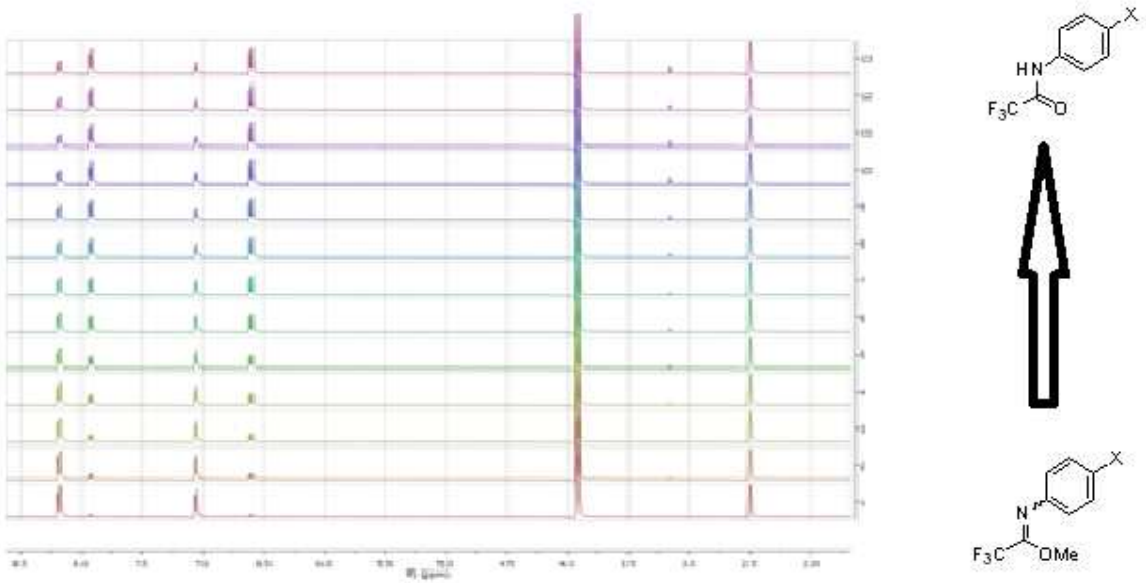


Figure 3.2: Figure depicting the change in ^{1}HNMR spectra of acetimidate in acidic conditions.

A plot of % imidate peak was plotted against time to get exponential graphs resembling Figure 3.3 below. From this graph and the equation $A= A_oe^{-kt}$ we can find the rate of reaction k and using these values for each case, X=NO_2, Cl, H, Me, OMe, we can compare the rates.

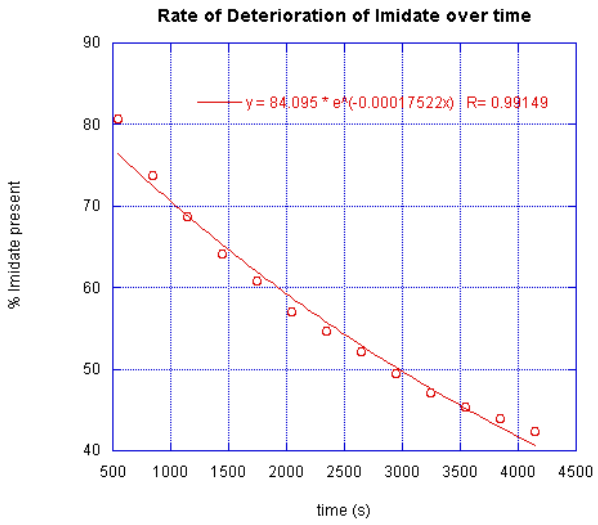


Figure 3.3: % imidate aromatic peaks over time.

Alternatively the natural log of the concentrations can be plotted against time to give a linear graph whose slope represents the rate.

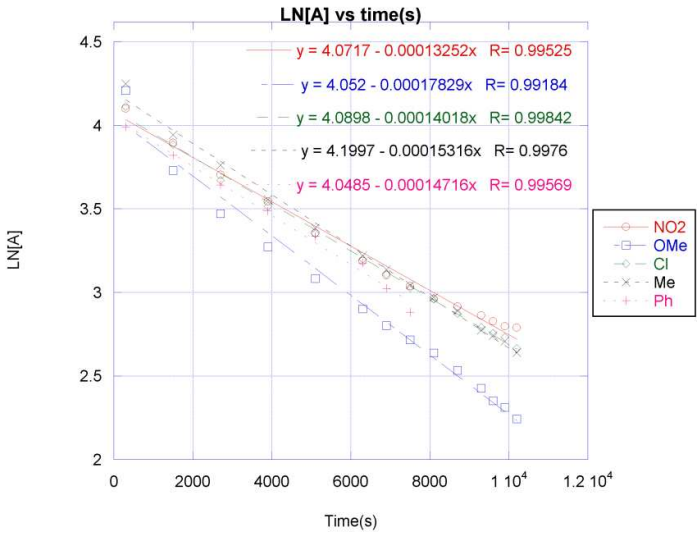


Figure 3.4: Plot of LN[concentration] versus time for the different analogues of trifluoroacetimidates.

The different rates were used to plot Hammet plot using known sigma values for the different substituents. The Hammett plot is a plot based on the equation

$$\sigma\rho = \log(k/k_o)$$

k_o is the reference reaction rate of the unsubstituted reactant and k that of a substituted reactant.

is that for any two reactions with two aromatic reactants only differing in the type of substituent the change in free energy of activation is proportional to the change in Gibbs free energy. This data was fitted into a straight line with a gradient of -8.37×10^{-2}. The gradient is also equal to the rho(ρ) value. The rho value informs of the kind mechanism that a reaction goes through.

Table 3.1 Reaction rates and sigma values for different aryl substituents

Substituents	**σ**	**Relative rates of disappearance**
OMe	-0.78	1.78 $X10^{-4}$
Me	-0.17	1.53 $X10^{-4}$
H	0	1.47 $X10^{-4}$
Cl	+0.23	1.40 $X10^{-4}$
NO2	+0.78	1.32 $X10^{-4}$

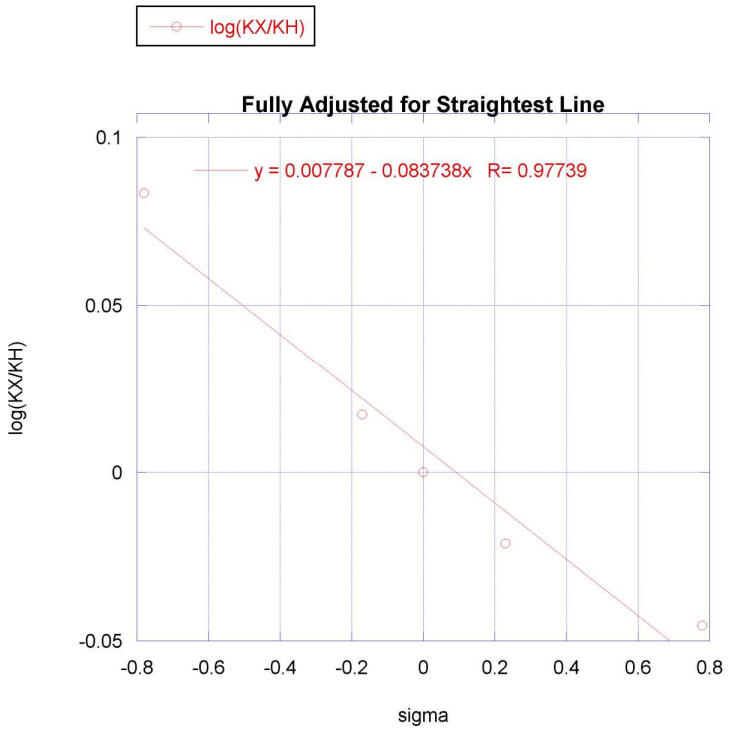
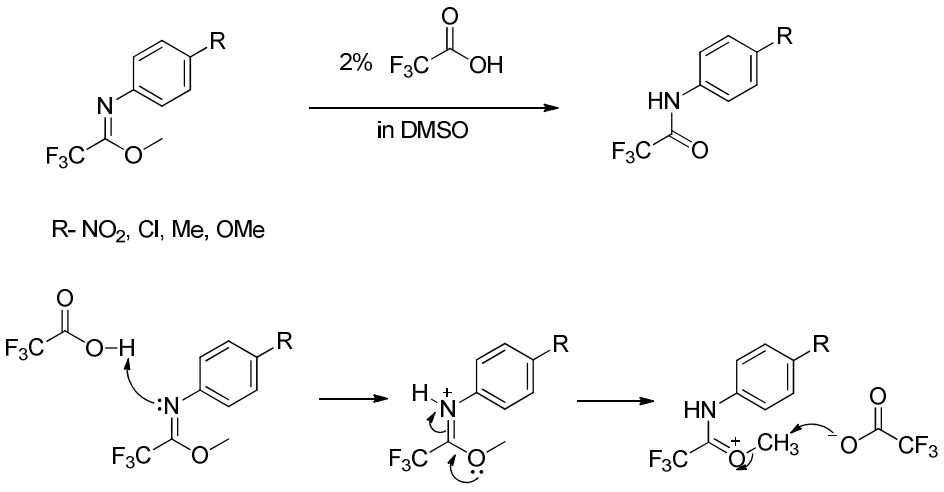


Figure 3.5 Hammett plot

According to Hammett's theory the value of ρ is positive, it means a negative charge builds up in the rate determining step and if the value of ρ is negative, it means there is an accumulation of positive charge during the rate determining step.(Jackson, 2004) When the value of ρ is small, it signifies that the substituent is far from the reaction center and this can be confirmed/confirms the existing proposed mechanism. When using σ neutral and the rho value is negative, it also means that the substituent is not conjugated to the reaction site. In the proposed mechanism, there is no conjugation of substituent to

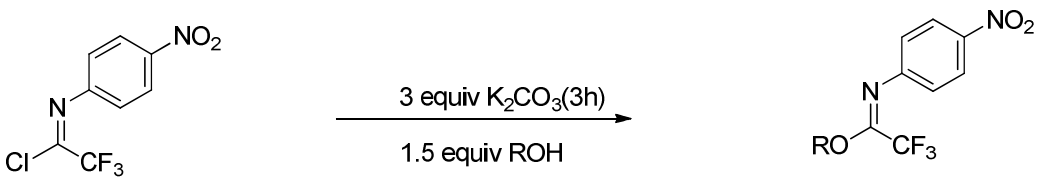
reaction site. With all the given information, we can conclude that the rate determining step of the rearrangement/ degradation of trifluoroacetimidates is the protonation.



Scheme 3.7: Proposed mechanism for the rearrangement of a trifluoroacetimidate to an amide in acidic conditions

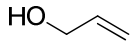
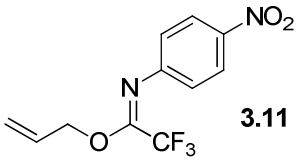
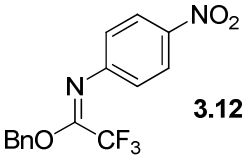
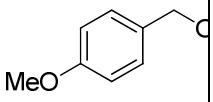
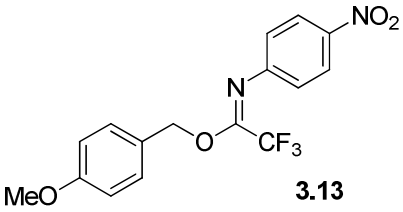
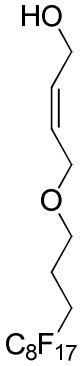
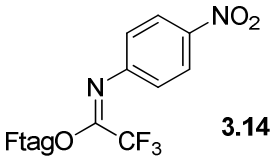
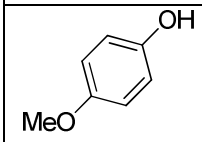
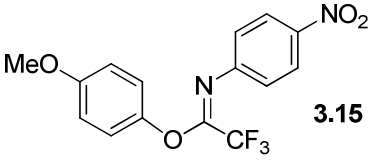
Synthesis of different N-(p-nitrophenyl) trifluoroacetimidate alkylating agents.

After the stability studies, it was very clear that the nitro analogues of the trifluoroacetimidate were the most stable. It also had the advantage of giving a product that was a solid at room temperature. Based on this finding, other trifluoroacetimidates were synthesized. These include the use of allyl alcohol, benzyl alcohol, F-tag alcohol, 4-methoxybenzyl, and 4-methoxyphenol. Some of the acetimidates were solid (crystalline) at room temperature. Their usefulness as alkylating agents was tested using a variety of substrates.



Scheme 3.7: General reaction for the formation of N-(p-nitrophenyl)trifluoroacetimidate

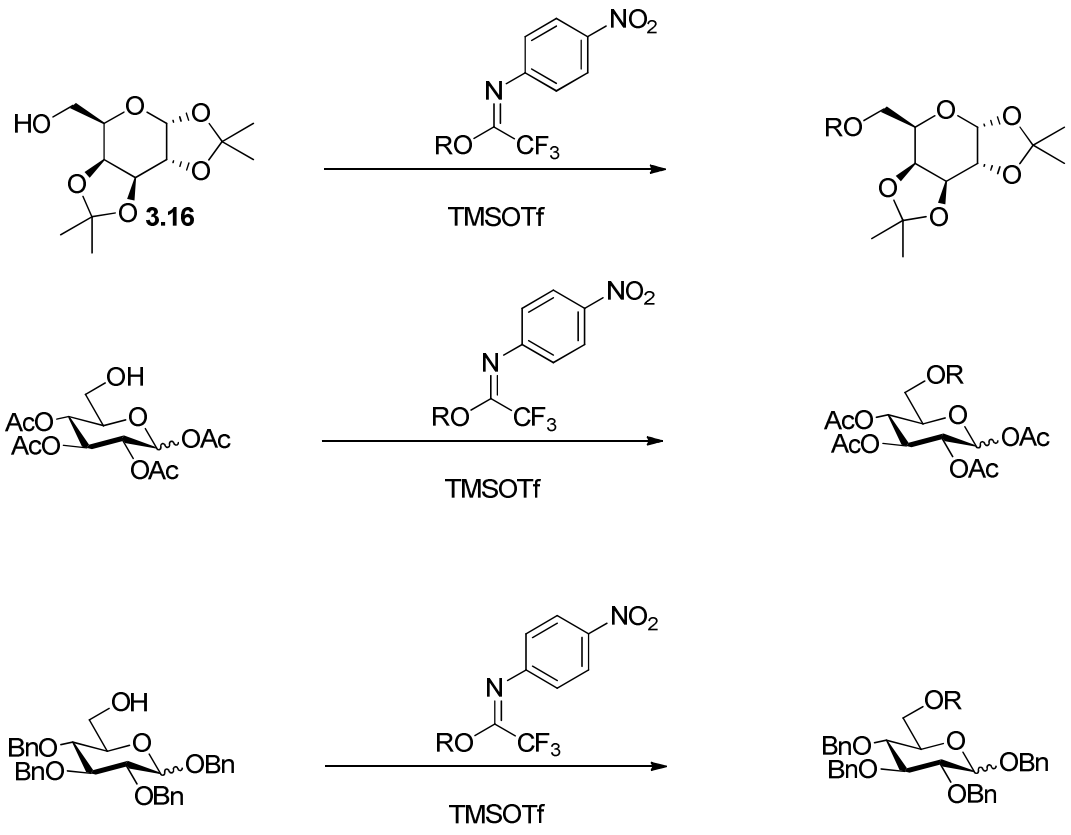
Table 3.2: Synthesis of N-(p-nitrophenyl) trifluoroacetimidates using different alcohols

ROH	Yield, Appearance	Product
	solid	 **3.11**
BnOH	solid	 **3.12**
	solid	 **3.13**
 (Ftag-OH)	liquid	 **3.14**
	solid	 **3.15**

The trifluoroacetimidate donors of allyl alcohol, benzyl alcohol and gave solid crystalline products that were stable under conditions of room temperature for at least 10 days. The limited use of such protecting group strategy has been mainly due to the lack of commercially available reagents. If these imidate donors are stable, they can be produced at the commercial scale and this can in turn solve some

problems in protecting group chemistry. For instance, while benzylating with NaH and benzyl bromide works, it can be problematic to use in conditions that are base sensitive and the Dudley reagent, which is also commercially available, works in neutral conditions but requires high temperatures, extended reaction time and the yields are not great.

To show the superiority of the benzyl trifluoroacetimidate (and other trifluoroacetimidates), these were reacted with a glycosyl acceptor. Conditions explored were glycosylation with TMSOTf, $Zn(OTf)_2$ and $Yb(OTf)_2$. TMSOTf shows a superior reactivity resulting in yields 70-90% while $Zn(OTf)_2$ gave 74% yield. $Yb(OTf)_2$ gave the least yields with 70% yield. Even though all the acetimidates in Table 3.2 have the capacity to alkylate, more effort was concentrated on the allylating and benzylating agents(**3.11** and **3.12)**. Three types of 6-OH free sugar alcohols were used; simple diacetal, fully benzylated and fully acetylated glucose acceptors.



Scheme 3.8: Application of N-(p-nitrophenyl) trifluoroacetimidate in alkylating sugar alcohols.

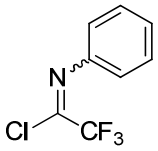
Herein we have reported the introduction of new alkylating/arylation agent that is catalyzed at mildy acidic conditions at room temperature. As well as high yields, these trifluoroacetimidates are, in most cases crystalline and very stable for storage, and this makes it a good candidate for commercialization.

EXPERIMENTAL SECTION (CHAPTER 3)

Synthesis of Trifluoroacetimidoyl Chlorides:

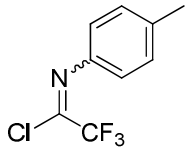
100-mL round bottom flask magnetic stir bar was charged with Ph_3P (17 g, 62 mmol), Et_3N (3.65 mL, 26.5 mmol), CCl_4 (21.1 mL, 220 mmol), and trifluoroacetic acid (1.7 mL,22 mmol). After the solution was stirred for about 10 min (ice bath), the aryl amine (26.5 mmol) was added. The mixture was then refluxed in the microwaved at 80^{o}C, 300W for 60 minutes. The resulting mixture was mixed with hexane, and washed several times with the same solvent. The filtrate was concentrated, and the residue was distilled.

N-Phenyl-2,2,2-trifluoroacetimidoyl Chloride (3.1)



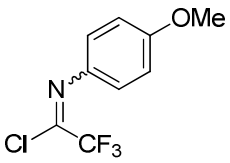
The aryl amine used is aniline and the product was a clear to yellow oil produced at 90%. Distillation conditions, 10 Torr, 54-56^{o}C. ^{1}H-NMR ($CDCl_3$): δ 7.08-7.12 (m, 2H, Ph), 7.26-7.49 (m, 3H, Ph). ^{13}C-NMR($CDCl_3$: δ 116.9 (q, $Jc\text{-}_F$ = 277.0 Hz, CF3), 120.7, 127.4 (2 C), 129.1 (2C) 131.9.9 (q, $J_{C\text{-}C\text{-}F}$ = 43.0 Hz), 143.5.

N-(p-Tolyl)-2,2,2-trifluoroacetimidoyl Chloride (3.2)



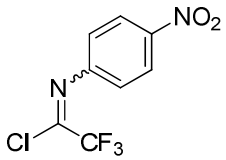
The aryl amine used is p-toluidine and the product was a yellow oil produced at 91%. Distillation conditions, 15 Torr, 90-92°C. ^{1}H-NMR ($CDCl_3$): δ 2.37(s, 3 H, Me), 7.02-7.10 (m, 2H, Ar), 7.20-7.27 (m, 2H,Ar). ^{13}C-NMR: δ 20.9, 117.0 (q, $Jc\text{-}_F$ = 277.0 Hz, CF3), 121.3(2 C), 129.7 (2C) 130.5 (q, $J_{C\text{-}C\text{-}F}$ = 42.9 Hz), 137.9, 140.6

N-(p-Anisyl)-2,2,2-trifluoroacetimidoyl Chloride (3.3)



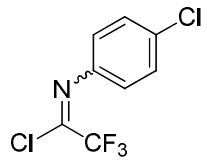
The aryl amine used is p-anisidine and the product was reddish yellow oil produced at 90%. Distillation conditions, 14 Torr, 96-98°C. ^{1}H-NMR ($CDCl_3$): δ 3.85 (s, 3 H, OMe), 6.93-7.02 (m, 2 H, Ar), 7.30-7.37 (m, 2 H,Ar). ^{13}C-NMR($CDCl_3$: δ 55.2,114.2(2C), 117.0 (q, $Jc\text{-}_F$ = 276.6 Hz, CF3), 124.4 (2 C), 127.9 (q, $J_{C\text{-}C\text{-}F}$ = 43.1 Hz), 135.3, 169.6

N-(p-Nitrophenyl)-2,2,2-trifluoroacetimidoyl Chloride (3.4)



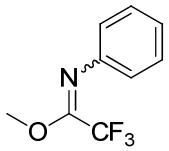
The aryl amine used is p-nitroaniline and the product was yellow oil produced at 92%. Distillation conditions, 5 Torr, 110-115°C.^{1}H-NMR ($CDCl_3$): δ 7.10-7.13 (m, 2H, Ar), 8.25-8.34 (m, 2 H,Ar). ^{13}C-NMR($CDCl_3$: δ 116.5 (q, $Jc\text{-}_F$ = 278.2 Hz, CF3), 120.4(2C), 125.0 (2 C), 135.7 (q, $J_{C\text{-}C\text{-}F}$ = 43.8 Hz), 146.2, 149.0

N-(p-Chlorophenyl)-2,2,2-trifluoroacetimidoyl Chloride (3.5)



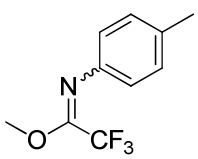
The aryl amine used is p-chloroaniline and the product was yellow oil produced at 92%. Distillation conditions, 5 Torr, 110-115°C.^{1}H-NMR ($CDCl_3$): δ 7.10-7.13 (m, 2H, Ar*)*, 8.25-8.34 (m, 2 H,Ar). ^{13}C-NMR($CDCl_3$: δ 116.5 (q, $Jc\text{-}_F$ = 278.2 Hz, CF3), 120.4(2C), 125.0 (2 C), 135.7 (q, $J_{C\text{-}C\text{-}F}$ = 43.8 Hz), 146.2, 149.0

Methyl 2,2,2-trifluoro-N-phenylacetimidate (3.6)



The aryl trifluoroacetimidoyl halide used was N-Phenyl-2,2,2-trifluoroacetimidoyl Chloride. Potassium carbonate was added to a mixture of methanol and acetone. The reaction was stirred for 5 minutes and the imidoyl chloride added. Reaction was allowed to stir for 30 minutes and then filtered and concentrated. Flash chromatography using 100 hexanes gave the product as a clear oil. ^{1}H NMR (600 MHz, CDCl3) δ (t, 2H, Ar)7.34-7.31, (s, 1H, Ar) 7.12, (d, 2H, Ar) 6.85-6.84, (s, 3H, OMe) 3.95. ^{13}C NMR (151 MHz, CDCl3) δ 144.33, 128.70, 123.89, 119.57, 50.92.

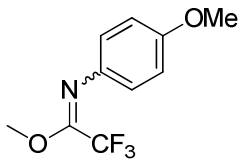
Methyl 2,2,2-trifluoro-N-(p-tolyl)acetimidate (3.7)



The aryl trifluoroacetimidoyl halide used was N-(p-tolyl)-2,2,2-trifluoroacetimidoyl Chloride. % yield. Potassium carbonate was added to a mixture of methanol and acetone. The reaction was stirred for 5

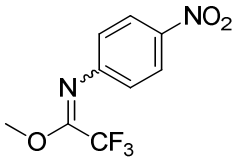
minutes and the imidoyl chloride added. Reaction was allowed to stir for 30 minutes and then filtered and concentrated. Flash chromatography using 100 hexanes gave the product as a yellowish oil. HNMR data showed the presence of two similar compounds and since there was only one TLC peak, the compounds were inseparable.

Methyl 2,2,2-trifluoro-N-(p-anisyl)acetimidate (3.8)



The aryl trifluoroacetimidoyl halide used was N-(p-anisyl)-2,2,2-trifluoroacetimidoyl Chloride. % yield. Potassium carbonate was added to a mixture of methanol and acetone. The reaction was stirred for 5 minutes and the imidoyl chloride added. Reaction was allowed to stir for 30 minutes and then filtered and concentrated. Flash chromatography using 100 hexanes gave the product as a yellowish oil. ^{1}H NMR (600 MHz, CDCl3) δ (d, 2H, Ar) 6.86- 6.85, (d, 2H, Ar) 6.78-6.77, (s, 3H, Me) 3.92, (s, 3H, Me) 3.80. ^{13}C NMR (151 MHz, CDCl3) δ 156.33, 137.46, 120.78, 120.76, 120.74, 120.73, 120.71, 120.70, 120.69, 120.68, 114.28, 114.03, 114.02, 114.01, 114.00, 55.42, 55.41, 55.40, 55.40.

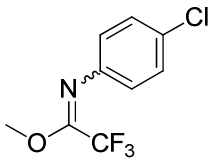
Methyl 2,2,2-trifluoro-N-(p-nitrophenyl)acetimidate (3.9)



The aryl trifluoroacetimidoyl halide used was N-(p-nitrophenyl)-2,2,2-trifluoroacetimidoyl Chloride. Potassium carbonate was added to a mixture of methanol and acetone. The reaction was stirred for 5 minutes and the imidoyl chloride added. Reaction was allowed to stir for 30 minutes and then filtered and concentrated. Flash chromatography using 100 hexanes gave the product as yellowish solid at 70% yield. ^{1}HNMR (600MHz, $CDCl_3$) δ 8.20 (d, J = 8.9Hz, 2H) 6.92(d, J = 8.9Hz, 2H) 3.98(s, 3H) ^{13}CNMR

(151 MHz, CDCl3) δ 150.44, 144.25, 124.78, 124.77, 124.76, 120.14, 120.13, 120.12, 120.11, 116.94, 115.04, 55.64.

Methyl 2,2,2-trifluoro-N-(p-chloroaniline)acetimidate (3.10)

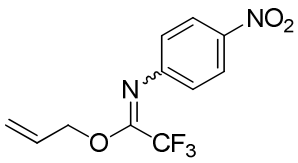


The aryl trifluoroacetimidoyl halide used was N-(p-anisyl)-2,2,2-trifluoroacetimidoyl Chloride. Potassium carbonate was added to a mixture of methanol and acetone. The reaction was stirred for 5 minutes and the imidoyl chloride added. Reaction was allowed to stir for 30 minutes and then filtered and concentrated. Flash chromatography using 100 hexanes gave the product as a pale yellow oil at 75% yield. ^{1}H NMR (600 MHz, CDCl3) δ (d, 2H, Ar) 7.30-7.29, (d, 2H, Ar) 6.79-6.77, (s, 3H, OMe) 3.95. ^{13}C NMR (151 MHz, CDCl3) δ 142.93, 129.30, 128.84, 128.83, 128.82, 128.81, 128.80, 128.80, 128.79, 121.02, 120.99, 120.98, 120.98, 117.02, 115.12, 55.17, 29.72.

Synthesis of trifluoroacetimidate alkylating agents

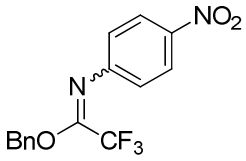
The general procedure for the synthesis of alkyl N-(4-nitrophenyl) trifluoroacetimidate: using acetone and a drop of water and 3 equivalents of potassium carbonate , excess alcohol was added at room temperature. N-(4-nitrophenyl) trifluoroacetimidoyl chloride was added and stirred at room temperature at 3 hours. The solution was filtered and evaporated under reduced pressure. Flash chromatography was used to purify the product.

Allyl 2,2,2-trifluoro-N-(4-nitrophenyl)acetimidate (3.11)



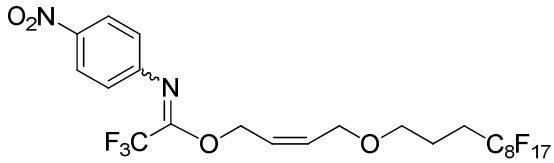
^{1}H NMR (600 MHz, CDCl3) δ (d, 2H, Ar) 8.21-8.19, (d, 2H, Ar) 6.92-6.91, (m, 1H, CH=) 6.07-6.02, (d, 1H, =CH_2) 5.48-5.45, (d, 1H, =CH_2) 5.40-5.38, (d, 2H, OCH_2) ^{13}C NMR (151 MHz, CDCl3) δ 150.55, 146.05, 130.66, 125.00, 120.27, 115.22, 75.20, 69.40

Benzyl 2,2,2-trifluoro-N-(4-nitrophenyl)acetimidate (3.12)



^{1}H NMR (600 MHz, CDCl3) δ (d, 2H, Ar) 8.21-8.19,(m, 5H, Bn) 7.45-7.41, (d, 2H, Ar) 6.92-6.91, (s, 2H, CH_2-Ph) 5.36. ^{13}C NMR (151 MHz, CDCl3) δ 134.21, 128.75, 124.81, 120.05, 70.41

4-[3-(perfluorooctyl)propyloxy]-cis-2-butenyl 2,2,2-trifluoro-N-(4-nitrophenyl)acetimidate (3.13)



^{1}H NMR (600 MHz, CDCl3) δ (d, 2H, Ar) 8.21-8.19, (d, 2H, Ar) 6.92-6.91, (m, 2H, CH=CH) 5.90-5.84, (d, 2H, CH_2C=) 4.93-4.91, (d, 2H, CH_2) 4.14-4.13, (t, 2H, CH_2) 3.53-3.51, (m, 2H) 2.20-2.18, (m, 2H) 1.90-1.87. ^{13}C NMR (151 MHz, CDCl3) δ 150.24, 132.11, 124.79, 120.04, 69.04, 66.70, 64.49, 20.83

CHAPTER 4. BIOSYNTHESIS AND CHEMICAL SYNTHESIS OF KDO

Introduction to bacteria and bacterial cell structure

Bacteria are single celled organisms without organelles or an organized nucleus. Some bacteria are pathogenic and some are symbiotic. It is widely known that more than 90% of the cells in or on a human body are bacterial cells. It is therefore important for human cells to be able to distinguish one bacterium cell from the other and to know when there is an infection and when there is not. The part of the bacteria structure responsible for cell recognition is found in the bacterial cell wall.

Some bacteria possess a thick cell wall, they give a purple color when stained with a dye known as crystal violet and are, therefore, known as Gram-positive (after the Danish bacteriologist who developed this staining procedure). In contrast, other bacteria have a relatively thin cell wall consisting of a fewer layers; an outer lipid membrane containing lipopolysaccharides (LPS) and lipoproteins; they do not stain purple with crystal violet and are known as Gram-negative.

The LPS of Gram negative bacteria contains lipid A which consists of a glucosamine disaccharide. Lipid A is closest part of the LPS to the bacterial membrane. It is usually connected to one or more units of unusual sugars such as 2-Keto-3-deoxy-D-manno-octulosonic acid (Kdo) and heptose forming the inner core. The outer core is made sugars such as glucose, mannose and rhamnose. The outer most layer is the O-specific chain which also contains repeating subunits of common sugars.

Upon introduction of bacteria to a host organism, the cells of the host organism recognize the LPS of the bacteria and can decide whether an immune response is necessary or not. Figuring out how much of the LPS plays a part in cell-cell recognition has been a subject of many inquiries. Does the host organism require the whole LPS structure or is the O-specific chain necessary? Is it the O-specific chain or the core or lipid A that is necessary. LPS has been isolated but it is impossible to isolate parts of the LPS(Silipo et al., 2005). It is for this reason that chemical synthesis of parts of the LPS is necessary. Many different

groups have and are synthesizing different parts of the LPS of different bacteria. In this project the lipid A and part of the inner core of the *Xanthomonas campestris pv. campestri (Xcc)* bacteria is being synthesized.

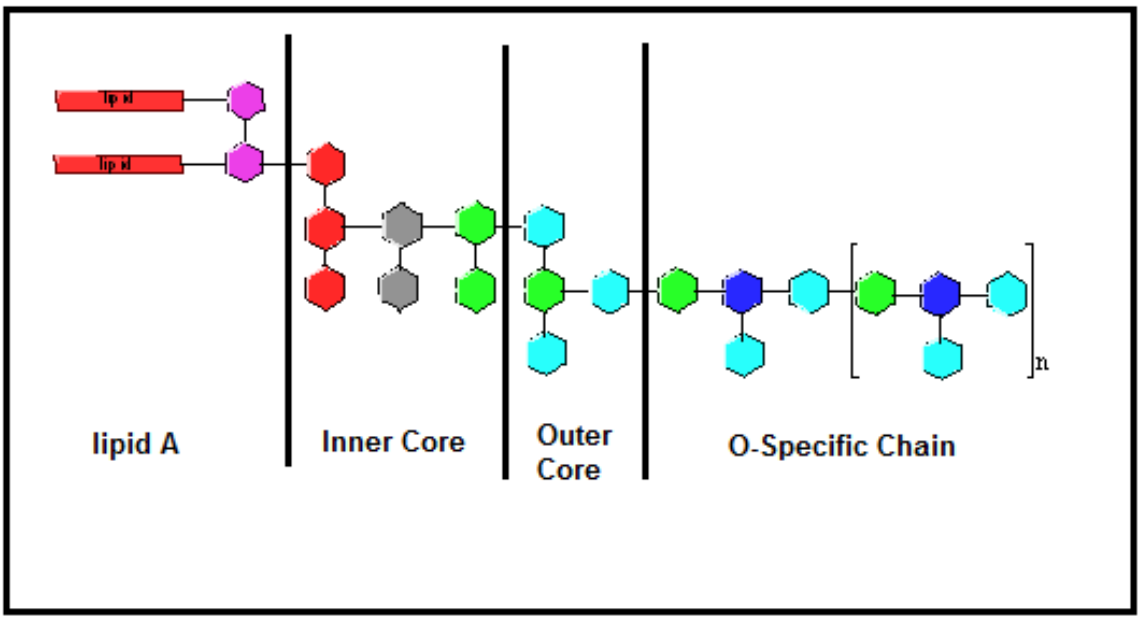


Figure 4.1: Representation of a Gram negative bacteria lipopolysaccharide

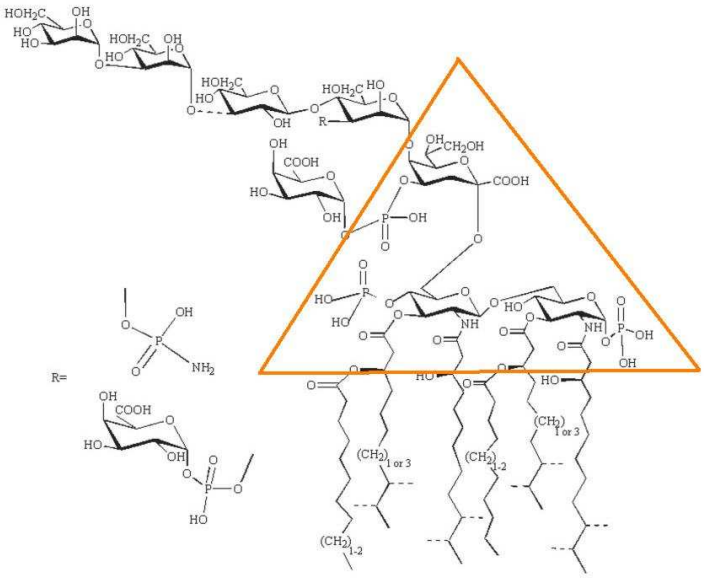


Figure 4.2: LPS structure of Xcc bacteria

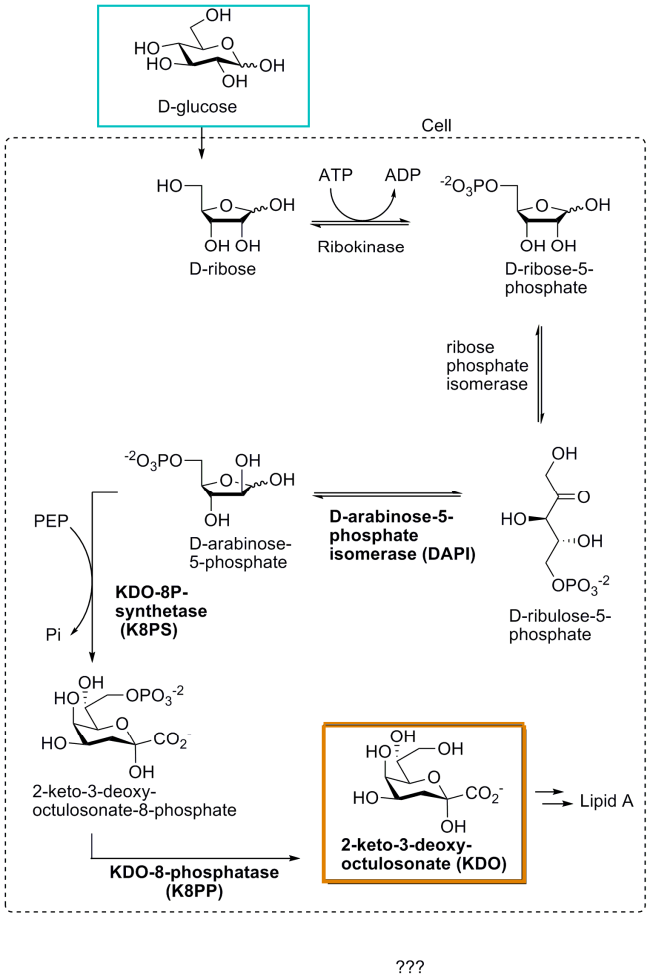
The structure of the LPS for the Xcc bacteria starts with lipid A connected to KDO and this is the target of this project. It is therefore necessary to obtain KDO. Unfortunately KDO is a very rare and

expensive sugar. Current prices are as high as US$35.60 per mg and buying KDO would not practical for this project. Many groups have tried to chemically synthesize KDO with some success.(Ichiyanagi, Sakamoto, Ochi, & Yamasaki, 2009; Liu, 1997; Ramage, Rose, & MacLeod, 1988; Waaglund & Claesson, 1992; Winzar, Philips, & Kiefel) They are all not efficient and still expensive. In most cases there are too many steps and the yields are low. There exists one short and relatively high yielding synthetic pathway to KDO.(Winzar et al.) This method uses oxaloacetic acid and D-arabinose as starting material. It also uses $NiCl_2$, a carcinogenic salt.

As a first alternative, one of these chemical syntheses of KDO was attempted. It was clear very early on that it was not going to work because the scheme involved highly reactive reagents which require small scale reactions, and many reactions steps overall which would mean that half way through the scheme, there would not be enough product to proceed. This coupled with the fact that the KDO would still need to be protected and further manipulated into a glycosyl donor, meant that chemical synthesis would not work. Since E. *coli* already produce KDO on their own, the idea to manipulate some cells into overexpressing KDO was pursued. To that end, some colleagues developed a method to bioengineer E.*coli* cells into overexpressing KDO.

Biosynthesis and extraction of 3-deoxy-d-*manno*-oct-2-ulosonic acid (KDO)

KDO was synthesized from D-glucose using bioengineered *E. coli* cells. Glucose rather than D-arabinose was beneficial since it is a more readily available and cheaper starting material. The following figure shows a schematic of the reactions leading to the formation of KDO; the bolded steps represent the steps that were manipulated/non-native to the cell (Camci-Unal, Mizanur Rahman, Chai, & Pohl Nicola, 2012). The following figure shows a schematic of the reactions leading to the formation of KDO; the bolded steps represent the steps that were manipulated/non-native to the cell.

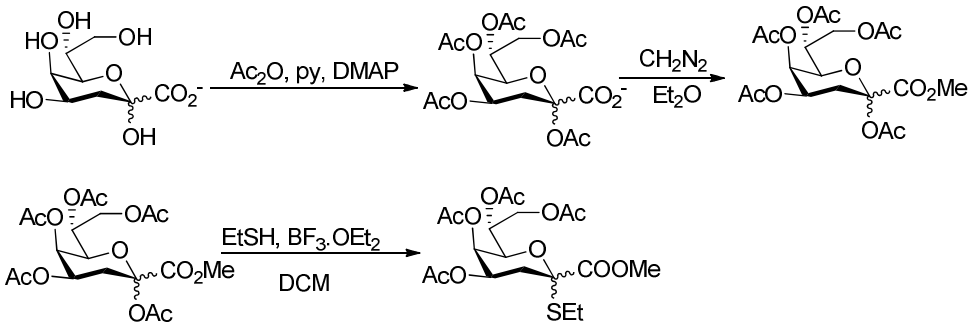


Scheme 4.1: Biosynthetic pathway of KDO from glucose

After obtaining the engineered cells the thiobarbituric acid (TBA) assay (Ghalambor & Heath, 1963; Warren, 1963) was used to confirm that the cells were indeed producing KDO. The challenge in this whole process was the extraction of KDO from fermentation broth. After lyophilization of the broth, the resulting solid was acetylated to enhance extraction from the aqueous media. At this point thin layer chromatography still showed a lot of spots and ^{1}HNMR was very messy. Esterification of the product was then carried though the NMR had all necessary peaks on the spectra; it was too crowded and unclean. Attempts to further separate product from the mixture resulted in more and more peaks being discovered.

Different kinds of separation methods including charcoal column (Carlson, 1966; Morales, Sanz, Olano, & Corzo, 2006; Saleem, Dhasan, & Rafiullah, 2006), using baker's yeast to digest the excess

glucose, anion exchange column but none has been very successful. It must be noted that the cells were engineered to be resistant to the antibiotic carbenicilin.

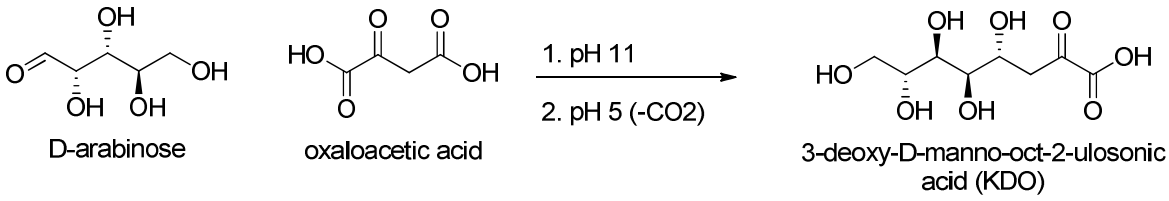


Scheme 4.2: Synthesis of the KDO donor.

Chemical synthesis of KDO

Chemical synthesis is not an easy task. It is on its own, a synthetic achievement. It is however very difficult to expect the product of such chemical syntheses to be applied in building block synthesis. Synthesizing KDO in such a manner involves many reactions steps that have to be done in very small quantities. Overall yields are very low and are often in the milligram scale. It is necessary to use a method that involves shorter steps and can be carried out in bigger scale.

Cornforth(Cornforth, Firth, & Gottschalk, 1958) introduced a methodology for a relatively big scale synthesis of KDO. It uses D-arabinose and oxaloacetic acid. The downside to this method is that oxaloacetic acid is not a cheap reagent and the method uses $NiCl_2$ which is a carcinogenic salt. This method also has limitations with purification. It requires the use of anion exchange chromatography, HPLC and this means that KDO cannot be easily produced in the multi-gram scale.



Scheme 4.3: Chemical synthesis of KDO

Chemical versus Biosynthetic pathway

The biosynthetic pathway offers a way to mass produce KDO and uses very safe reagents. It does however take a long time to get to the KDO and involves a lot of preparation and hard work. Cornforth's Chemical synthesis on the other hand is less labor intensive and can also mass produce KDO, but it involves dangerous and expensive reagents. Both method's biggest bottleneck is the purification step. A new and more efficient way to purify KDO would be an advantageous thing to find and this may solve the high cost associated with KDO production.

EXPERIMENTAL SECTION (CHAPTER 4)

Biosynthetic Procedure

Make LB Agar solution and add carbenicilin. Spread it over petri dishes and put some cell solutions. Let it sit in a 37 ^{o}C oven and allowed to grow for less than 18 hours. Using sterilized toothpicks, colonies of cells were added to a solution of LB broth containing carbenicilin and stirred over 12 hours. The cells were centrifuged down and put in a fermentation medium containing potassium phosphates, ammonium and magnesium sulfates. (Lundgren & Boddy, 2007). Glucose was used as a food source for the cells. After about an hour, optical density between 0.6 and 0.8 at 600nm, the cells were induced with 1M isopropyl β-D-1-thiogalactopyranoside (IPTG) and the cells fed with glucose solution every 24 hours for 96 hours. The fermentation medium was centrifuged and the supernatant was evaporated and lyophilized to afford a solid. TBA Assay performed to confirm the presence of KDO. This was then separated either by acetylation or different kinds of columns.

TBA Assay

(Ghalambor & Heath, 1963; Karkhanis, Zeltner, Jackson, & Carlo, 1978; Warren, 1963)

Add 1 mL of 0.2N H_2SO_4 to a test tube containing 2 mg of KDO material. Heat the reaction mixture at 100^o C for 30 min, cool and centrifuge the mixture at maximum speed in a clinical centrifuge for 5 min and pipet 0.5 ml as a clear solution into another test tube. Add 0.25 mL of 0.04M HIO_4 in 0.125 N H_2SO_4 and let the mixture stand at room temperature for 20 min.

Add 0.25 mL of 2.6 % $NaAsO_2$ in 0.5 N HCl, vortex, and wait until the brown color disappears. Add 0.5 of 0.6% thiobarbituric acid, vortex and heat the mixture at 100^o C for 15 min. While hot add 1 mL DMSO. Let the mixture cool to room temperature and read its optical density at 548nm against a blank treated the same except without KDO.

Chemical Synthesis of KDO

D-Arabinose (500 mg, 3.3 mmol) was added to a solution of Na_2CO_3 (860 mg, 8.1 mmol) in H_2O (8 mL). Oxalacetic acid (525 mg, 4.0 mmol) was added portion wise over 5 min, and the solution was adjusted to pH 11 using NaOH (10 M). After stirring for 2 h at room temperature the solution was acidified to pH 5 using acetic acid, $NiCl_2$ (7.5 mg, 0.03 mmol) added, and the mixture was heated at 50 °C for 1 h. After cooling to room temperature the reaction was neutralized to pH 8 with ammonium hydroxide. The solution was concentrated under reduced pressure and the product mixture was isolated by anion exchange chromatography using Bio-Rad® formate resin (AG1-X8), washing first with H_2O and then eluting with ammonium bicarbonate (0.5 M). Fractions containing KDO were visualized as bluish-grey spots on silica gel TLC plates ($CHCl_3$–MeOH–H_2O, 5:5:1) by staining with ethanol-anisaldehyde–sulfuric acid dip. The eluent was concentrated under reduced pressure, and then freeze-dried. The lyophilized residue was purified using reversed-phase (C18) silica gel with H_2O as the mobile phase. In both methods, there is clearly evidence for the presence of KDO by TBA Assay and the spotting on TLC plate. Separation is the main problem

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTION

The synthesis of the LPS fragment is still under way. The glucosamine building blocks are a challenge and are not as widely used as other building blocks, for instance glucose. This is because the amine group on the glucosamine makes it have complicated chemistry. First, the amine group has to be protected first and while this can be easily achieved with protecting groups like phthalimide, their lability reduces their usefulness. Other more stable protecting group such as trichloroacetamide groups are problematic since they require multiple steps to protect and single step methods are low yielding and demand long reaction times. Low glycosylation yields associated with glucosamine are due to the formation of a stable oxazoline intermediate. Careful protecting group manipulation can achieve a severely unstable oxazoline by, for instance, using another ring, as in the case of butane diacetal protection. This can increase the glycosylation yield but the need to hydrolyze the diacetal in acidic conditions makes this method not favorable in the presence of a glycosidic bond. Thioglycoside are very efficient alternatives to the popular trichloroacetimidates since they reduce reaction steps and increase overall yields, but their smell and lack of variety of homogenous promoter systems makes them less than desirable for application in automated solution phase synthesis. They are still a very viable donor-type for KDO.

The failure to sufficiently purify KDO is the main reason for the lack of progress in this project. Chemically KDO can be mass produced in a matter of hours but using the biosynthetic protocol, KDO can be mass produced in a week. Even though the time difference is vast, biosynthesis is still favorable since it uses cheap and benign reagents such as glucose and common salt solution. Chemical synthesis on the other hand uses very expensive reagents, arabinose and oxaloacetic acid. It also uses $NiCl_2$. Using a KDO donor is not expected to be an easy task, owing to its 2-deoxy nature and lack of any stereochemical helpers to direct the stereochemistry of the glycosyl bond. KDO also contains a carboxylic functional group as C-1 and this would also present problems when considering that for most glycosyl, the intermediate is an oxocarbenium ion. All these studies and more can only be possibly with the ability to mass produce and purify KDO.

Future directions in this project include finding a better method to purify KDO. The glucosamine building blocks have been sufficiently studied and there are several options that can result in a building block in a couple of days with very high yielding steps. More trials of the glucosamine disaccharide could be performed on the automated synthesizer using the differently protected building blocks synthesized. The LPS fragment thus synthesized can then be used in biological studies to see if it shows the same reactivity as the full LPS structure extracted.

The stability studies show predictable preliminary results. After the experiments are replicated, this will bring this project to a close and the alkylating agents discovered can provide a very good alternative to current allylating/benzylating methods. These new alkylating agents are not only high yielding and easy to use, but they are also stable and can be produced and stored for extended periods of time.

Glycobiology is still lagging in progress. The invention of automated synthesis will go a long way into producing libraries upon libraries of glycans. The main problem faced by the field now is with the building block synthesis. Every day newer and shorter paths that are cheaper and more environmentally friendly are being discovered. These include using microwaves radiation, ultrasound and developing crystallization protocols.

APPENDIX A: THE DEVELOPMENT OF N-ARYL TRIFLUOROACETIMIDATE-BASED BENZYL AND ALLYL PROTECTING GROUP REAGENTS

Manuscript submitted to Tetrahedron Letters : Compounds **5** and **6** were synthesized by both Daniel Kabotso and I. Daniel Kabotso contributed the Mass Spectroscopy data. Compound **11** and all relevant were produced by Daniel Kabotso. Daniel Kabotso was contributed in the writing of the manuscript.

Sinele B. Tsabedze[a], Daniel E. K. Kabotso [b], Nicola L. B. Pohl[a,b,] ∗

[a]*Department of Chemistry, Hach Hall, Iowa State University, Ames IA 50011, USA*
[b]*Department of Chemistry, Simon Hall, Indiana University, Bloomington IN 47405, USA*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

ABSTRACT

An exploration of the role of *para*-substituents on the balance between stability and reactivity of *N*-phenyl trifluoroacetimidates prompted the discovery of new reagents for the addition of allyl and benzyl protecting groups, namely *O*-allyl and *O*-benzyl *N*-(*p*-nitrophenyl)trifluoroimidates. These compounds are stable and crystalline at ambient temperature, making them ideal alternatives to existing benzylating and allylating reagents used under acidic conditions.

Keywords:
trifluoroacetimidate
benzylation
allylation
protecting groups

∗ Corresponding author. Tel.: +1-812-855-0298; fax: +1-812-855-8300; e-mail: npohl@indiana.edu

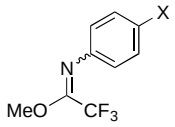
Introduction

The development of protecting group chemistries has allowed the synthesis of increasingly complex structures with dense functional group patterns, especially in the realm of carbohydrate synthesis.[1a,b] Amongst hydroxyl protecting groups, much effort has focused on allyl and benzyl groups.[2a-j] Unfortunately, the introduction of these protecting groups often involves employment of harsh acidic/basic conditions that can be problematic when other fragile groups are present in the compound being protected.[2i] Benzylation is one of the more common permanent protecting groups employed in oligosaccharide synthesis owing to its stability under both the acidic and basic conditions employed in typical glycosylation/deprotection reactions. A popular benzylating protocol uses benzyl bromide in the presence of sodium hydride and catalytic tetrabutylammonium iodide (TBAI).[2j] The utility of this reaction is limited when there are already base sensitive functional groups in the substrate such as acetyl groups. For neutral conditions, benzylation in the presence of silver oxide[2i] can be successful, but the need for freshly prepared silver oxide to make this reaction reliable complicates the procedure. To employ acidic rather than basic conditions, benzyl trichloroacetimidate[2k] has been used, but this reagent usually requires in situ preparation due to its high reactivity. In the realm of glycosylation chemistry, several analogues of the *N*-aryl trifluoroacetimidates have been used as glycosyl donors to circumvent the high reactivity of the trichloroacetimidates.[3] The advantage of using these donors as opposed to the widely used trichloroacetimidate donors is that they are more stable and still very efficient and can thus be used in conditions where the less stable trichloroacetimidates fail.[3] Given these precedents, we decided to explore the possibility of modulating the reactivity of *N*-phenyl trifluoroacetimidates to find a balance between the stability and reactivity of this leaving group for use in reagents designed for benzylation and

allylation reactions. Herein we report structure/function studies with these *N*-phenyl trifluoroacetimidates and the discovery of a leaving group that provides stable and crystalline reagents for the facile addition of benzyl and allyl groups under acidic conditions.

Method

To find a more stable acetimidate, a systematic method to investigate the effect of changing a *para*-substituent on the *N*-phenyl group on the stability of the acetimidate under acidic conditions was sought. To start the study, a variety of *N*-phenyl trifluoracetimidates were made attached to methanol rather than benzyl for simplicity in the analysis by ^{1}H NMR (Figure 1). Methyl 2,2,2-trifluoro-*N*-aryl acetimidates were synthesized by reacting methyl alcohol with *N*-aryl trifluoroacetimidoyl chlorides[6] under basic conditions. Several bases such as NaOMe, NaH and K_2CO_3 were tested for their viability in this synthesis; K_2CO_3 was ultimately chosen for its ease of use while maintaining excellent (85-90%) yields.



1 X = NO_2 (90%)
2 X = Cl (87%)
3 X = H (89%)
4 X = OMe (90%)

Figure 1. Synthesis yields of *para*-substituted *O*-methyl *N*-aryl triflouroacetimidates.

After synthesis and subsequent purification of each compound **1**-**4**, a sample of 40 µmol of acetimidate was treated to 2% trifluoroacetic acid in DMSO-d_6 and allowed to react over the course of at least 120 min. Recordings of the reaction were taken by ^{1}H NMR every 5 min to determine which donor maintained the integrity of its structure the longest. The rate of transformation was quantified as the percent acetimidate peak divided by the total amount of aromatic peaks (starting material and product aromatic peaks). The difference between the

aromatic peaks of the imidate and the degraded product is very distinct, making it very easy to quantify the rate of transformation.

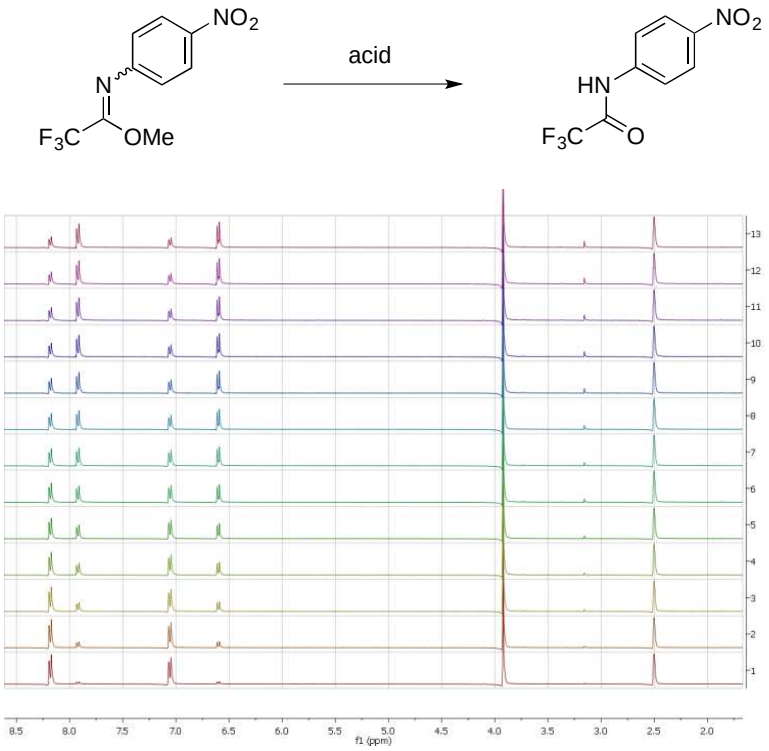


Figure 2. ^{1}H NMR depicting the change in structure of acetimidate **1** under acidic conditions.

Results and Discussion

The percent imidate peak was plotted against time to get exponential graphs resembling Figure 3 below. From such graphs, the comparative rates of degradation were obtained. A comparison of the nitro-substituted acetimidate **1** to the other analogs showed marked stability under acidic conditions. Compound **1** formed needle-like light yellow crystals at ambient temperature.

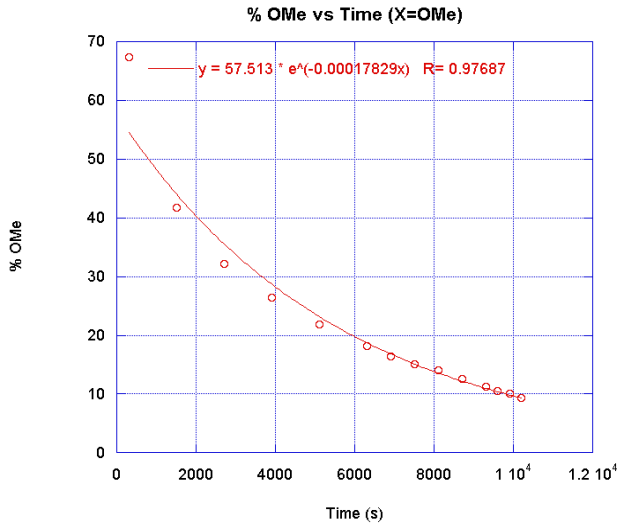


Figure 3. An example of a graph showing the % imidate aromatic peaks by ^{1}H NMR over time for compound **4**. Additional graphs are available in the supporting information.

Given the superior stability of the nitro-substituted *N*-phenyl trifluoroacetimidate, presumably because of the inductive effect of the electron-withdrawing group making the nitrogen less basic, we next tested its reactivity as a leaving group in the context of a benzylation reaction. Obviously, to be a successful leaving group, the substituted *N*-phenyl trifluoracetimidate could not be too stable and thereby require harsh conditions for its transfer to a hydroxyl as a protecting group. A benzylating agent using the unsubstituted *N*-phenyl trifluoroacetimidoyl had already been reported,[4e] but this reagent, like its trichloroacetimidate counterpart,[2e,k] is also not particularly stable and therefore requires in situ preparation or stringent storage conditions. Prompted by the superior stability of the nitro derivatives in the simple methanol study above, benzyl derivative **5** was synthesized in excellent (>90%) yield (Figure 4). Interestingly, the reagent was crystalline and proved to be stable at ambient temperature for at least 10 days—properties that could make it more viable for commercial production than benzyl trichloroacetimidate.

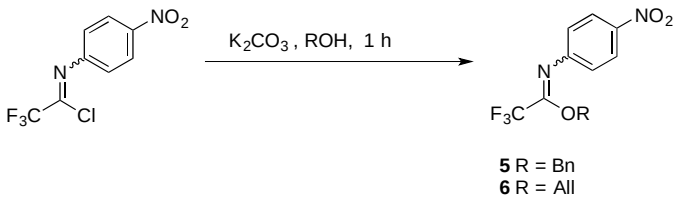
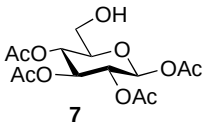
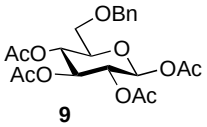
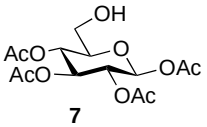
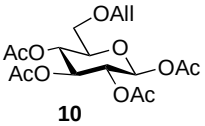
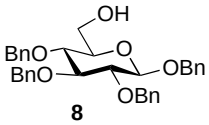
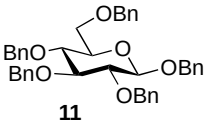
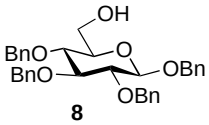
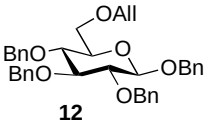


Figure 4. Synthesis of benzyl and allyl *N*-(nitrophenyl) trifluoroacetimidates.

An initial trial reaction showed that the new nitro-derivative **1** could indeed serve as a benzylation reagent. Given this promising initial data, the allyl version **6** was also made. The reaction also proceeded in excellent yield to provide a crystalline product. These two new reagents were then used in reactions to protect alcohols **7** and **8**, representative of deactivated and activated carbohydrate hydroxyls, to form the known products **9**,[7a] **10,**[7b] and **11**[7c] and the new product **12**. The benzylation and allylation reactions were first tried with $Zn(OTf)_2$ and $Yb(OTf)_2$. Though successful, the yields in these conditions were not satisfactory (~60%) compared to later reactions with TMSOTf (Table 1). All reactions were complete within 1 hour compared to longer traditional methods and the yields were improved compared to using the less stable trichloroacetimidates.

Table 1

Reactions of 1.2 equivalents *O*-allyl and *O*-benzyl *N*-(nitrophenyl)trifluoroimidates with different alcohols using catalytic amounts of TMSOTf as a promoter. All reactions were complete within 1 hour using CH_2Cl_2 as the solvent.

Alcohol	Imidate	Product	Yield
 **7**	**5**	 **9**	83%
 **7**	**6**	 **10**	80%
 **8**	**5**	 **11**	85%
 **8**	**6**	 **12**	81%

Conclusion

After comparative monitoring of the rates of reaction associated with different *para*-substituents on *N*-aryl trifluoroacetimidates, the nitro-substitution was found to provide a perfect balance between stability and reactivity to aid in the development of new reagent for the addition of benzyl and allyl protecting groups. These benzylating and allylating reagents are crystalline and stable at ambient temperatures for days, thereby simplifying their use, and react with alcohols in high yields with catalytic amounts of TMSOTf comparable to the less stable allyl and benzyl trichloroacetimidate reagents. These new reagents should thereby provide attractive alternatives to the standard trichloroacetimidate reagents for acid-catalyzed additions of benzyl and allyl protecting groups.

Acknowledgements

This work was supported in part by the U.S. National Science Foundation (CHE-0911123/1261046) and the Joan and Marvin Carmack fund.

References

1. (a) Guo, J.; Ye, X-S. *Molecules* **2010,** *15*, 7235-7265; (b) Greene, T. W.; Wuts, P. G. M. Greene *Protective Groups in Organic Synthesis*, 4th ed.; Wiley: New York, 2006.
2. (a) Winzar, R.; Philips, J.; Kiefel, M. J. *Synlett*, 2010, (4), 583-586; (b) Rawal, G. K.; Rani, S.; Kumari, N.: Vankar, Y. D. *J. Org. Chem*, **2009**, *74* (15), 5349-5355. (c) Poon, K. W. C.; Dudley, G.B. *J. Org. Chem*, **2006**, *71* (10), 3923-3927; (d) Okada, Y.; Ohtsu, M.; Bando, M.; Yamada, H. *Chem. Lett.* **2007**, *36* (8), 992-993; (e) Iversen, T.; Bundle, D. R. *J. Chem. Soc., Chem. Commun.* **1981**, (23) 1240-1241; (f) Cao, Y.; Okada, Y.; Yamada, H. *Carbohydr. Res.* **2006**, *341*, 2219-2223; (g) Waaglund, T.; Claesson, A. *Acta Chem. Scand.* **1992**, *46*, 73-76; (h) Sivik, M. R. *e-EROS Encycl. Reagents Org. Synth.* **2001**, DOI:10.1002/047084289X.ra066 (i) Berry, J.M.; Hall, L.D. *Carbohydr. Res.* **1976**, *47*, 307. (j) Czernecki, S.; Georgoulis, C.; Provelenghiou, C.; Fusey, G. *Tetrahedron Lett.* **1976**, *17* (39), 3535-3536. (k) Wessel, H.-P.; Iversen, T.; Bundle, D.R. *J. Chem. Soc. Perkin Trans. 1*. **1985**, 2247-2250.
3. Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405-2407.
4. (a) Dunkerton, L. V.; Adair, N. K.; Euske, J. M.; Brady, K. T.; Robinson, P. D. *J. Org. Chem*. **1988**, *53*, 845–850; (b) Wieczorek, E.; Thiem, *J. Carbohydr. Res*. **1998**, *307*, 263–270; (c) Ferrier, R. J.; Prasad, N. J. *J. Chem. Soc.* **1969**, 570–575; (d) Kinfe, H. H.; Mebrahtu, F. M.; Sithole, K. **2011**, *346,* 2528-2532.

5. (a) Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M., *Tetrahedron Lett.* **2002,** *43*, 5573-5577; (b) Bedini, E.; Carabellese, A.; Schiattarella, M.; Parrilli, M., *Tetrahedron* **2005,** *61*, 5439-5448; (c) Huchel, U.; Tiwari, P.; Schmidt, R. R., *J. Carbohydr. Chem. 29*, 61-75; (d) Li, Y.; Wei, G.; Yu, B., *Carbohydr. Res.* **2006,** *341* (16), 2717-2722; (e) Okada, Y.; Ohtsu, M.; Bando, M.; Yamada, H., *Chem. Lett.* **2007,** *36* (8), 992-993; (f)Yu, B.; Tao, H., *Tetrahedron Lett.* **2001,** *42* (12), 2405-2407.

6. Tamura, K.; Mizukami, H.; Maeda, K.; Watanabe, H.; Uneyama, K. *J. Org. Chem.* **1993**, *58*, 32-35.

7. (a) Bessel, E. M.; Westwood, J. H. *Carbohydr. Res.* **1972**, *25*, 11-21; (b) Giordano, M.; Iadonisi, A. *Tetrahedron Lett.* **2013**, *54*, 1550-1552; (c) Gomberg, M.; Buchler, C. C.; *J. Am. Chem. Soc*. **1921**, *43*, 1904–1911.

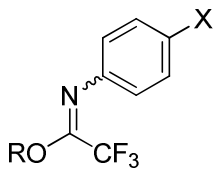
SUPPORTING INFORMATION

The development of N-aryl trifluoroacetimidate-based benzyl and allyl protecting group reagents

Sinele B. Tsabedze[a], Daniel E. K. Kabotso [b], and Nicola L. B. Pohl[a,b,] ∗

[a]*Department of Chemistry, Hach Hall, Iowa State University, Ames IA 50011, USA*
[b]*Department of Chemistry, Simon Hall, Indiana University, Bloomington IN 47405, USA*

General procedure for synthesis of alkyl 2,2,2-trifluoro-*N*-arylacetimidates.



Excess alcohol (at least 5 equiv., allyl alcohol or benzyl alcohol) with excess potassium carbonate (at least 5 equiv.) was stirred at room temperature for 5-10 minutes. Aryl trifluoroacetimidoyl chloride (1 equiv., made according to reference 6 and purified by distillation[1] or basic alumina) was added and the reaction stirred at ambient temperature for at most 1 h. The solution was filtered using filter paper and the filtrate concentrated under high vacuum. Leaving the reaction overnight produced an undesired diacetal side product. Separation using the Biotage® SP1 Flash + purification unit using the FLASH 12+M cartridge Medium Pressure Liquid Chromatography (MPLC) (0%-20% ethyl acetate in hexane) afforded the product, which was recrystallized from 10% ethyl acetate/hexane to provide a crystalline solid.

∗ Corresponding author. Tel.: +1-812-855-0298; fax: +1-812-855-8300; e-mail: npohl@indiana.edu

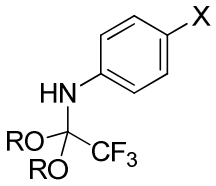
General procedure for alkylation with trifluoroacetimidates.[2]

To one equivalent of the alcohol, **7** or **8**, TMSOTf (10 mol%) in anhydrous CH_2Cl_2 (5 mL) were added and the reaction stirred under nitrogen and cooled to 0 °C for 10 min. The imidate (1.2-2 equiv.) dissolved in anhydrous CH_2Cl_2 (5 mL) was added to the alcohol/promoter mixture and the reaction allowed to stir at 0 °C for 1 h.

Synthesis of methyl 2,2,2-trifluoro-*N*-arylacetimidates.

The general procedure above was applied. The amount of time for reaction was between 10-30 minutes for all cases. In the case of X=NO_2, the desired product was significantly more polar than the cases where X= H, OMe or Cl. Different chromatography conditions were necessary: in the case where X=NO_2 the desired product was isolated with a gradient column of 0-20% ethyl acetate in hexane. For the other cases, 100% hexane was sufficient to separate the desired product.

Formation of an undesired diacetal.

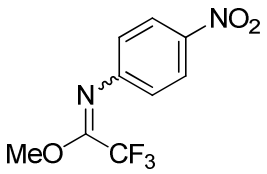


An undesired diacetal product forms easily and very fast (within hours of the commencement of the reaction). The reaction should be closely monitored and stopped before most of the desired product forms the undesired diacetal product.

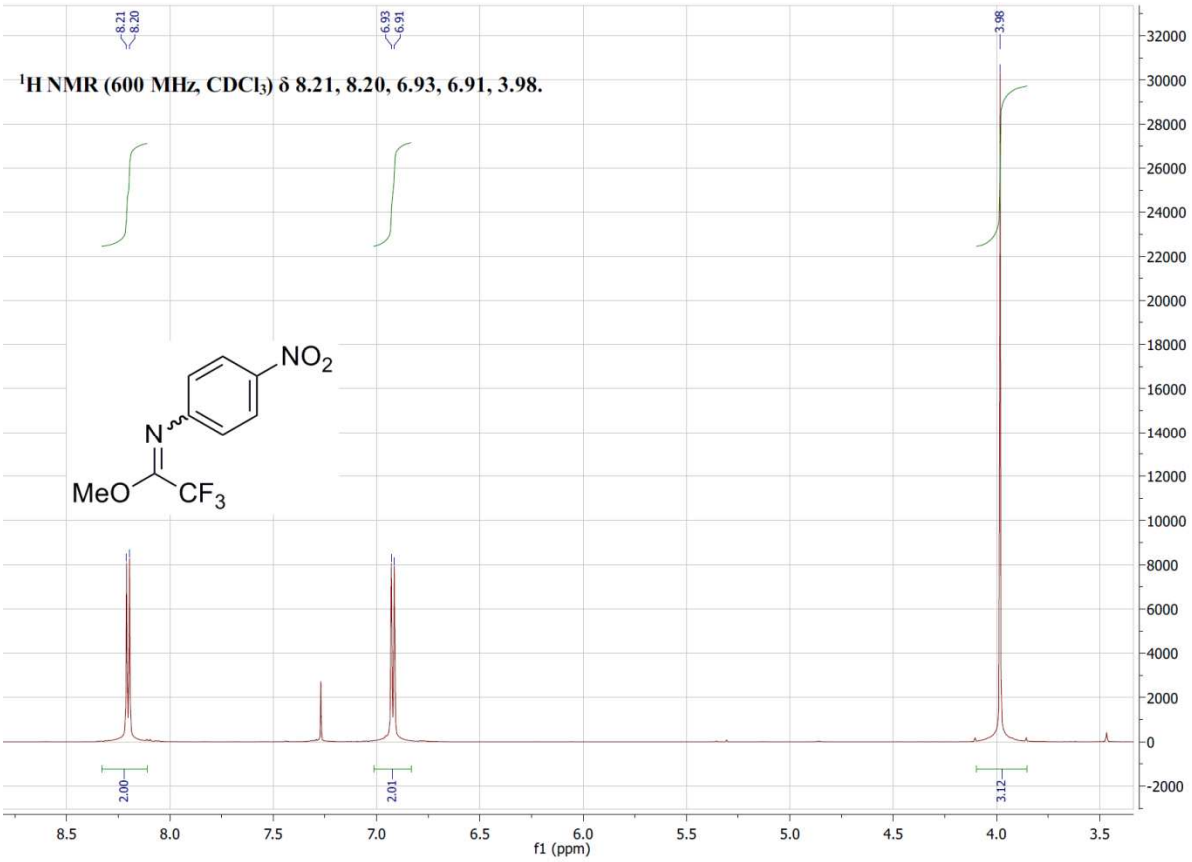
General procedure for alkylation with trifluoroacetimidates.

To one equivalent of the alcohol, **7** or **8**, (50-100 mg scale), TMSOTf (10 mol %) in anhydrous CH_2Cl_2 (5 mL) was added and the reaction stirred under nitrogen and cooled to 0 °C for 10 min. The imidate (1.2-2 equiv.) dissolved in anhydrous CH_2Cl_2 (5 mL) was added to the alcohol/promoter mixture and the reaction allowed to stir at 0 °C for 1 h. The reaction was quenched by adding a few drops of triethylamine. The reaction mixture was diluted with CH_2Cl_2 and washed with water. The aqueous layer was extracted twice with CH_2Cl_2 and the combined organic layers dried with anhydrous $MgSO_4$. The product was purified using either Biotage® SP1 Flash+ purification unit or the FLASH 12+M cartridge or medium pressure liquid chromatography MPLC, (Teledyne ISCO, Teledyne Technology Company, 4700 Superior St., Lincoln, NE, 68504, www.isco.com) and then recrystallized either from ethanol or methanol.

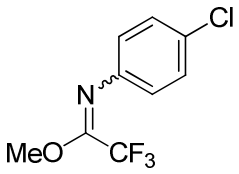
Methyl 2,2,2-trifluoro-*N*-(*p*-nitrophenyl)acetimidate (1).



^{1}H NMR (600 MHz, $CDCl_3$) δ (2H, d, Ar) 8.21, 8.20, (2H, d, Ar) 6.93, 6.91, (3H, s, OMe) 3.98; **^{13}C NMR** (151 MHz, $CDCl_3$) δ 150.44, 146.88, 146.64, 144.25, 124.78, 124.75, 120.13, 120.12, 116.94, 115.04, 55.64.

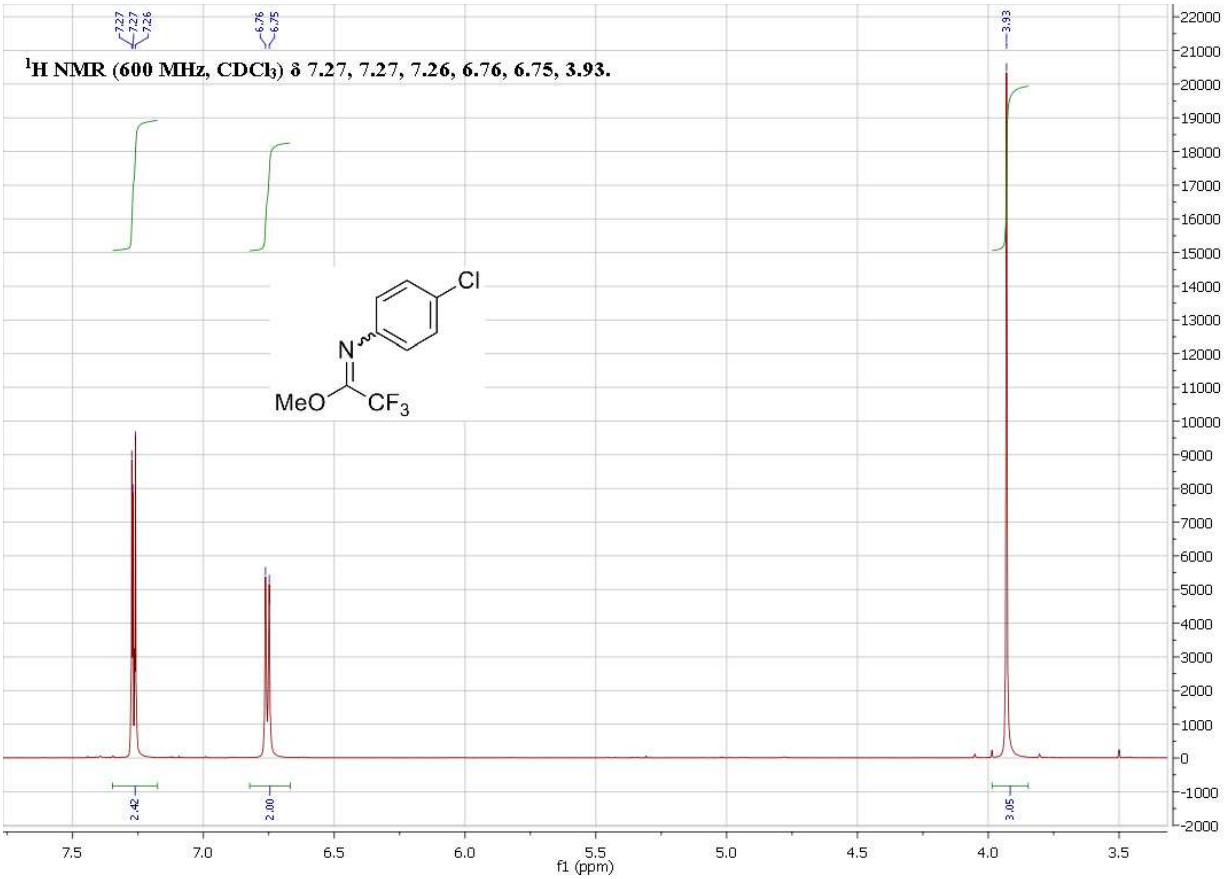


Methyl 2,2,2-trifluoro-*N*-(*p*-chlorophenyl)acetimidate (2).

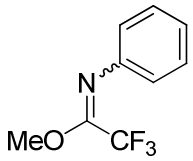


^{1}H NMR (600 MHz, $CDCl_3$) δ 7.27- 7.26 (2H, d, Ar), 6.76-6.75 (2H, d, Ar), 3.93 (3H, s, OMe);

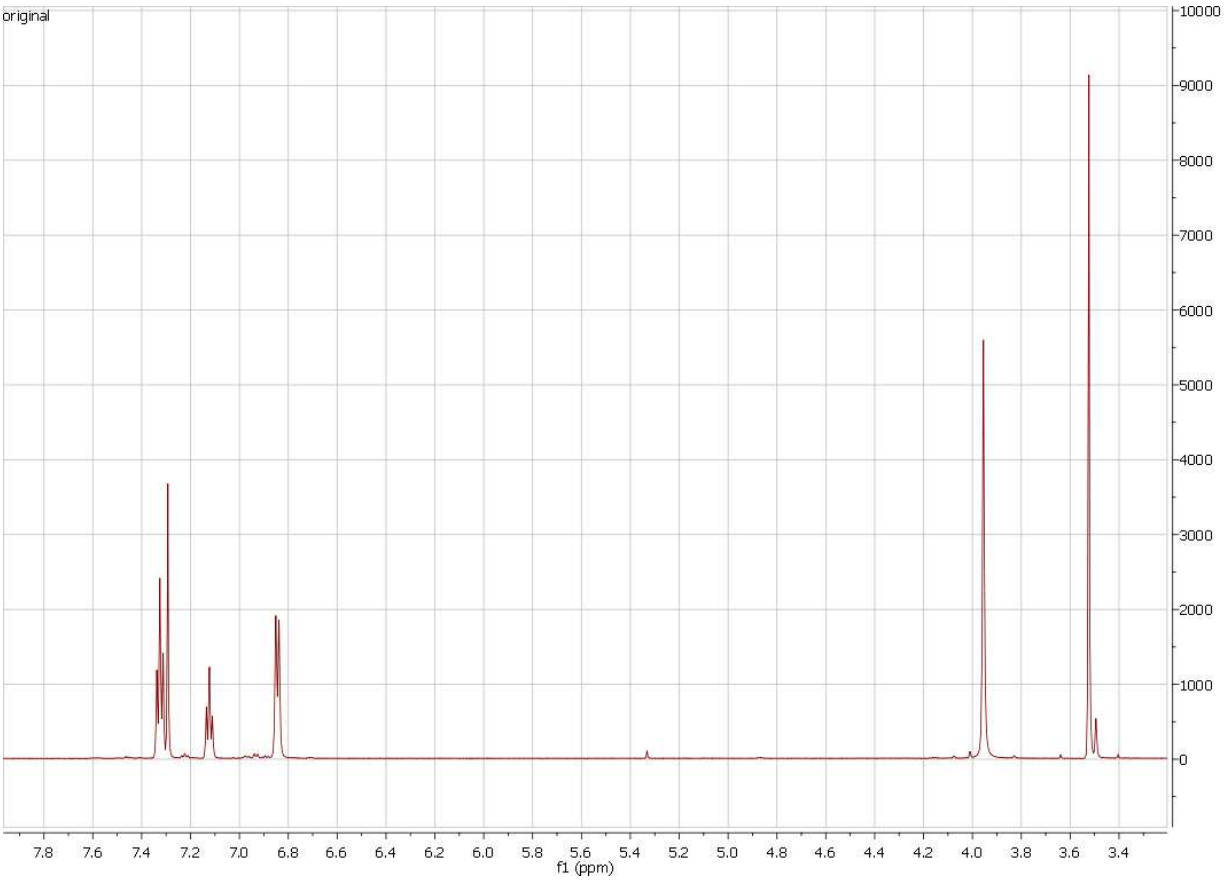
^{13}C NMR (151 MHz, $CDCl_3$) δ 142.93, 129.30, 120.99, 117.02, 115.12, 77.23, 77.02, 76.81, 55.16, 29.72.



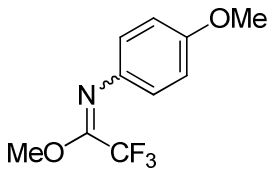
Methyl 2,2,2-trifluoro-*N*-phenylacetimidate (3).



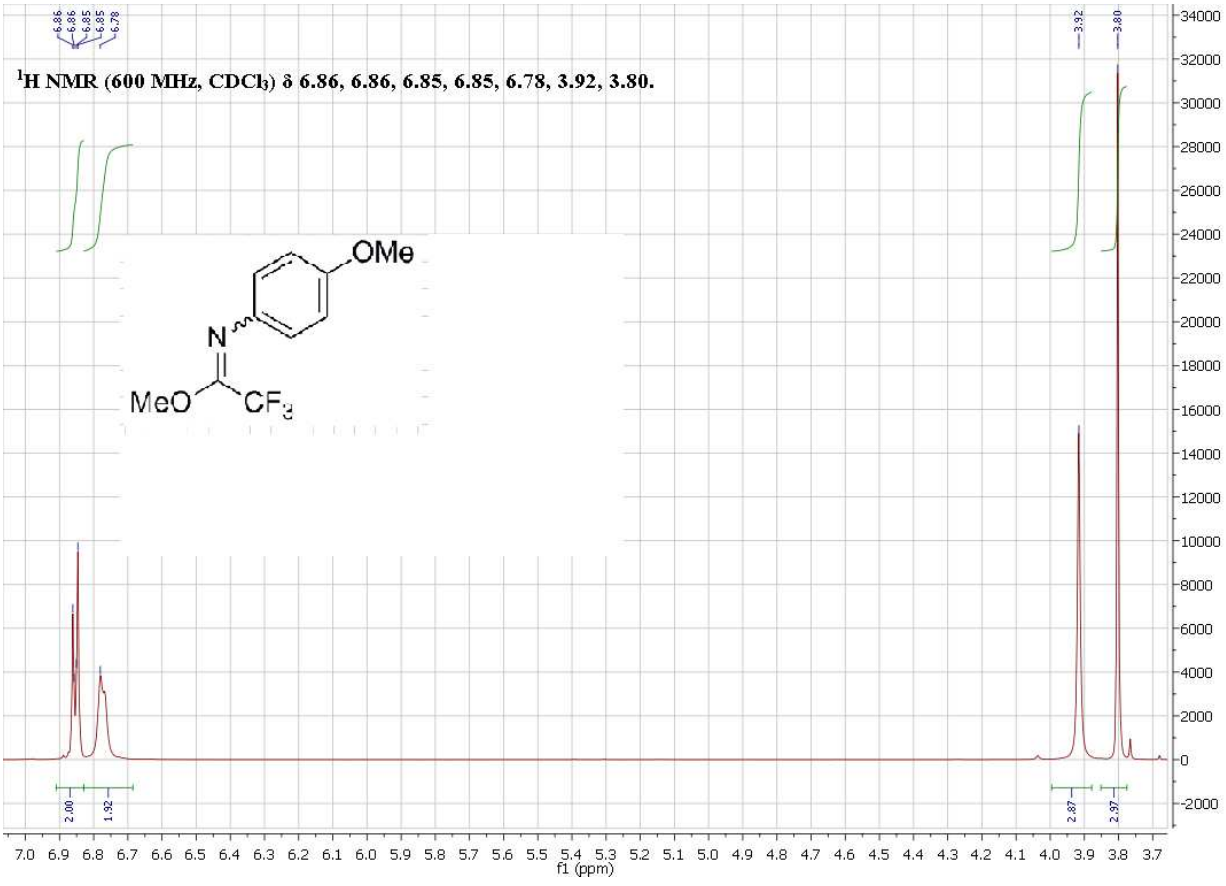
^{1}H NMR (600 MHz, $CDCl_3$) δ (2H, t, Ar)7.32-7.29, (1H, t, Ar) 7.11-7.10, (2H, d, Ar) 6.83-6.81, (2H, s, OMe) 3.93; **^{13}C NMR** (151 MHz, $CDCl_3$) δ 144.33, 128.71, 123.89, 119.57, 77.23, 77.02, 76.81, 50.92.



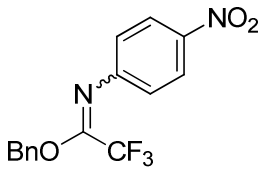
Methyl 2,2,2-trifluoro-*N*-(*p*-methoxyphenyl)acetimidate (4).



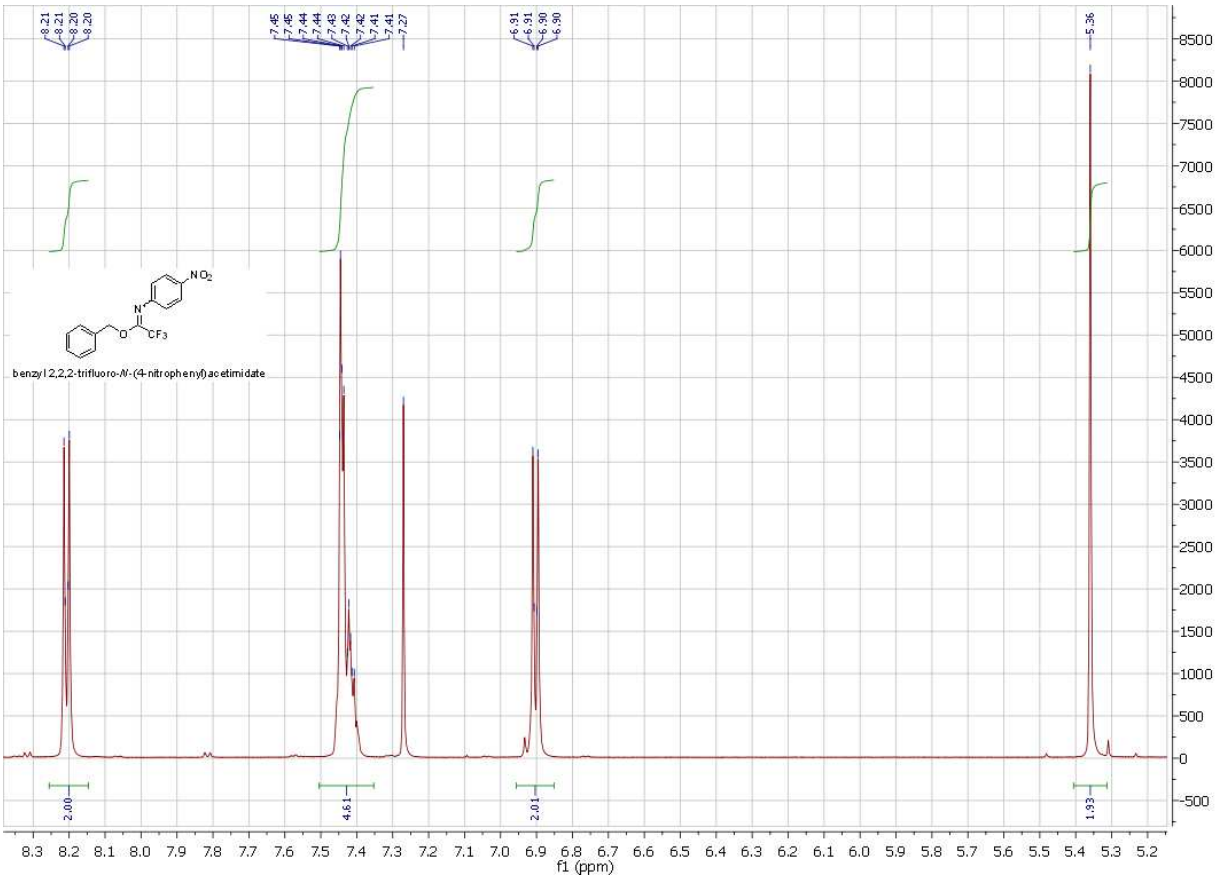
^{1}H NMR (600 MHz, $CDCl_3$) δ (2H, d, Ar)6.86- 6.85, (2H, m, Ar)6.78, (3H, s, PhO**Me**)3.92, (3H, s, OMe) 3.80; **^{13}C NMR** (151 MHz, $CDCl_3$) δ 156.33, 137.46, 120.71, 118.96, 114.28, 114.02, 77.23, 77.02, 76.81, 55.50, 55.41, 54.86.

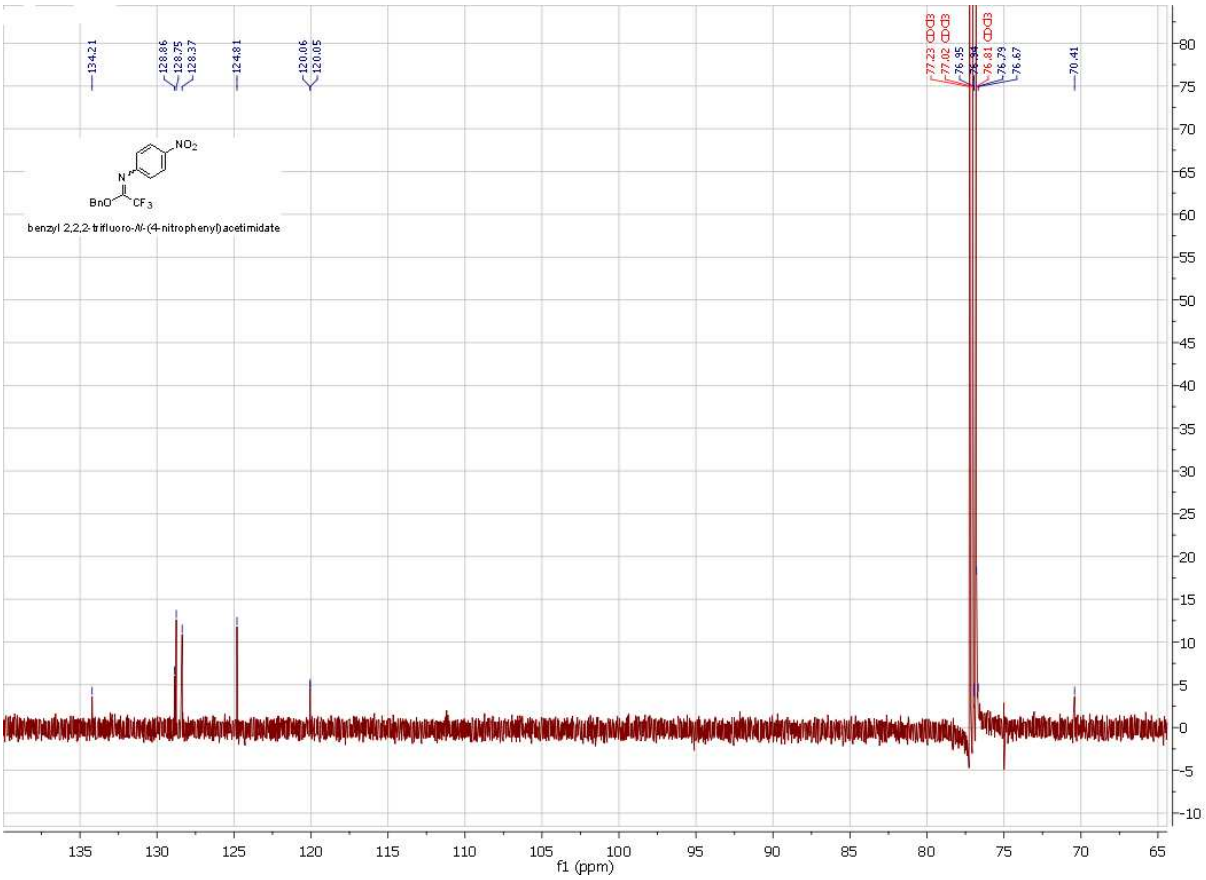


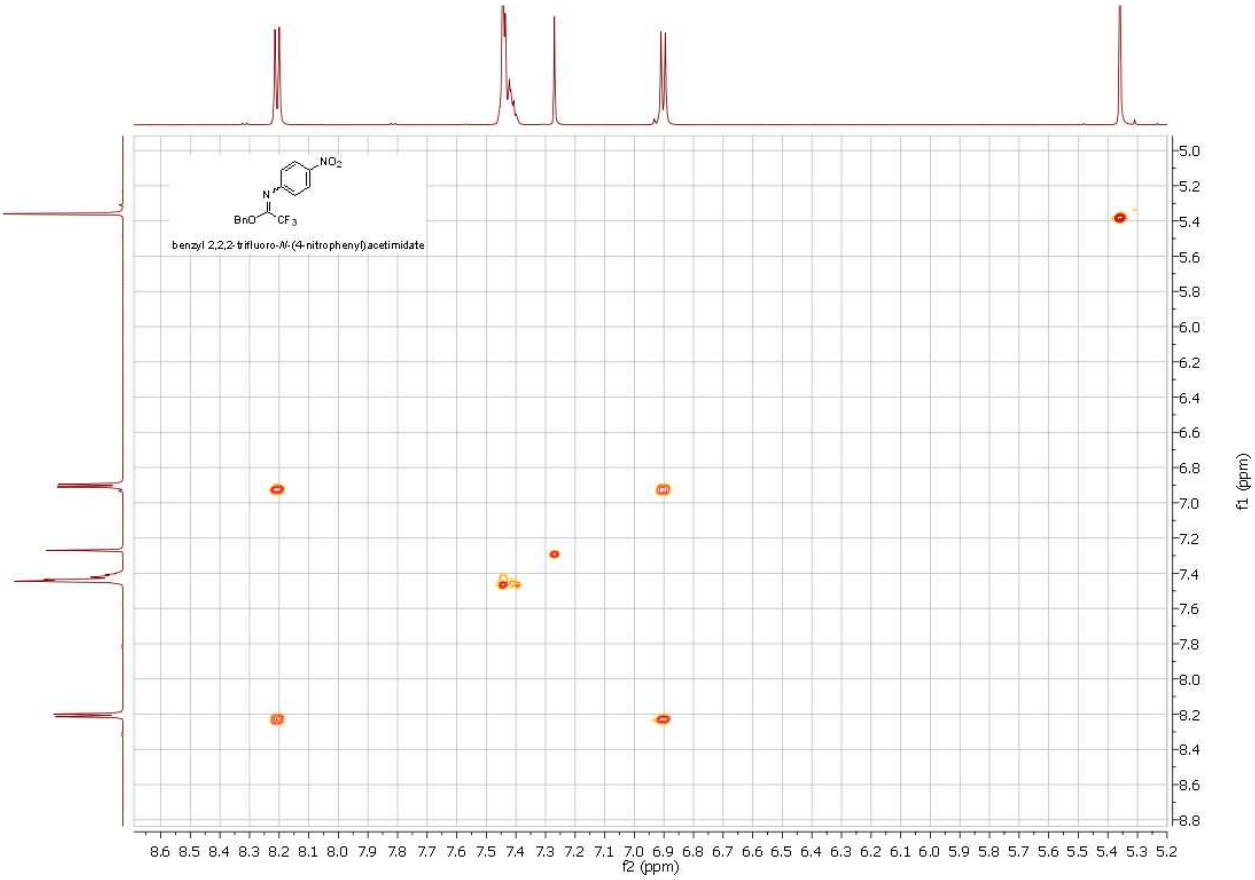
Benzyl 2,2,2-trifluoro-*N*-(4-nitrophenyl)acetimidate (5).



^{1}H NMR (600 MHz, $CDCl_3$) δ (d, 2H, Ar) 8.21-8.19,(m, 5H, Bn) 7.45-7.41, (d, 2H, Ar) 6.92-6.91, (s, 2H, CH_2-Ph) 5.36; **^{13}C NMR** (151 MHz, CDCl3) δ 134.21, 128.75, 124.81, 120.05, 70.41; **HRMS**: (M+H) calcd for $C_{15}H_{12}O_3N_2F_3$ 325.0795, found 325.0792; **m.p**.: 111-112 °C.

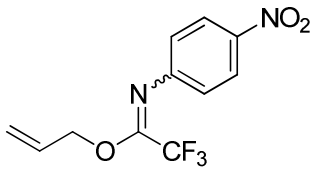




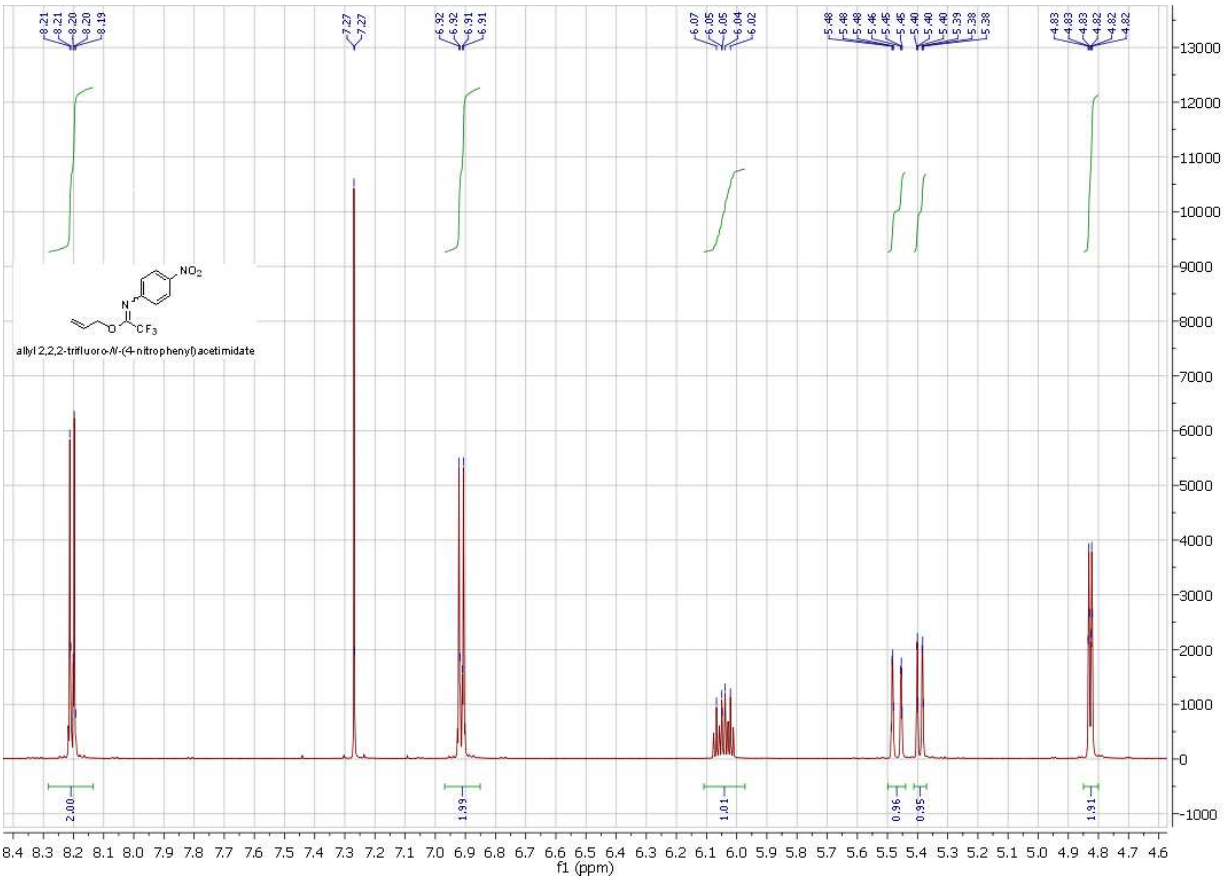


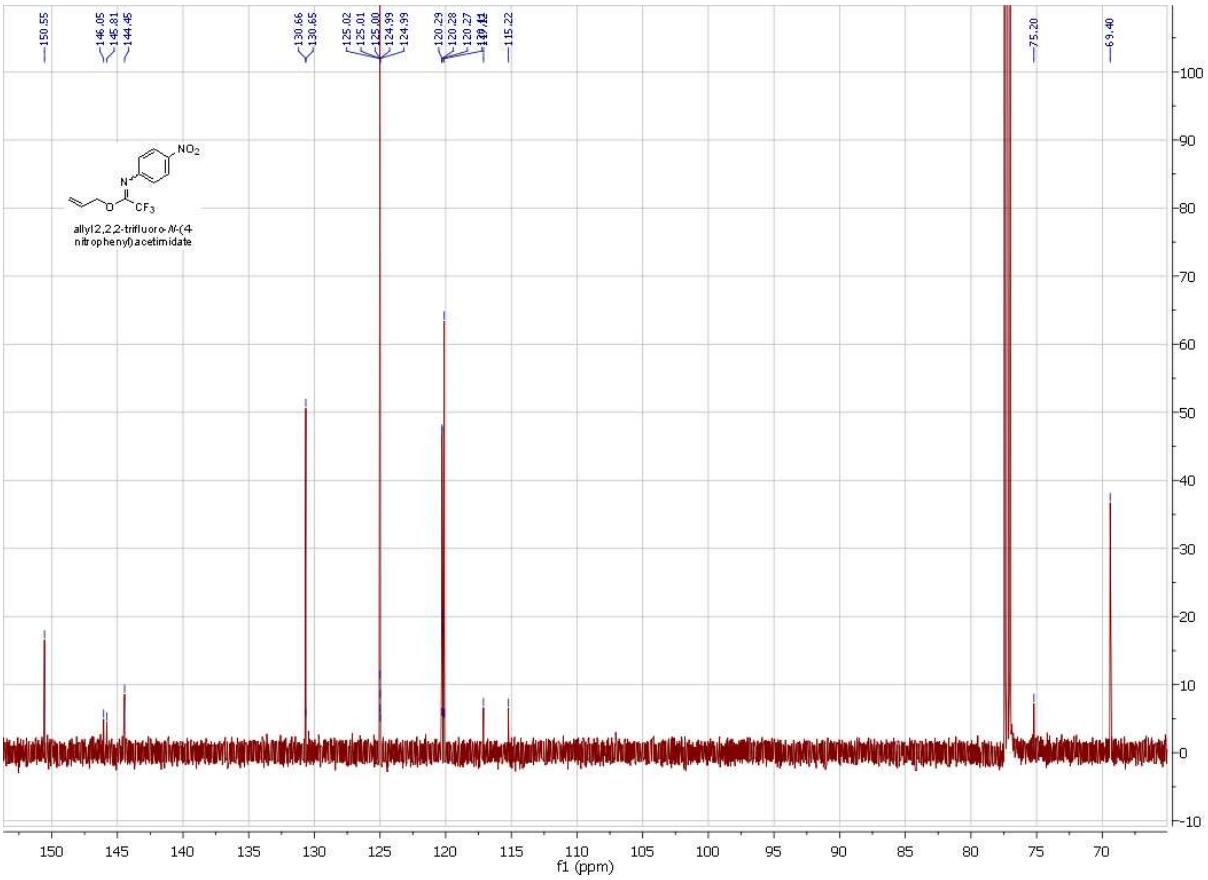
^{1}H NMR COSY, 600 MHz, $CDCl_3$

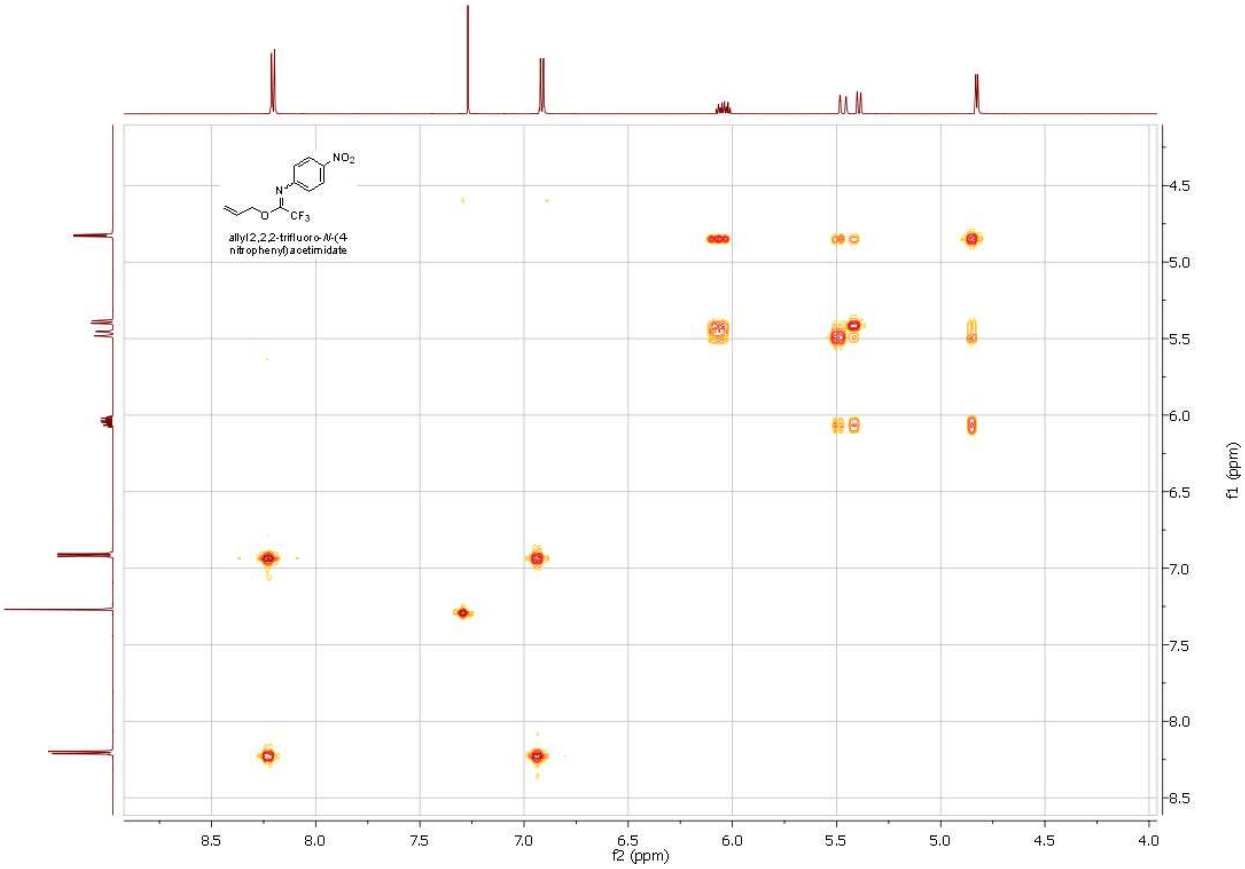
Allyl 2,2,2-trifluoro-*N*-(4-nitrophenyl)acetimidate (6).



^{1}H NMR (600 MHz, $CDCl_3$) δ (d, 2H, Ar) 8.21-8.19, (d, 2H, Ar) 6.92-6.91, (m, 1H, CH=) 6.07-6.02, (d, 1H, $=CH_2$) 5.48-5.45, (d, 1H, $=CH_2$) 5.40-5.38, (d, 2H, OCH_2); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 150.55, 146.05, 130.66, 125.00, 120.27, 115.22, 75.20, 69.40; **HRMS**: (M+H) calcd for $C_{11}H_{10}O_3N_2F_3$ 275.0638, found 275.0647; **m.p**.: 59.5-61 °C.

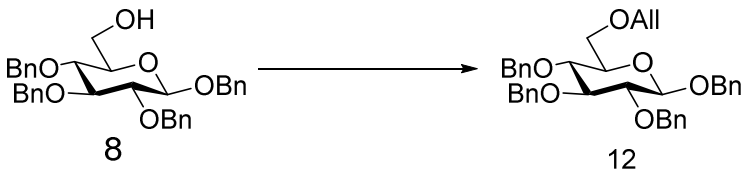




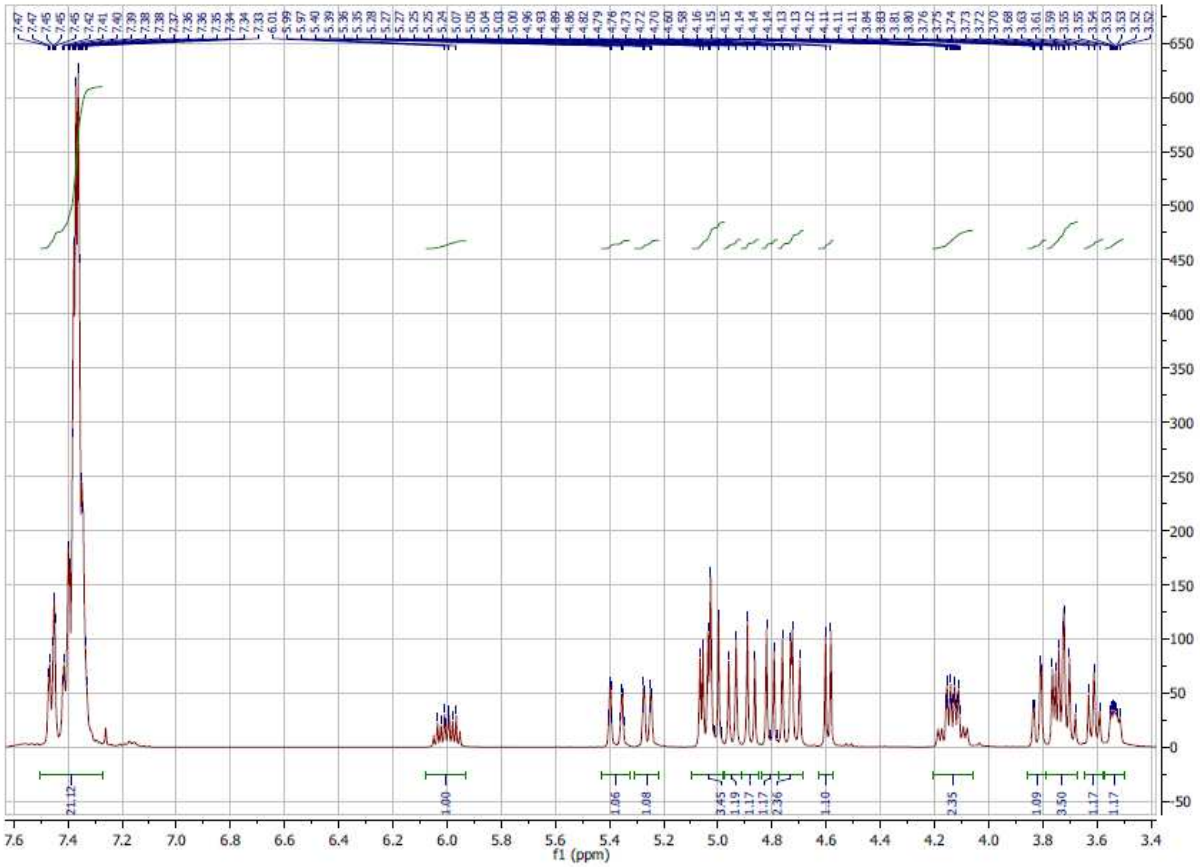


^{1}H NMR COSY, 600 MHz, $CDCl_3$

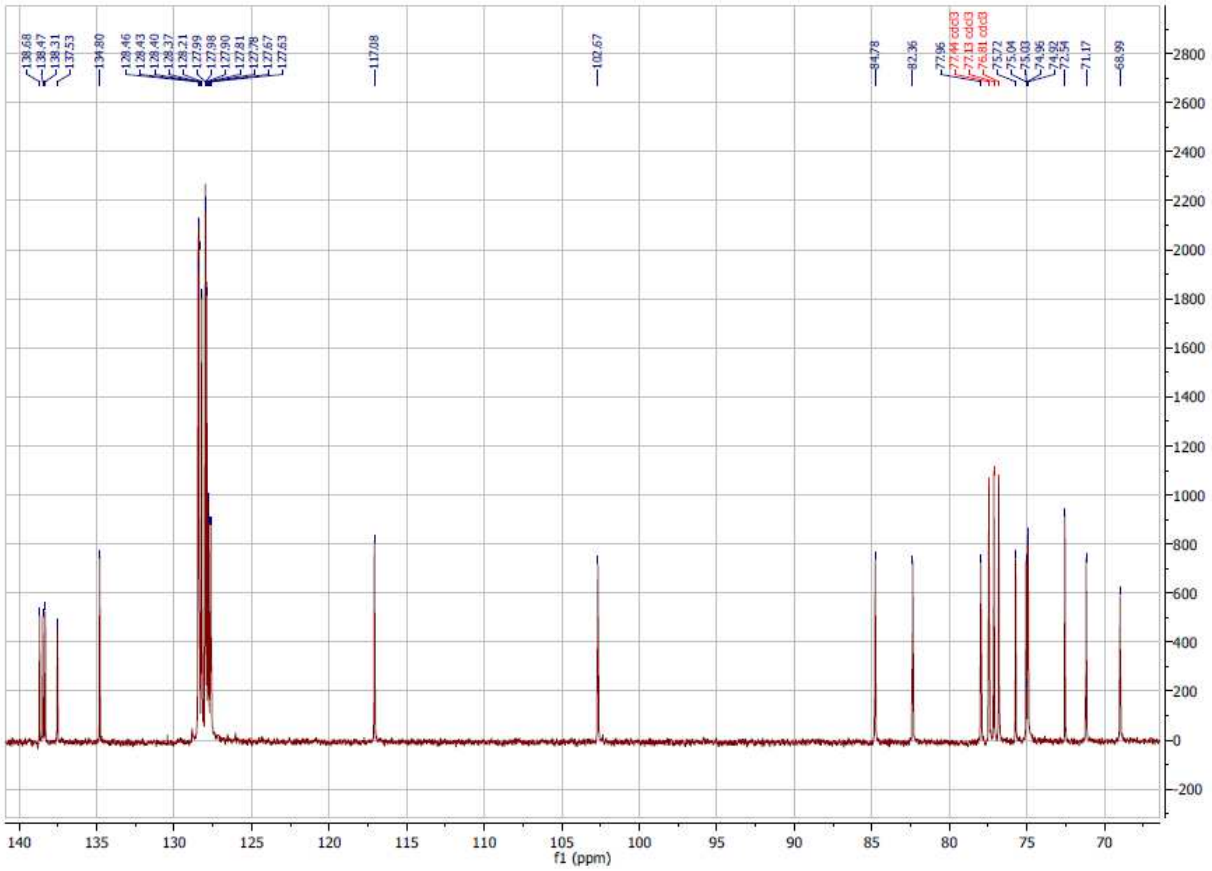
Synthesis of benzyl 6-*O*-allyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (12).



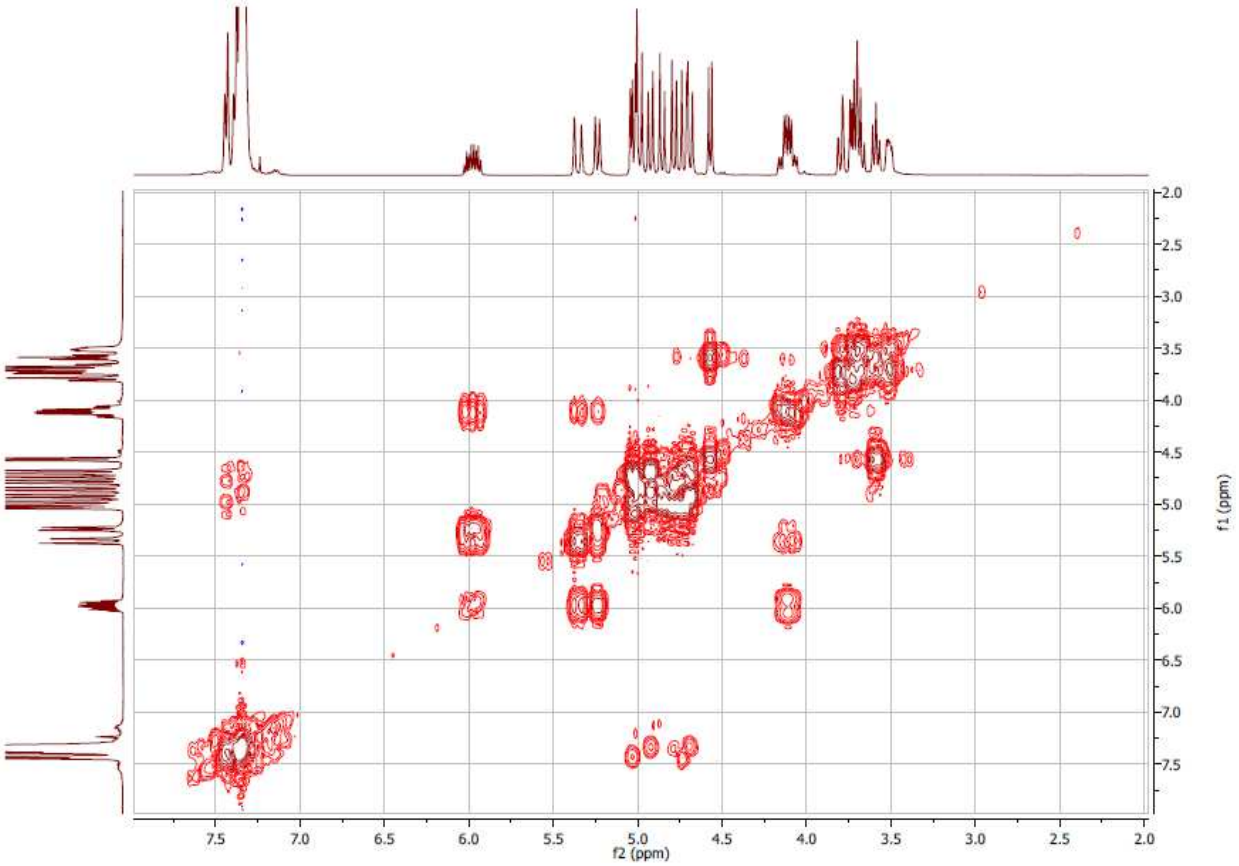
To a solution of benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**8**)[3] (100.0 mg, 185.0 µmol) in anhydrous CH_2Cl_2 (10 mL) was added TMSOTf (3.35 mL, 18.5 µmol). The reaction mixture was cooled to 0 °C and stirred under nitrogen for 10 min. Reagent **6** (60.9 mg, 222 µmol) dissolved in anhydrous CH_2Cl_2 (5 mL) was added and the reaction mixture allowed to stir at 0 °C. After TLC indicated complete conversion of the starting material to product (1 h), the reaction was quenched by the addition of a few drops of triethylamine. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with water (15 mL). The aqueous layer was extracted CH_2Cl_2 (2 x15 mL) and the combined organic layers dried over anhydrous $MgSO_4$. After filtration, the filtrate was concentrated and the crude purified with MPLC (Teledyne ISCO, Teledyne Technology Company, 4700 Superior St., Lincoln, NE, 68504, www.isco.com) using 5-10% ethyl acetate in hexane to give a white solid. The solid was recrystallized from methanol to give the desired product (86.8 mg, 81%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ (m, 20H, CH, Ar) 7.45-7.32, (m, 1H, CH=) 6.03-5.96, (d, 1H, $=CH_2$) 5.39-5.34, (d, 1H, $=CH_2$) 5.27-5.23, (d 6H, CH_2) 4.89-4.69, (d, 1H, CH) 4.60-4.58, (m, 2H CH_2) 4.19-4.08, (t, 2H, CH_2) 3.63-3.59, (t, 3H, CH) 3.58-3.52; **^{13}C NMR** (100 MHz, CDCl3) δ 138.68, 138.47, 138.31, 137.53, 134.80, 128.46-127.63117.08, 102.67,84.78, 82.36, 77.96, 75.72, 75.04, 75.03, 74.96, 74.92, 71.17, 68.99; **HRMS**: (M+Na) calcd for $C_{37}H_{40}O_6Na$ 603.2723, found 603.2725; **m.p**.: 81-82 °C.



^{1}H NMR for benzyl 6-O-allyl-2,3,4-tri-O-benzylglucopyranoside (**12**).



^{13}C NMR for benzyl 6-*O*-allyl-2,3,4-tri-*O*-benzylglucopyranoside (**12**).



^{1}H NMR COSY, 400 MHz, $CDCl_3$ (**12**).

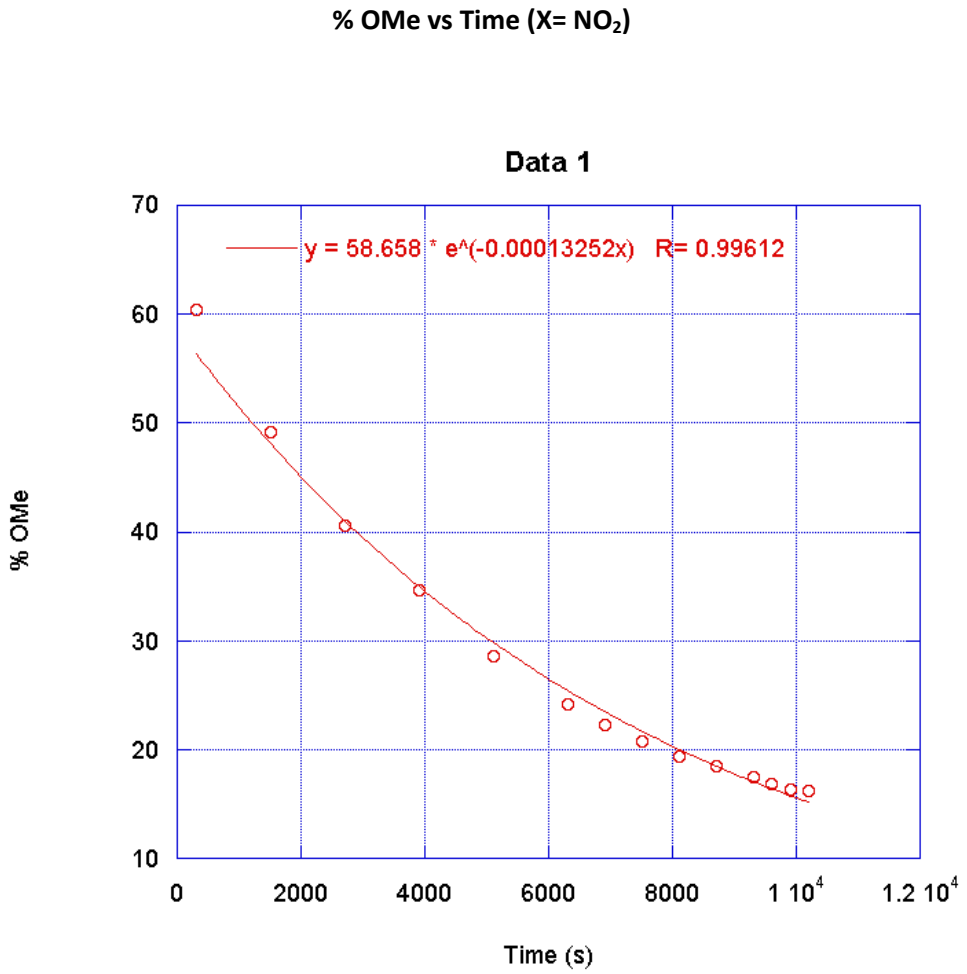


Figure S1. A graph showing the % imidate aromatic peaks by ^{1}H NMR over time for compound **1**.

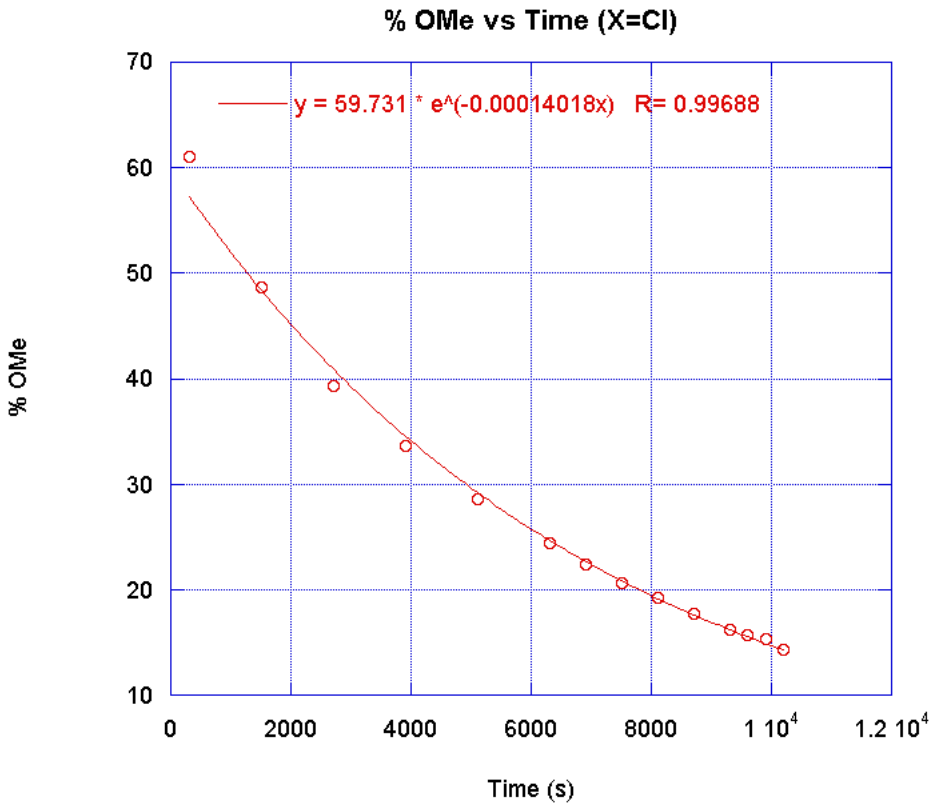


Figure S2. A graph showing the % imidate aromatic peaks by ^{1}H NMR over time for compound **2**.

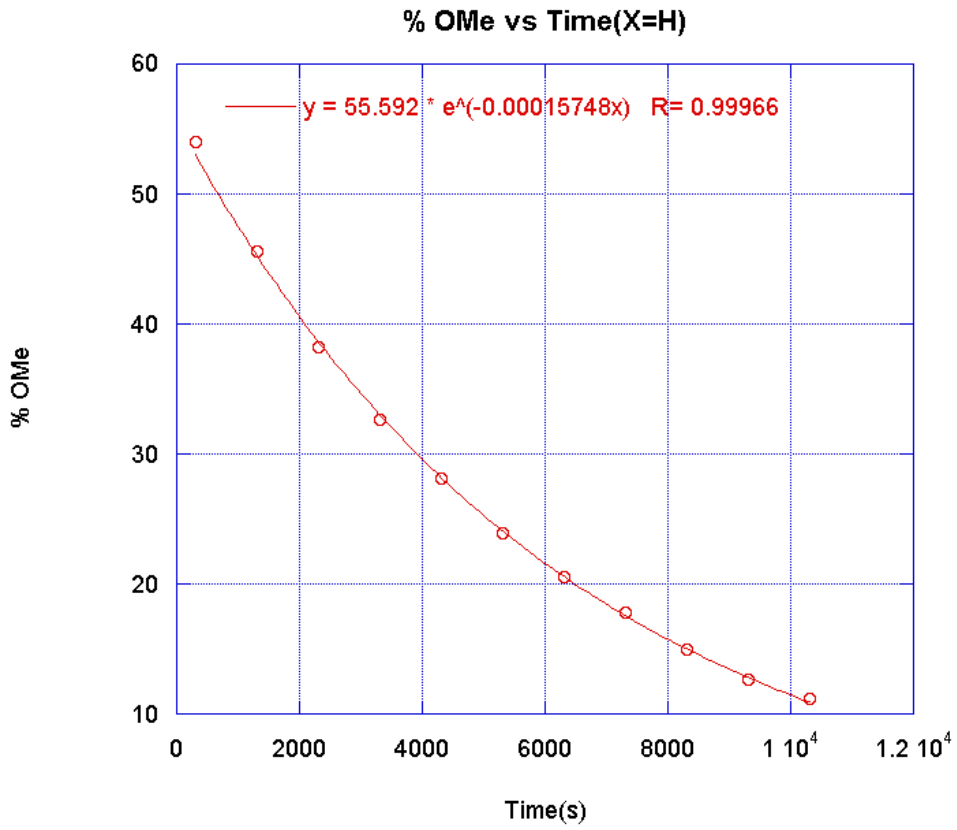


Figure S3. A graph showing the % imidate aromatic peaks by ^{1}H NMR over time for compound **3**.

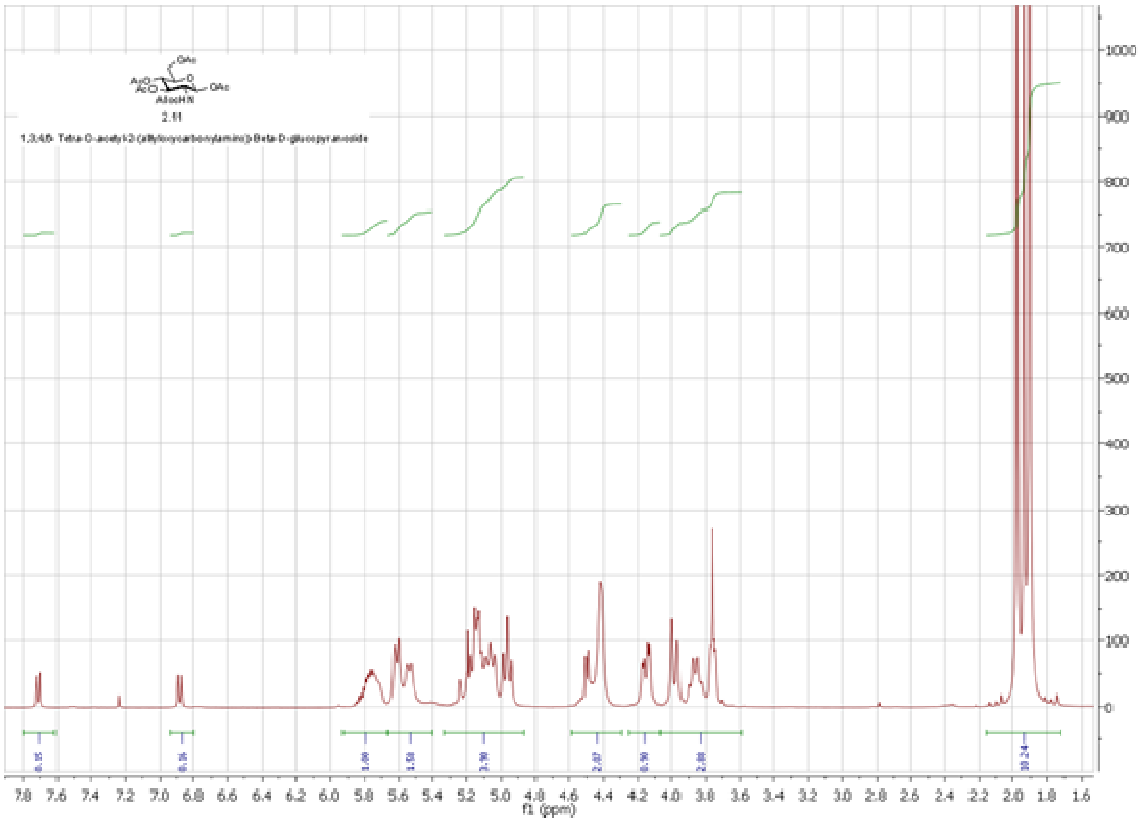
Table S1. Calculated values of the rate constant(k) for the degradation of compounds **1-4**.

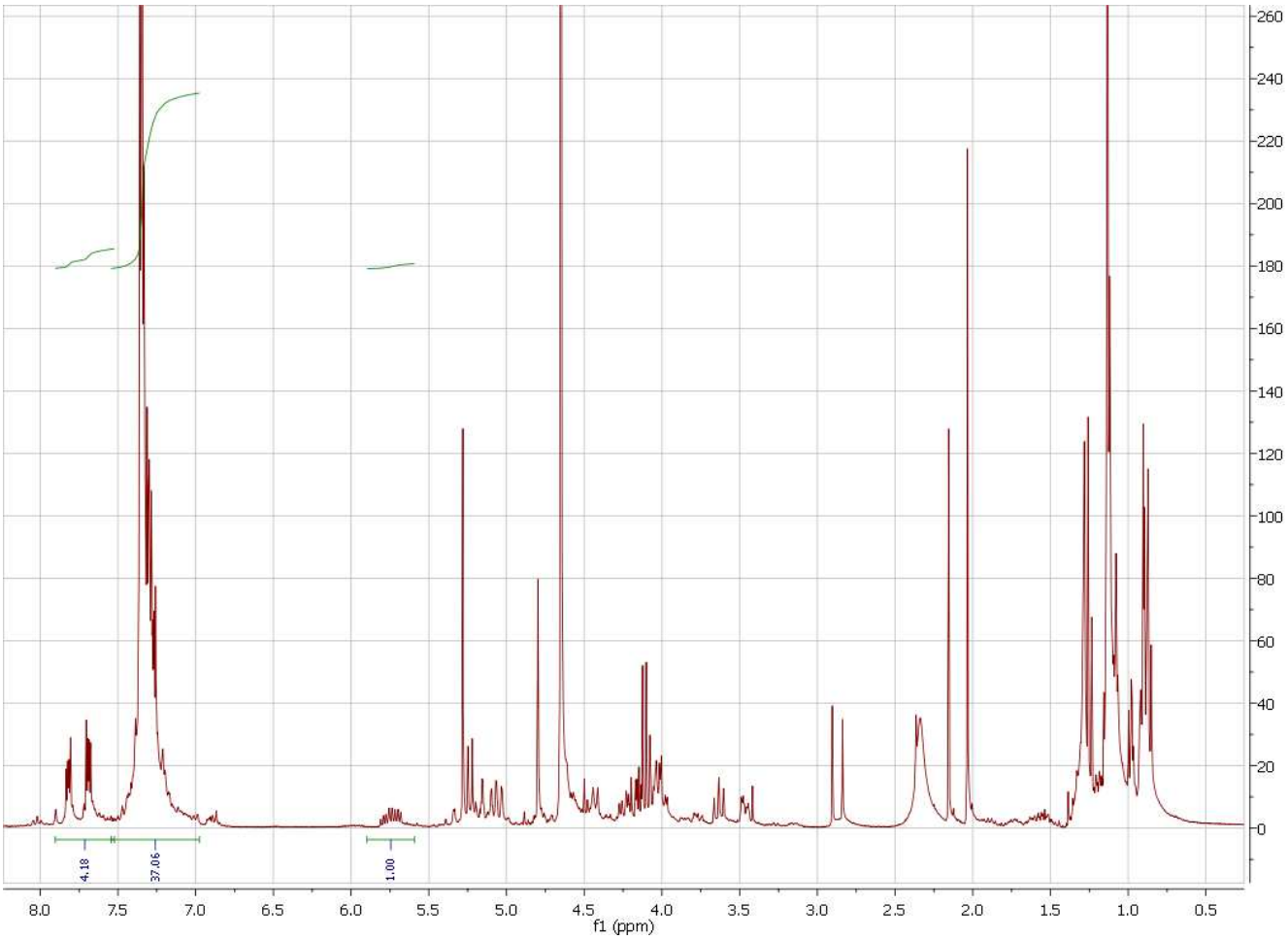
Imidate	**Rate Constant (k)(10^{-4} sec^{-1})**
1 (X= NO_2)	1.33
2 (X= Cl)	1.40
3 (X= H)	1.47
4 (X= OMe)	1.78

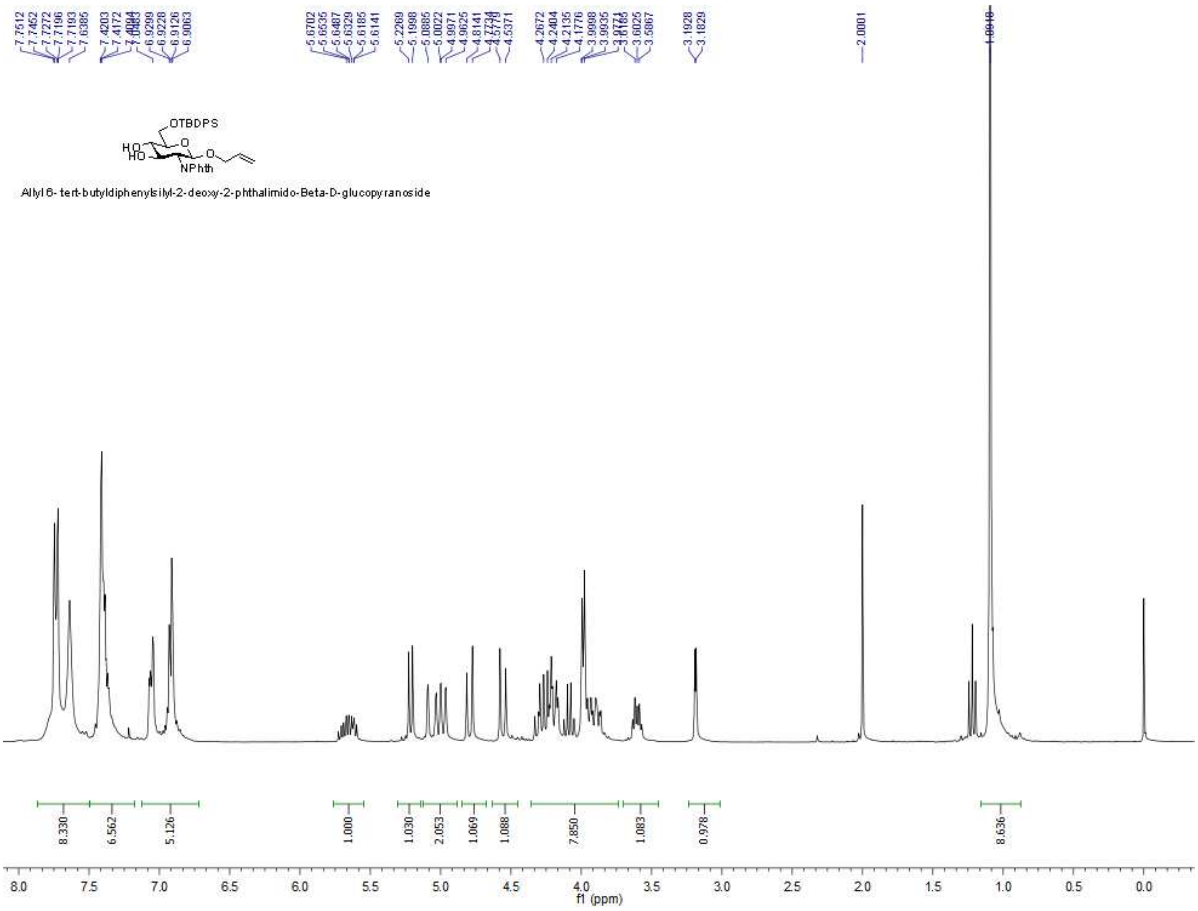
References.

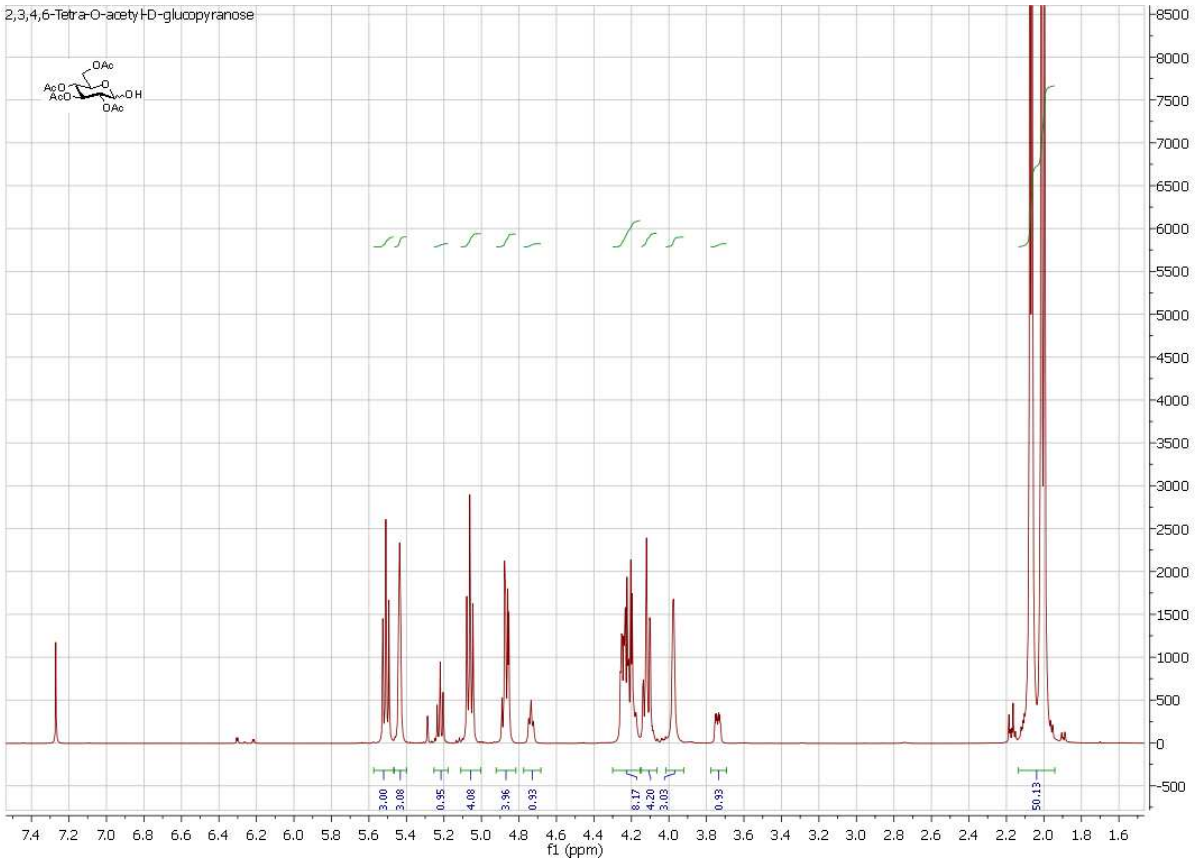
(1) Tamura, K.; Mizukami, H.; Maeda, K.; Watanabe, H. *J. Org. Chem.* **1993**, 32–35.
(2) Okada, Y.; Ohtsu, M.; Bando, M.; Yamada, H. *Chem. Lett.* **2007**, *36*, 992–993.
(3) Lu, W.; Navidpour, L.; Taylor, S. D. *Carbohydr. Res.* **2005**, *340*, 1213–1217.

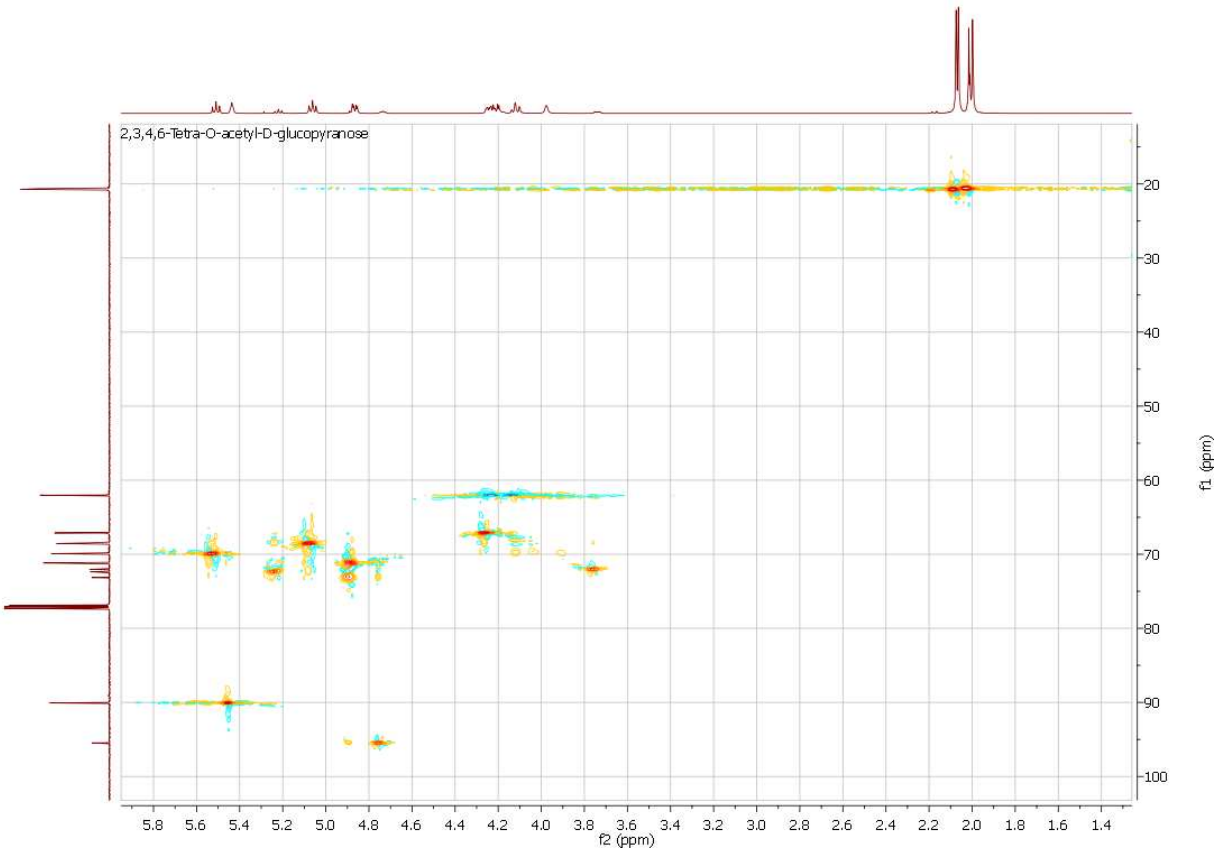
APPENDIX B. SPECTROSCOPIC DATA

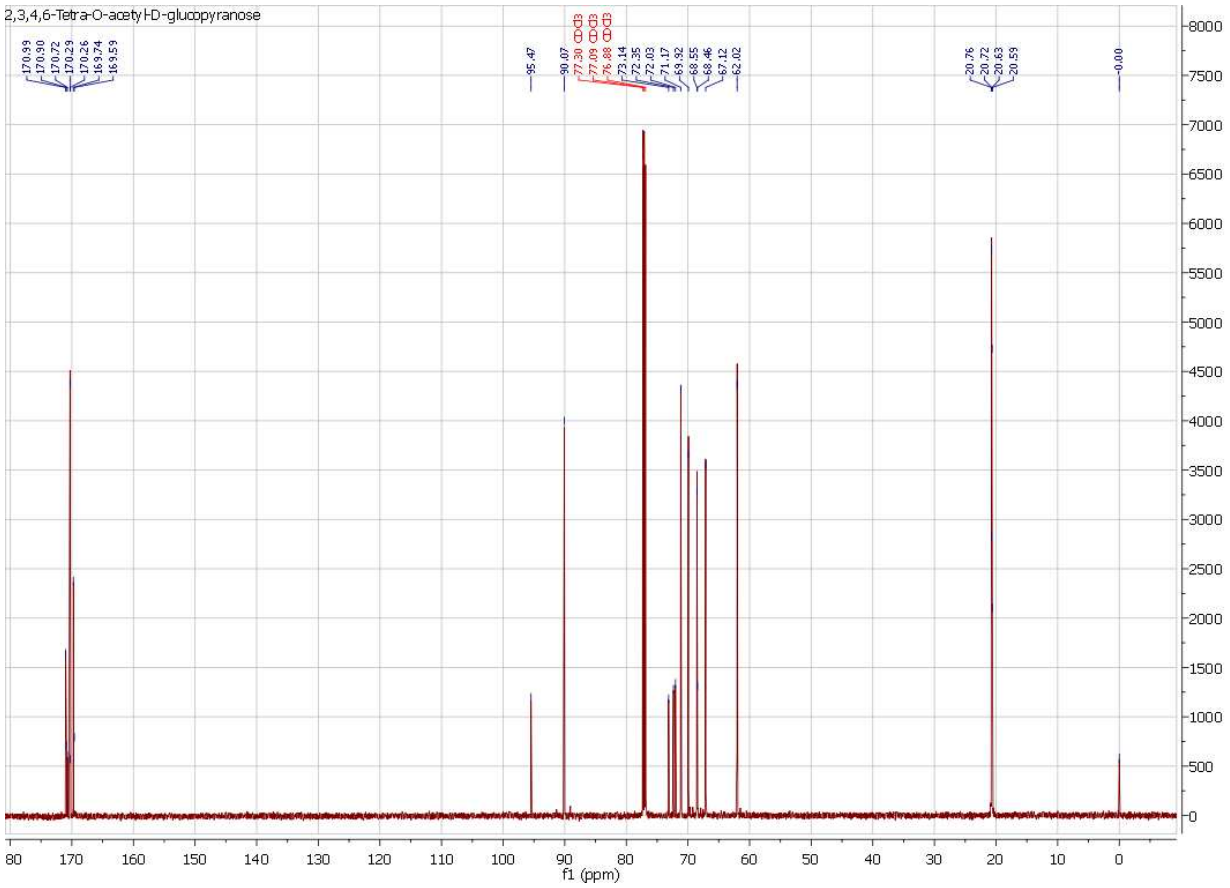


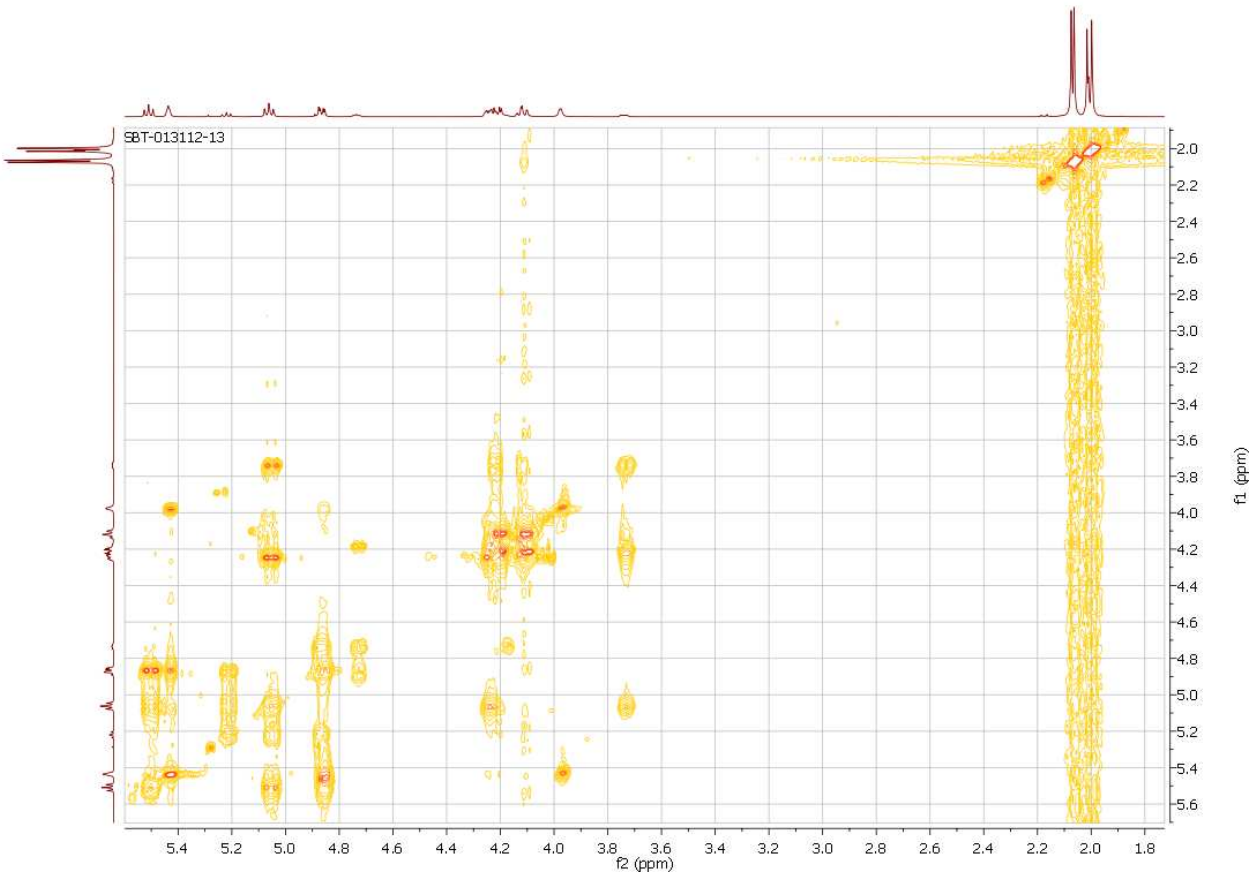




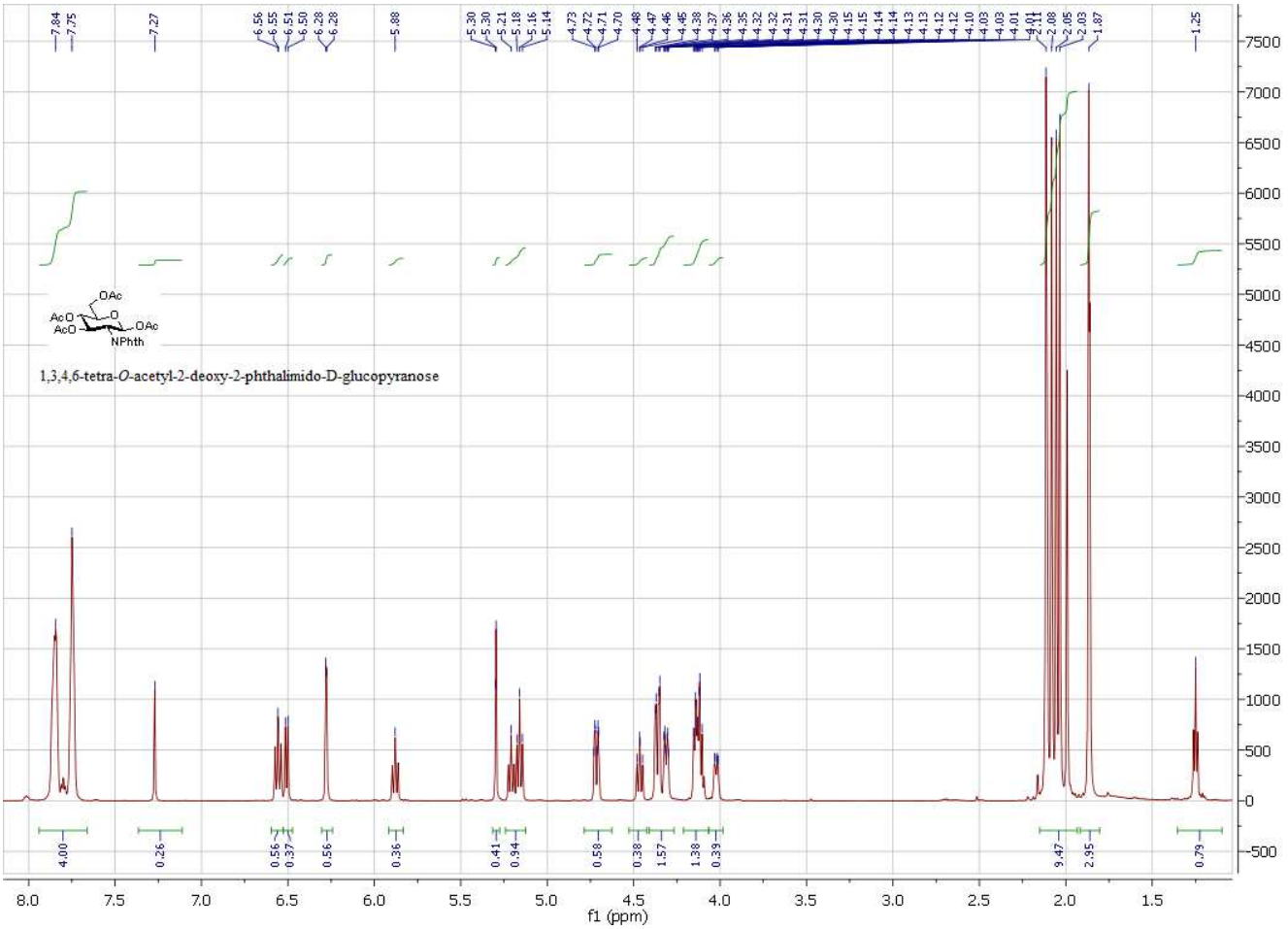


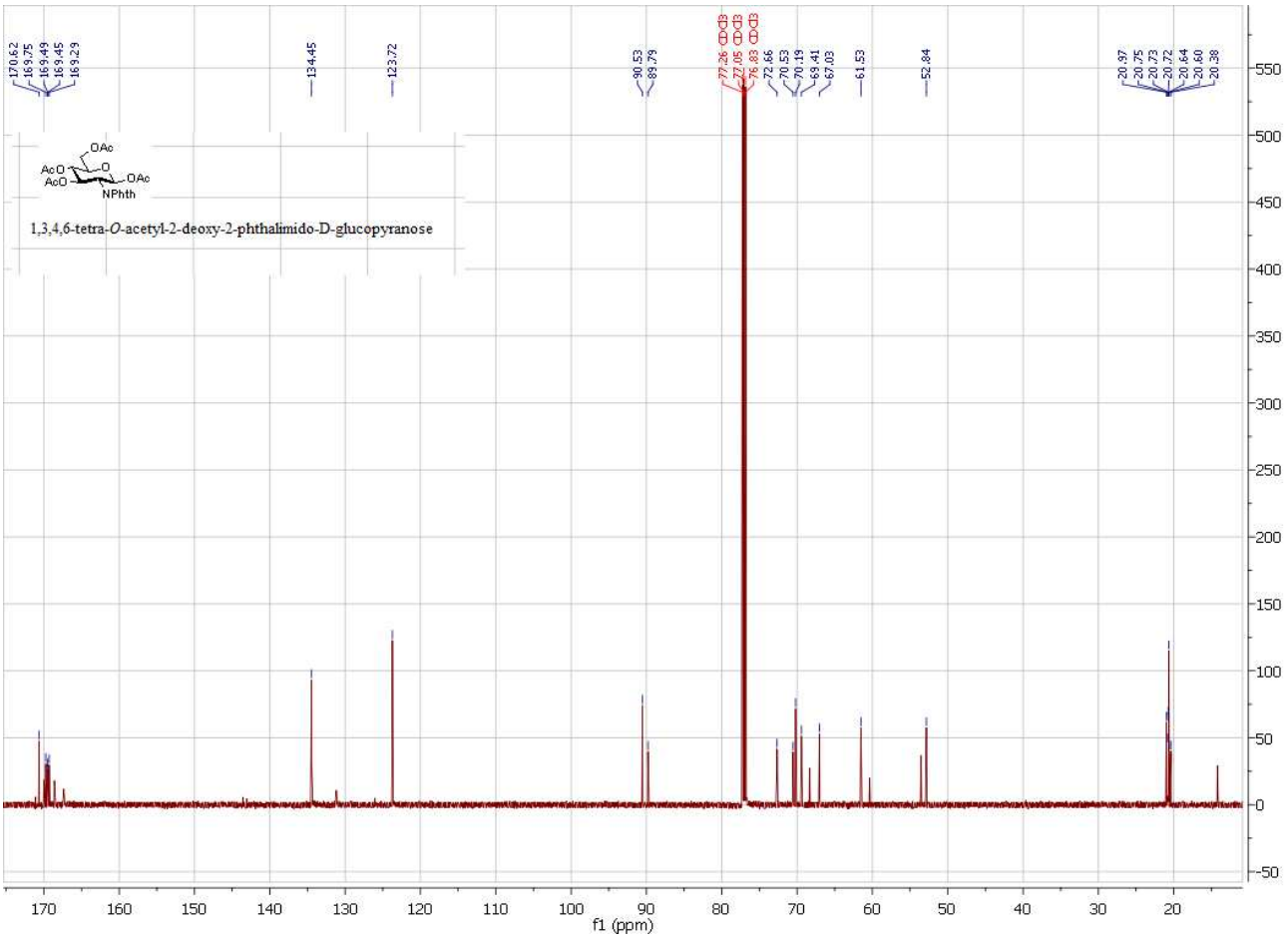


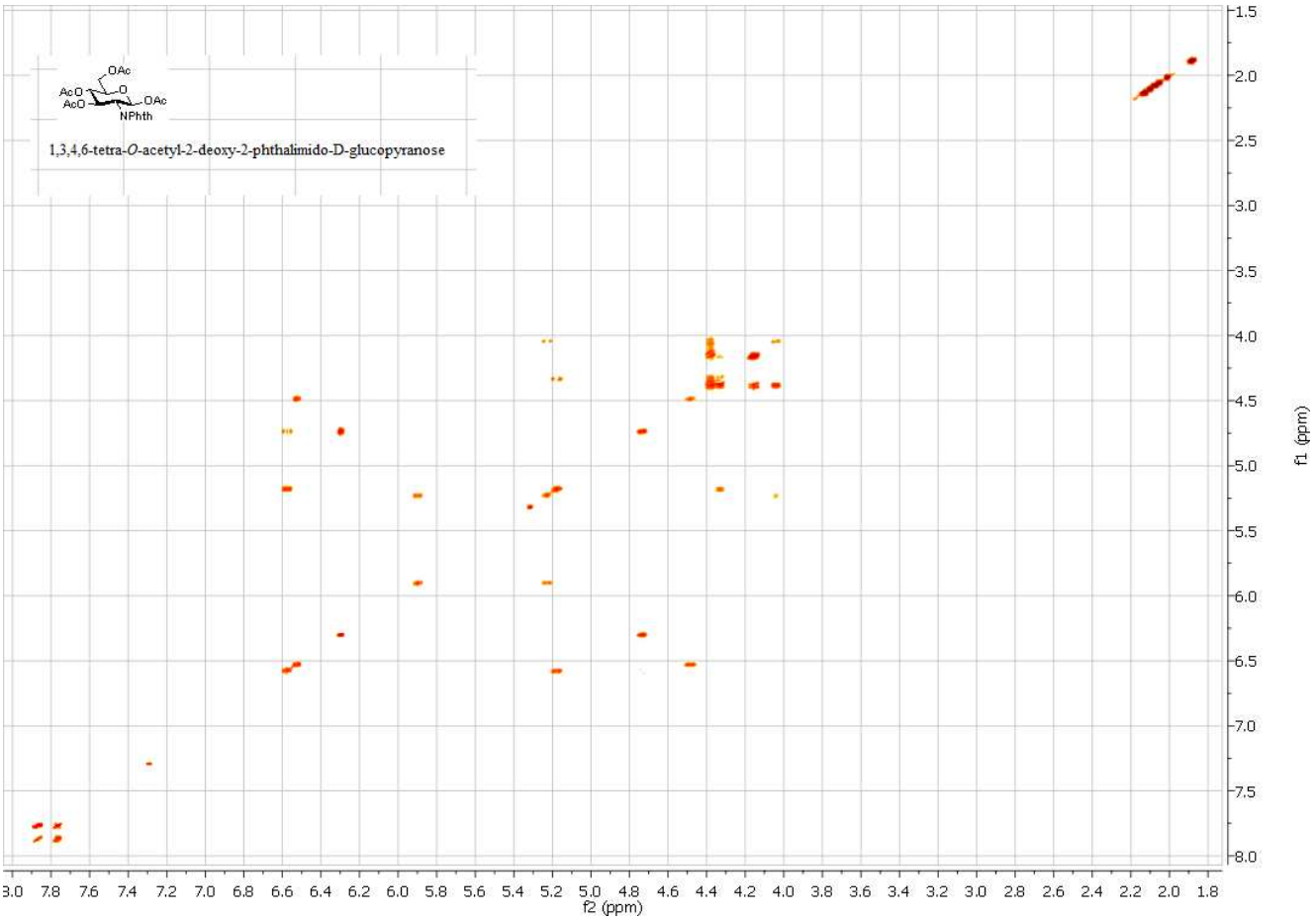




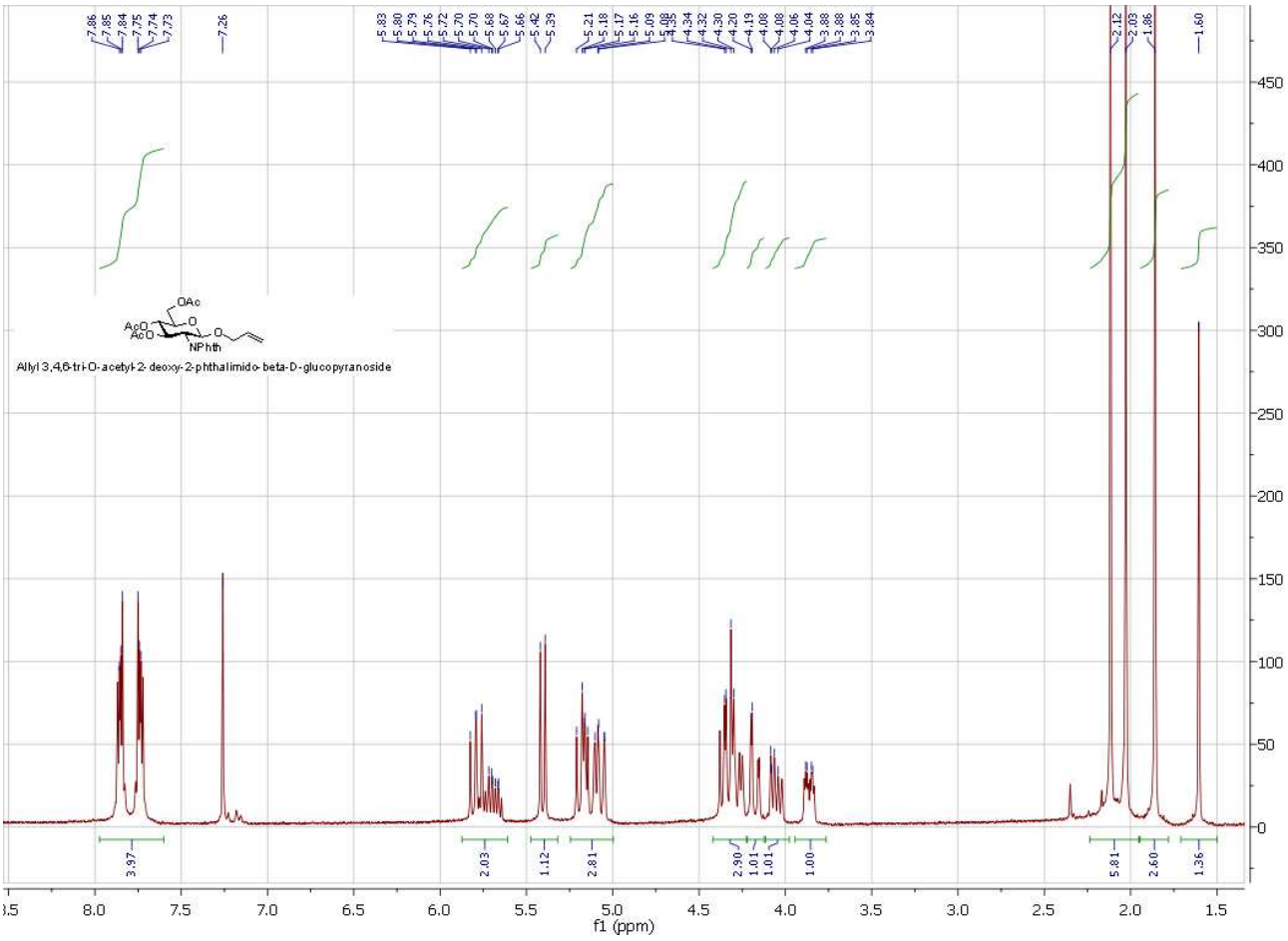
1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-D-glucose



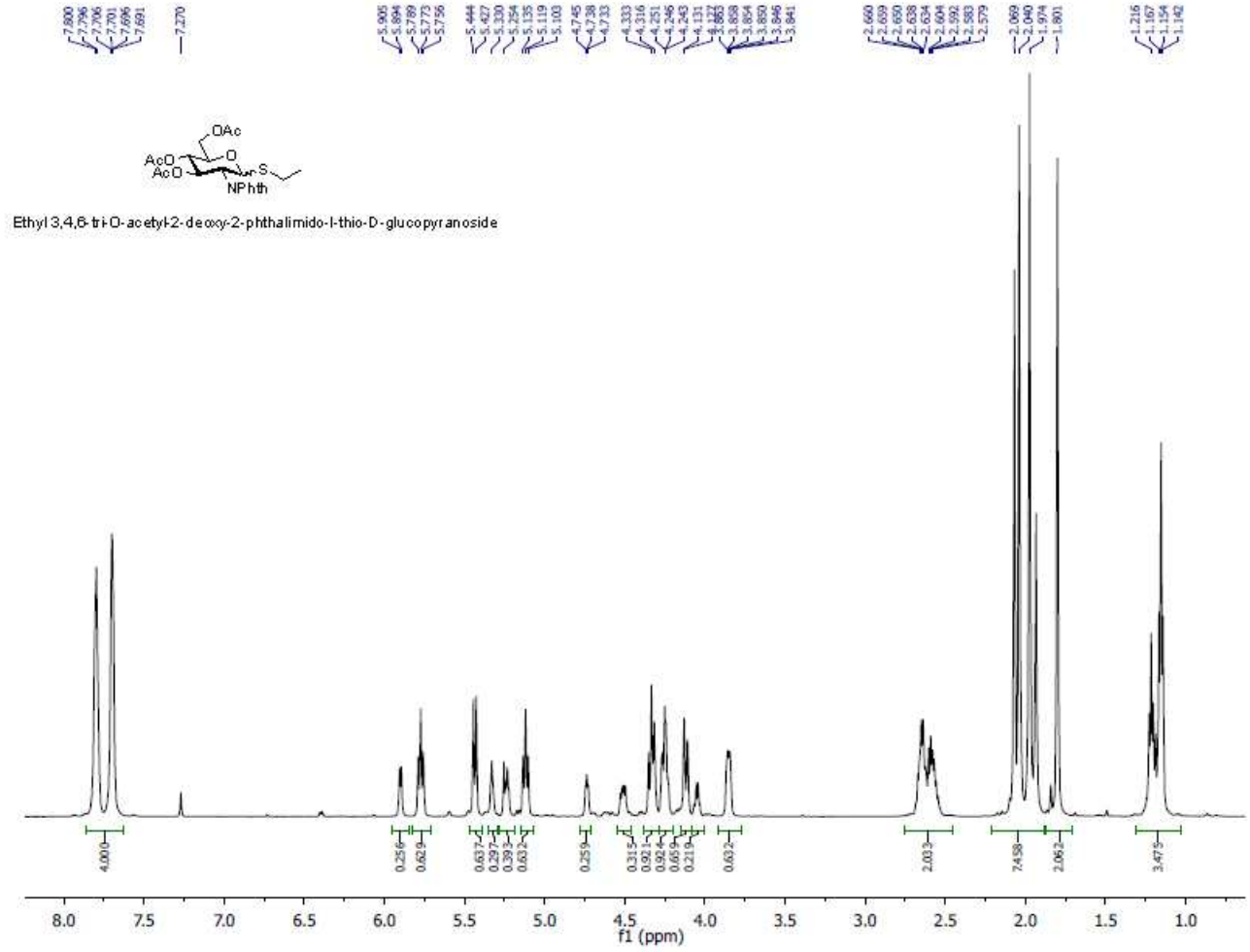


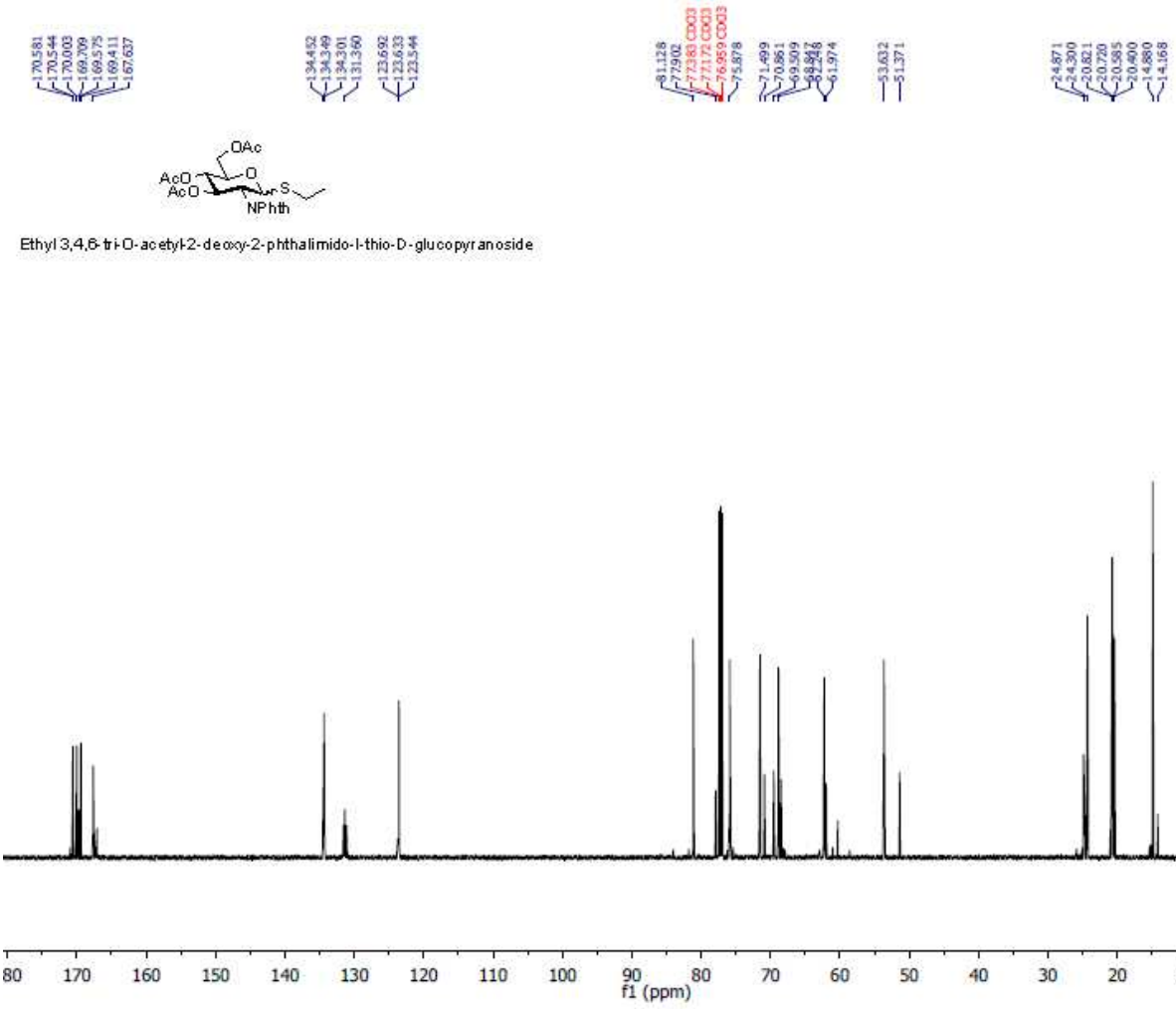


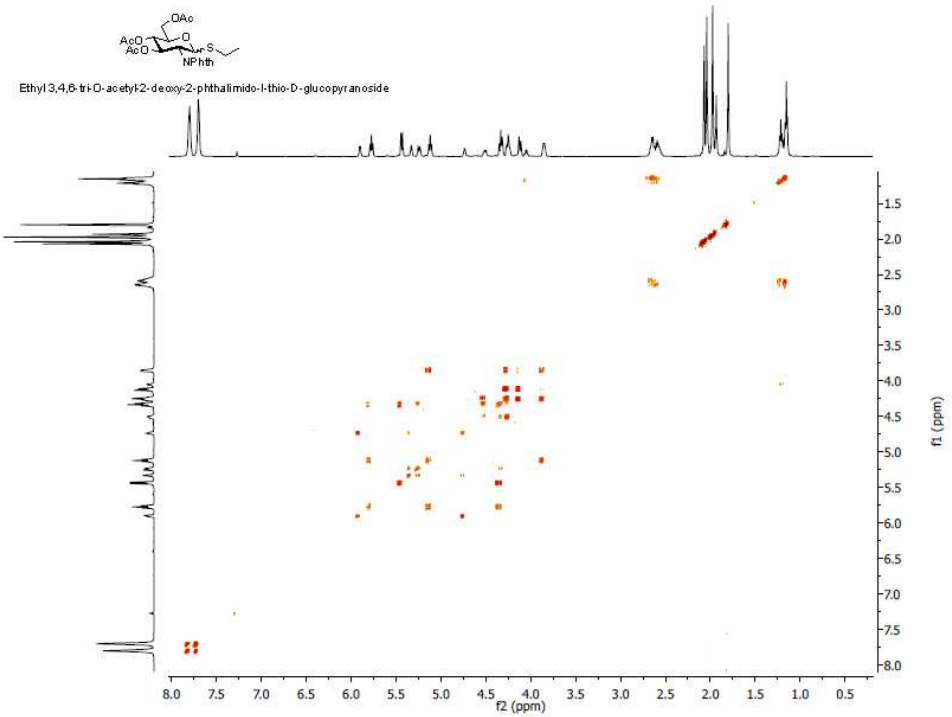
Allyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside

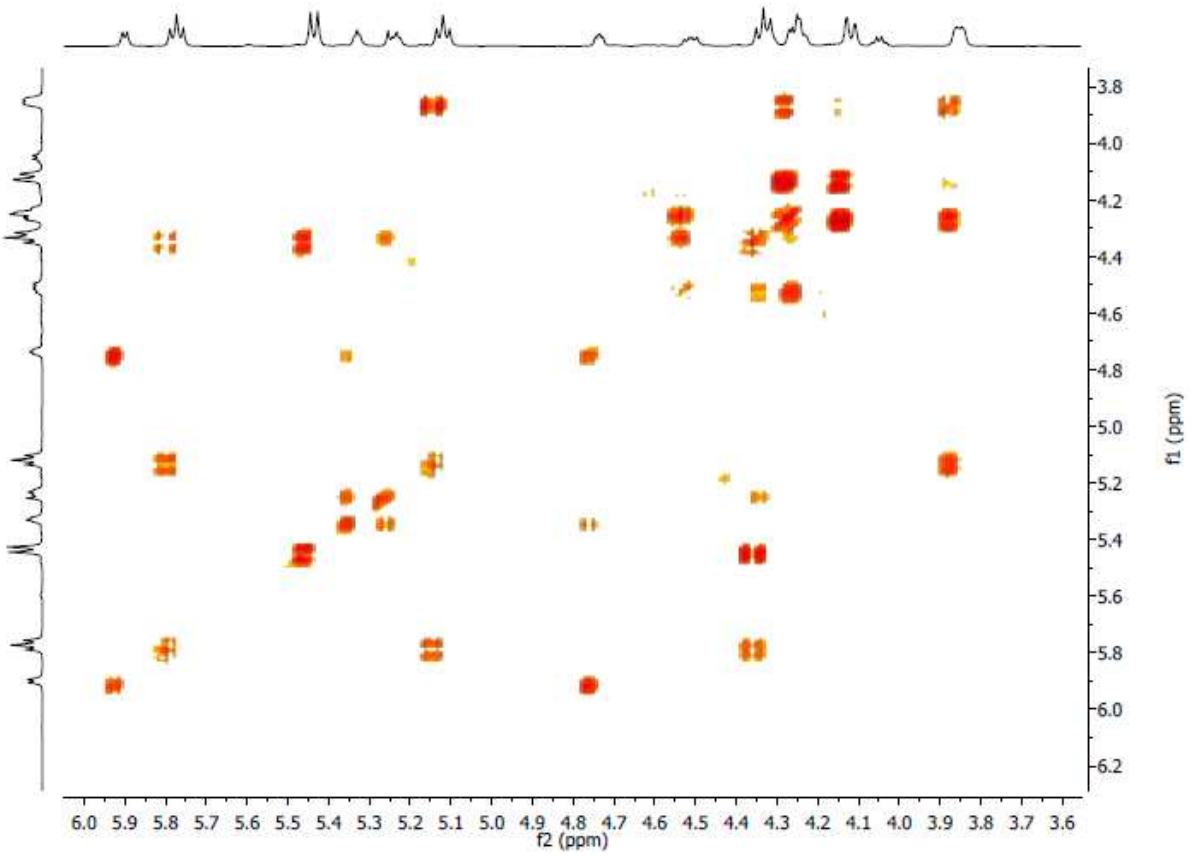


Ethyl 3,4,6-tri-O-ace~l-2-deoxy-2-phthalimido-l-thio-β-D-glucopyranoside

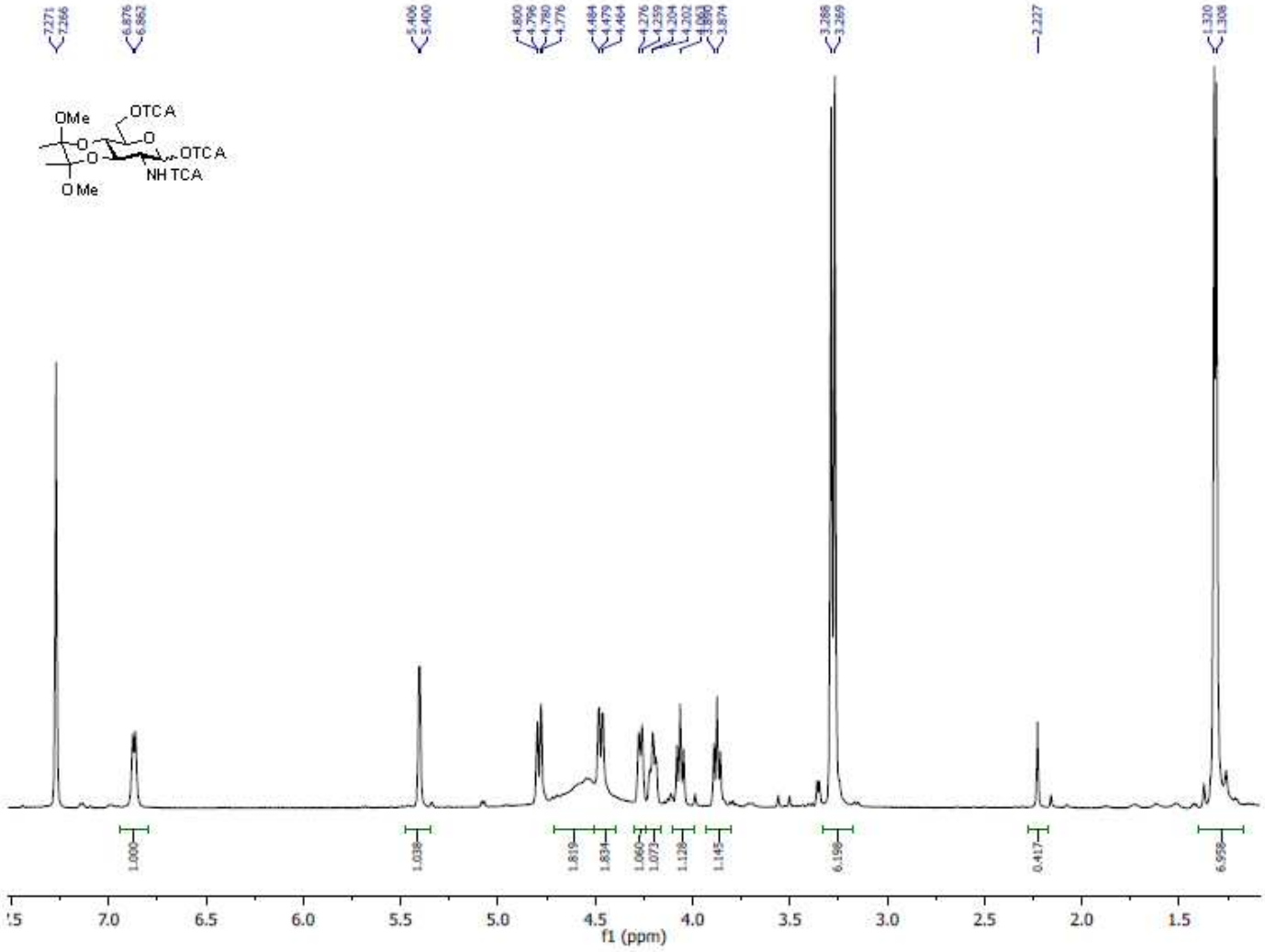


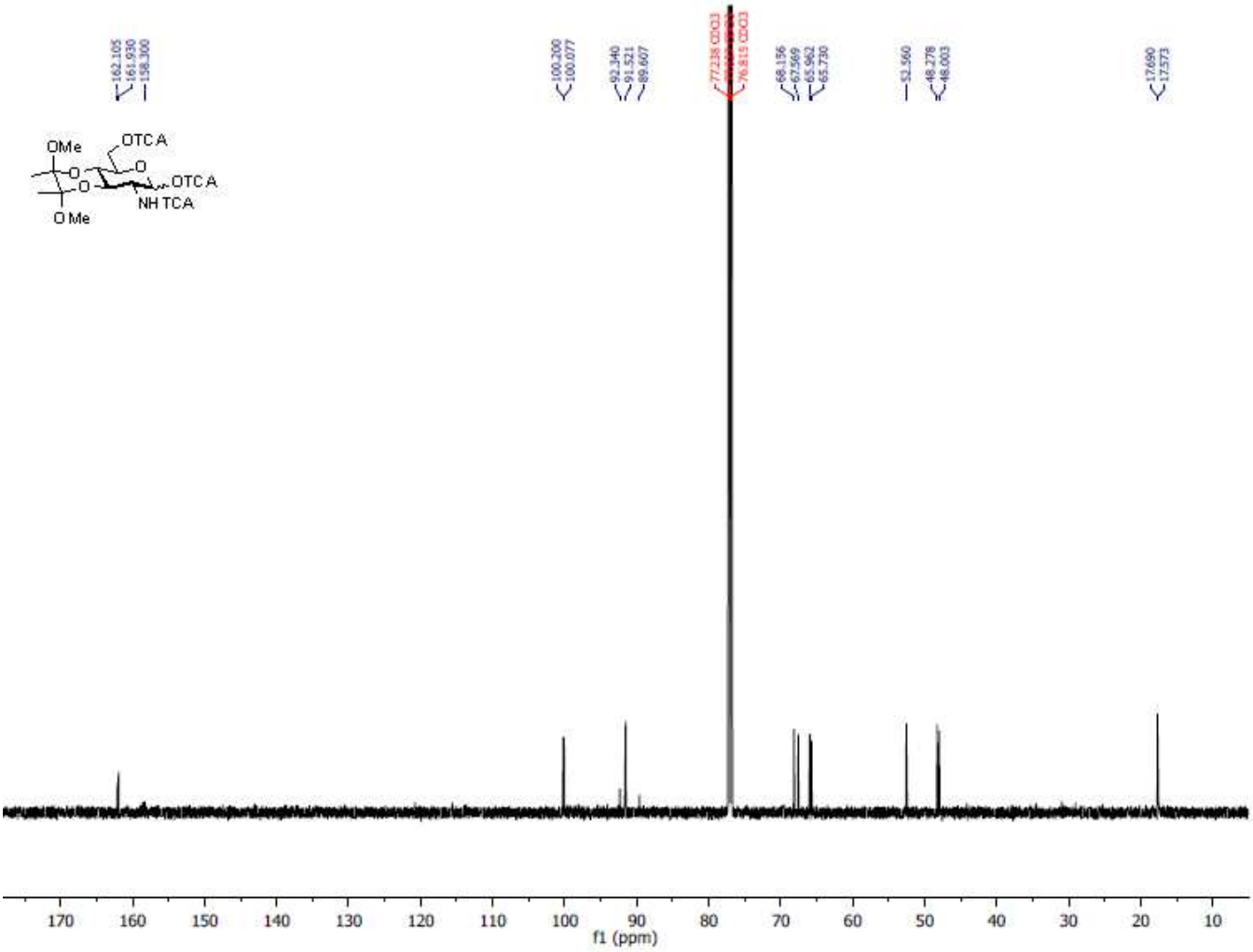


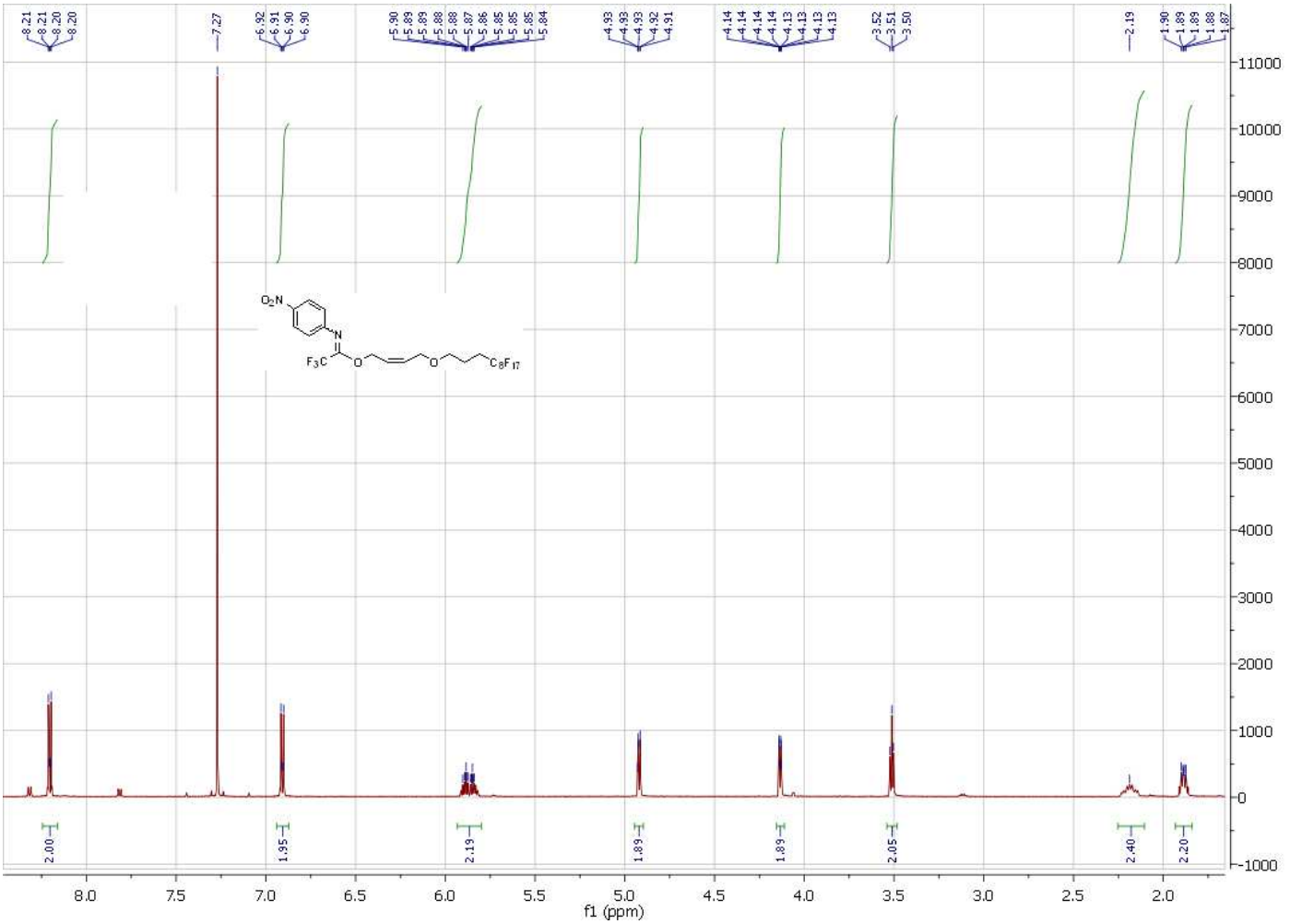


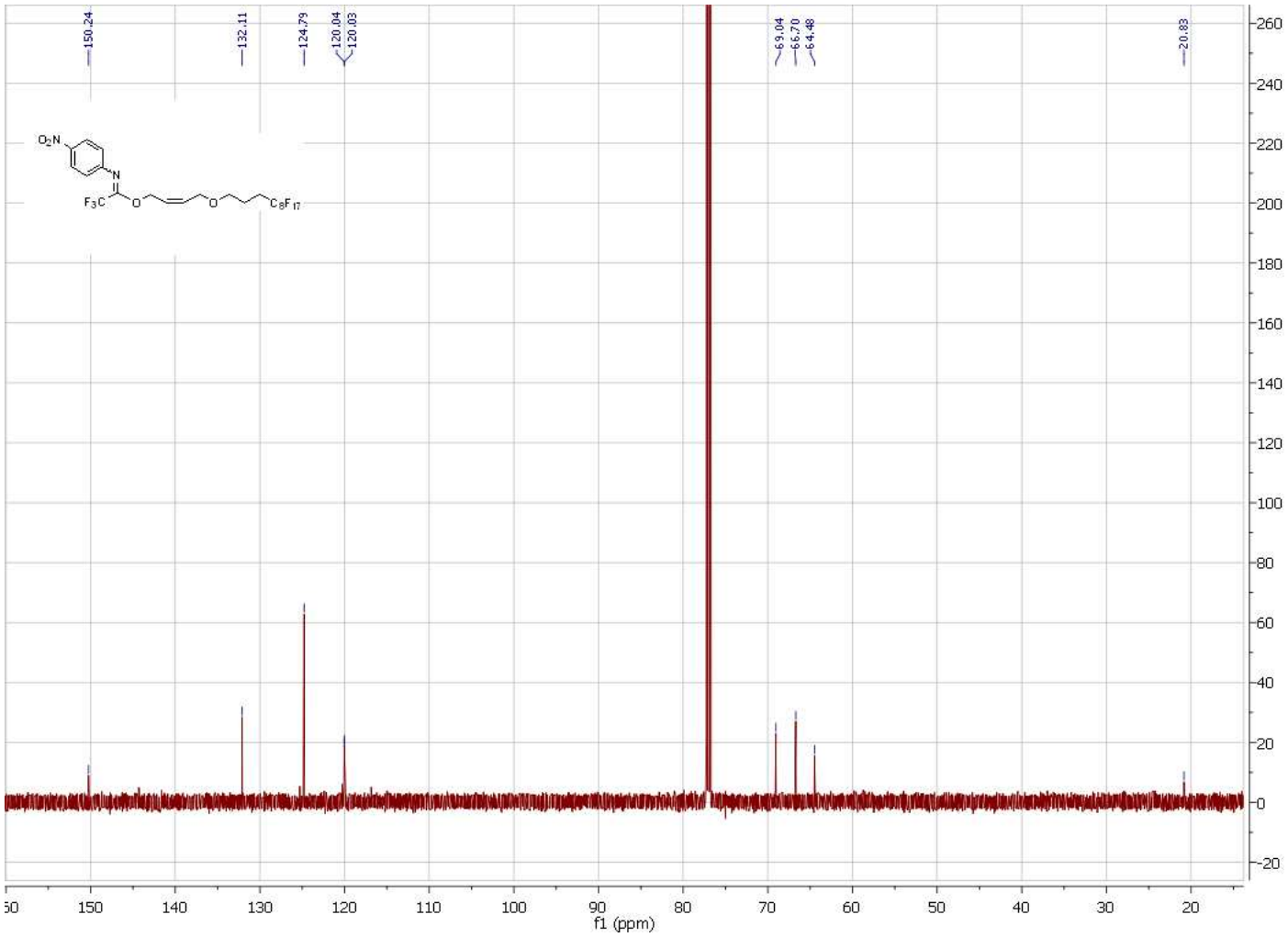


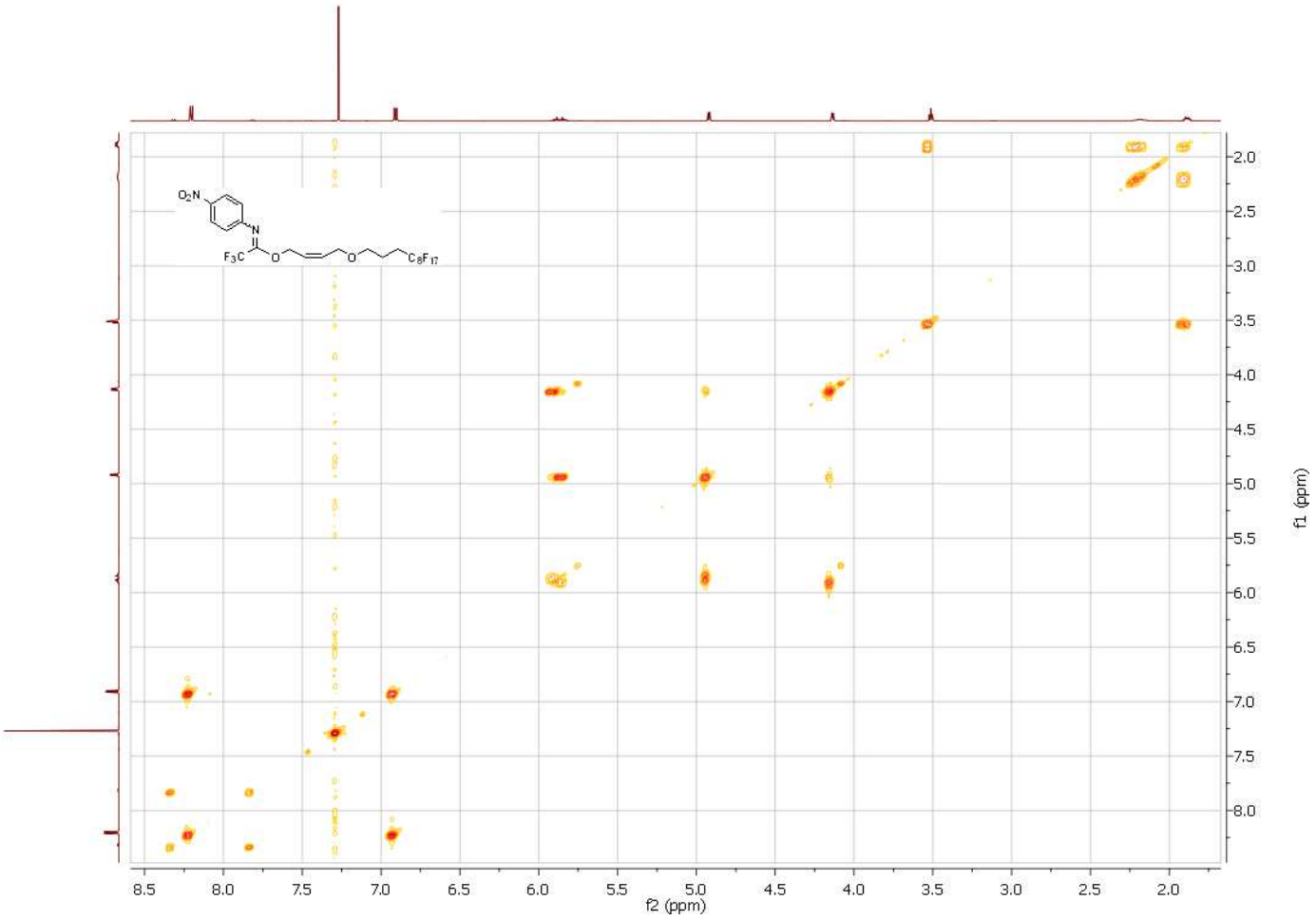
(2'S,3'S) Methyl- 3,4-O-(2',3'-dimethoxybutane-2',3'-diyl)1,6-di-O-trichloroacetyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside











ACKNOWLEDGEMENTS

I would like to thank God for all the people who were part of my journey. It was not easy but it was worth it. First and foremost, I thank God for my major professor, Dr. Nicola L.B. Pohl for all the encouragement and for all the advice and understanding throughout my stay at Iowa State University. I am also grateful for the assistance of the Iowa State University faculty especially Dr. Emily Smith, Dr. Yan Zhao, Dr. Reuben Peters, Dr. Jason Chen and Dr. Malika Jeffries-El who served as my Program of Study Committee.

Life would not have been the same without the presence of past and presence Pohl group members. Special thanks go to each and every one of them for being a constant support through the tough times and the good times. It was inspiring to get to know and work alongside some of the great minds of our times. I am also grateful for the whole Chemistry Department, from other graduate students, to the people in the instrument rooms and all the professors who were my supervisors when I was a teaching assistant. I am also thankful for my African family in Ames and my church family.

Last but not least I would like to thank my 'families.' The entire Tsabedze clan (especially Lindiwe, Thokozani, Tsatsekile, Mcinseli, Gcabho, Happiness and Mduna.) in Swaziland and the Larsons (Dan, Helen and Jane) in Minnesota. You have all made huge sacrifices on my behalf and I hope to repay you in kind in the near future. Thank you for always routing for me. This work is dedicated to the memory of my mother, Mary Skhabalanjani Dlamini (1957-2007.)

BIBLIOGRAPHY

1. **Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M., Efficient activation of glycosyl N-(phenyl)trifluoroacetimidate donors with ytterbium(III) triflate in the glycosylation reaction. *Tetrahedron Lett.* 2002, *43* (32), 5573-5577.**

2. **Aly, M. R. E.; Schmidt, R. R., New diacyl-amino protecting groups for glucosamine. *Eur. J. Org. Chem.* 2005, (20), 4382-4392.**

3. **Artner, D., Stanetty, C., Mereiter, K., Zamyatina, A., & Kosma, P. Crystal and molecular structure of methyl L-glycero-α-D-manno-heptopyranoside, and synthesis of 1→7 linked L-glycero-D-manno-heptobiose and its methyl α-glycoside. *Carbohydr. Res.*, *346*(13), 1739-1746.**

4. **Baeschlin, D. K.; Chaperon, A. R.; Green, L. G.; Hahn, M. G.; Ince, S. J.; Ley, S. V., 1,2-Diacetals in synthesis: total synthesis of a glycosylphosphatidylinositol anchor of Trypanosoma brucei. *Chem.--Eur. J.* 2000, *6* (1), 172-186.**

5. **Bedini, E.; Carabellese, A.; Schiattarella, M.; Parrilli, M., First synthesis of an α-D-Fucp3NAc containing oligosaccharide: a study on D-Fucp3NAc glycosylation. *Tetrahedron* 2005, *61* (23), 5439-5448.**

6. **Bedini, E., Comegna, D., Di Nola, A., & Parrilli, M. (2008). Selective acetolysis of 6-deoxy-sugar oligosaccharide building blocks governed by the armed-disarmed effect. *Tetrahedron Lett.*, *49*(16), 2546-2551.**

7. **Berens, U.; Leckel, D.; Oepen, S. C., Transacetalization of Diethyl Tartrate with Acetals of α-Dicarbonyl Compounds: A Simple Access to a New Class of C2-Symmetric Auxiliaries and Ligands. *J. Org. Chem.* 1995, *60* (25), 8204-8.**

8. **Best, W. M.; Dunlop, R. W.; Stick, R. V.; White, S. T., All about 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucosyl trichloroacetimidate. *Aust. J. Chem.* 1994, *47* (3), 433-40.**

9. **Bindschadler, P.; Dialer, L. O.; Seeberger, P. H., Synthesis of differentially protected glucosamine building blocks and their evaluation as glycosylating agents. *J. Carbohydr. Chem.* 2009, *28* (7 & 8), 395-420.**

10. **Boutet, J.; Kim, T. H.; Guerreiro, C.; Mulard, L. A., Efficient route to orthogonally protected precursors of 2-acylamino-2-deoxy-3-O-substituted-β-D-glucopyranose derivatives and use thereof. *Tetrahedron Lett.* 2008, *49* (36), 5339-5342.**

11. **Braibante, M. E. F.; Braibante, H. T. S.; Morel, A. F.; Costa, C. C.; Lima, M. G., The solvent free preparation of β-amino esters α, β -unsaturated ketones and esters with domestic microwave oven. *J. Braz. Chem. Soc.* 2006, *17* (1), 184-188.**

12. **Camci-Unal, G., Mizanur Rahman, M., Chai, Y., & Pohl Nicola, L. B. Synthesis of a 3-deoxy-D-manno-octulosonic acid (KDO) building block from d-glucose via fermentation. *Org Biomol Chem*.**

13. **Carlson, D. M. (1966). Oligosaccharides isolated from pig submaxillary mucin. *J. Biol. Chem.*, *241*(12), 2984-2986.**

14. **Cao, Y.; Okada, Y.; Yamada, H., Facile and regioselective preparation of partly O-benzylated D-glucopyranose acetates via acid-mediated simultaneous debenzylation-acetolysis. *Carbohydr. Res.* 2006, *341* (13), 2219-2223.**

15. **Charonnat, J. A.; Steinberg, C. S., Microscale synthesis of 2,3:5,6-di-O-isopropylidene- α -D-mannofuranose ("diacetone mannose"). *J. Chem. Educ.* 1996, *73* (8), A170-A171.**

16. **Chen, X.; Varki, A., Advances in the Biology and Chemistry of Sialic Acids. *ACS Chem. Biol. 5* (2), 163-176.**

17. **Claffey, D. J., Casey, M. F., & Finan, P. A. (2004). Glycosylation of 1,4:3,6-dianhydro-D-glucitol (isosorbide). *Carbohydr. Res.*, *339*(14), 2433-2440.**

18. **Corey, E. J.; Achiwa, K., Oxidation of primary amines to ketones. *J. Amer. Chem. Soc.* 1969, *91* (6), 1429-32.**

19. **Cornforth, J. W., Firth, M. E., & Gottschalk, A. (1958). The synthesis of N-acetylneuraminic acid. *Biochem. J.*, *68*, 57-61.**

20. **Coutant, C.; Jacquinet, J.-C., 2-Deoxy-2-trichloroacetamido-D-glucopyranose derivatives in oligosaccharide synthesis: from hyaluronic acid to chondroitin 4-sulfate trisaccharides. *J. Chem. Soc., Perkin Trans. 1* 1995, (12), 1573-81.**

21. Cramer, F.; Pawelzik, K., Nucleophilic substitution at iminoesters. *Angew. Chem.* 1956, *68*, 649.

22. Debenham, J.; Rodebaugh, R.; Fraser-Reid, B., Recent advances in N-protection for amino sugar synthesis. *Liebigs Ann./Recl.* 1997, (5), 791-802.

23. de la Fuente, J. M.; Penades, S., Synthesis of Lex-neoglycoconjugate to study carbohydrate-carbohydrate associations and its intramolecular interaction. *Tetrahedron: Asymmetry* 2002, *13* (17), 1879-1888.

24. Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R., Glycosyl imidates. Part 77. N-trichloroethoxycarbonyl-glucosamine derivatives as glycosyl donors. *Carbohydr. Res.* 1996, *296*, 135-147.

25. Demchenko, A. V., General aspects of the glycosidic bond formation. *Handb. Chem. Glycosylation* 2008, 1-27.

26. Dondoni, A.; Merino, P., Chemistry of the enolates of 2-acetylthiazole: aldol reactions with chiral aldehydes to give 3-deoxy aldos-2-uloses and 3-deoxy 2-ulosonic acids. A short total synthesis of 3-deoxy-D-manno-2-octulosonic acid (KDO). *J. Org. Chem.* 1991, *56* (18), 5294-301.

27. Garegg, P. J. (2004). Synthesis and reactions of glycosides. *Adv. Carbohydr. Chem. Biochem.*, *59*, 69-134.

28. Ghalambor, M. A.; Heath, E. C., The chemical synthesis of 2-keto-3-deoxyoctonate and its cleavage by a specific aldolase. *Biochem. Biophys. Res. Commun.* 1963, *11*, 288-93.

29. Glaudemans, C. P. J.; Fletcher, H. G., Jr., 2,3,4,6-Tetra-O-benzyl-ALPHA-D-glucopyranose. *Methods Carbohyd. Chem.* 1972, *6*, 373-6.

30. Gouliaras, C., Lee, D., Chan, L., & Taylor, M. S. Regioselective Activation of Glycosyl Acceptors by a Diarylborinic Acid-Derived Catalyst. *J. Am. Chem. Soc.*, *133*(35), 13926-13929.

31. Guchhait, G., & Misra, A. K. Efficient glycosylation of unprotected sugars using sulfamic acid: A mild eco-friendly catalyst. *Catal. Commun.*, *14*(1), 52-57.

32. Hanashima, S.; Tomiya, T.; Ishikawa, D.; Akai, S.; Sato, K.-i., Sialylation using N-glycolylneuraminyl phosphite donors to synthesize Neu5Gc-containing glycans. *Carbohydr. Res.* 2009, *344* (8), 959-965.

33. Hanessian, S., & Guindon, Y. (1980). Chemistry of the glycosidic linkage. Direct conversion of glycosides into 1-thioglycosides by use of [alkyl(or aryl)thio]trimethylsilanes. *Carbohydr. Res.*, *86*(2), C3-C6.

34. Hansen, S. G.; Skrydstrup, T., Studies directed to the synthesis of oligochitosans - preparation of building blocks and their evaluation in glycosylation studies. *Eur. J. Org. Chem.* 2007, (20), 3392-3401, S3392/1-S3392/17.

35. Harada, T., & Mukaiyama, T. (1981). A convenient synthesis of 2-deoxy-D-ribose. *Chem. Lett.*(8), 1109-1110.

36. Huchel, U.; Tiwari, P.; Schmidt, R. R., N-Aryl-O-glycosyl Haloacetimidates as Glycosyl Donors. *J. Carbohydr. Chem. 29* (2), 61-75.

37. Ichiyanagi, T.; Sakamoto, N.; Ochi, K.; Yamasaki, R., A chemical synthesis of 3-deoxy-D-manno-2-octulosonic acid from D-mannose. *J. Carbohydr. Chem.* 2009, *28* (2), 53-63.

38. Iversen, T.; Bundle, D. R., Benzyl trichloroacetimidate, a versatile reagent for acid-catalyzed benzylation of hydroxy-groups. *J. Chem. Soc., Chem. Commun.* 1981, (23), 1240-1.

39. Jackson, R. A. (2004). *Mechanisms in Organic Reactions*. Page 53-57

40. Jaipuri, F. A.; Pohl, N. L., Toward solution-phase automated iterative synthesis: fluorous-tag assisted solution-phase synthesis of linear and branched mannose oligomers. *Org. Biomol. Chem.* 2008, *6* (15), 2686-2691.

41. Kamkhachorn, T., Parameswar, A. R., & Demchenko, A. V. Comparison of the Armed/Disarmed Building Blocks of the D-Gluco and D-Glucosamino Series in the Context of Chemoselective Oligosaccharide Synthesis. *Org. Lett.*, *12*(13), 3078-3081.

42. Karkhanis, Y. D., Zeltner, J. Y., Jackson, J. J., & Carlo, D. J. (1978). A new and improved microassay to determine 2-keto-3-deoxyoctonate in lipopolysaccharide of gram-negative bacteria. *Anal. Biochem.*, *85*(2), 595-601.

43. Kimura, M., Mechanism of the Chapman rearrangement of N-arylbenzimidates on the basis of a molecular structure established by x-ray crystallography. *J. Chem. Soc., Perkin Trans. 2* 1987, (2), 205-9.

44. Kirihara, M.; Asai, Y.; Ogawa, S.; Noguchi, T.; Hatano, A.; Hirai, Y., A mild and environmentally benign oxidation of thiols to disulfides. *Synthesis* 2007, (21), 3286-3289.

45. Knowles, D. A.; Mathews, C. J.; Tomkinson, N. C. O., Oxidation of primary amines to ketones. *Synlett* 2008, (18), 2769-2772.

46. Larsen, K.; Olsen, C. E.; Motawia, M. S., Acid-catalyzed rearrangement of glycosyl trichloroacetimidates: a novel route to glycosylamines. *Carbohydr. Res.* 2008, *343* (2), 383-387.

47. Li, J. J., *Name Reactions: A Collection of Detailed Reaction Mechanisms*. 2002; p 416 pp.

48. Li, Y.; Wei, G.; Yu, B., Aryl C-glycosylation of phenols with glycosyl trifluoroacetimidates. *Carbohydr. Res.* 2006, *341* (16), 2717-2722.

49. Liu, W. Synthetic studies of 3-deoxy-d-manno-2-octulosonic acid (KDO), 3-deoxy-D-arabino-2-heptulosonic acid (DAH), and their 4-epimers through the radical chemistry of Barton esters (deoxy(manno)octulosonic acid, deoxy(arabino)heptulosonic acid, radical traps). 1997.

50. Love, K. R.; Seeberger, P. H., Solution Syntheses of Protected Type II Lewis Blood Group Oligosaccharides: Study for Automated Synthesis. *J. Org. Chem.* 2005, *70* (8), 3168-3177.

51. Lundgren, B. R.; Boddy, C. N., Sialic acid and N-acyl sialic acid analog production by fermentation of metabolically and genetically engineered Escherichia coli. *Org. Biomol. Chem.* 2007, *5* (12), 1903-1909.

52. Mamidyala, S. K.; Ko, K.-S.; Jaipuri, F. A.; Park, G.; Pohl, N. L., Noncovalent fluorous interactions for the synthesis of carbohydrate microarrays. *J. Fluorine Chem.* 2006, *127* (4-5), 571-579.

53. Mannerstedt, K.; Ekeloef, K.; Oscarson, S., Evaluation of thioglycosides of Kdo as glycosyl donors. *Carbohydr. Res.* 2007, *342* (3-4), 631-637.

54. Marino Albernas, J.; Verez Bencomo, V.; Gonzalez, L.; Perez, C. S.; Gonzalez-Abreu Castell, F.; Gonzalez Segredo, A., Chemical synthesis of an antigen for leprosy diagnosis. *Interferon Biotecnol.* 1987, *4* (1), 65-8.

55. Melean, L. G.; Love, K. R.; Seeberger, P. H., Toward the automated solid-phase synthesis of oligoglucosamines: systematic evaluation of glycosyl phosphate and glycosyl trichloroacetimidate building blocks. *Carbohydr. Res.* 2002, *337* (21-23), 1893-1916.

56. Mensah, E. A.; Nguyen, H. M., Nickel-catalyzed stereoselective formation of 1,2-cis-2-amino-2-deoxy glucosides and galactosides. *Abstracts of Papers, 240th ACS National Meeting, Boston, MA, United States, August 22-26, 2010*, ORGN-94.

57. Mensah, E. A.; Yu, F.; Nguyen, H. M., Nickel-Catalyzed Stereoselective Glycosylation with C(2)-N-Substituted Benzylidene D-Glucosamine and Galactosamine Trichloroacetimidates for the Formation of 1,2-cis-2-Amino Glycosides. Applications to the Synthesis of Heparin Disaccharides, GPI Anchor Pseudodisaccharides, and α-GalNAc. *J. Am. Chem. Soc. 132* (40), 14288-14302.

58. Morales, V., Sanz, M. L., Olano, A., & Corzo, N. (2006). Rapid separation on activated charcoal of high oligosaccharides in honey. *Chromatographia, 64*(3-4), 233-238.

59. Mukaiyama, T. (1982). Synthetic control in carbohydrate synthesis. *Kagaku, Zokan (Kyoto)*(96), 183-192.

60. Mukaiyama, T. (2004). Explorations into new reaction chemistry. *Angew. Chem., Int. Ed., 43*(42), 5590-5614.

61. Mukaiyama, T., Hashimoto, Y., & Shoda, S. (1983). Stereoselective synthesis of 1,2-cis-glycofuranosides using glycofuranosyl fluorides. *Chem. Lett.*(6), 935-938.

62. Mukaiyama, T., Murai, Y., & Shoda, S. (1981). An efficient method for glucosylation of hydroxy compounds using glucopyranosyl fluoride. *Chem. Lett.*(3), 431-432.

63. Mydock, L. K., & Demchenko, A. V. (2008). Application of the Superarmed Glycosyl Donor to Chemoselective Oligosaccharide Synthesis. *Org. Lett., 10*(11), 2107-2110.

64. Myszka, H.; Bednarczyk, D.; Najder, M.; Kaca, W., Synthesis and induction of apoptosis in B cell chronic leukemia by diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride and its derivatives. *Carbohydr. Res.* 2003, *338* (2), 133-141.

65. Nishikawa, T.; Urabe, D.; Tomita, M.; Tsujimoto, T.; Iwabuchi, T.; Isobe, M., One-Pot Transformation of Trichloroacetamide into Readily Deprotectable Carbamates. *Org. Lett.* 2006, *8* (15), 3263-3265.

66. Okada, Y.; Ohtsu, M.; Bando, M.; Yamada, H., Benzyl N-phenyl-2,2,2-trifluoroacetimidate: a new and stable reagent for O-benzylation. *Chem. Lett.* 2007, *36* (8), 992-993.

67. Panchadhayee, R.; Misra, A. K., N-Bromosuccinimide-Mediated Conversion of Allyl Glycosides to Glycosyl Hemiacetals. *J. Carbohydr. Chem. 29* (2), 76-83

68. Patil, V. J., A simple access to trichloroacetimidates. *Tetrahedron Lett.* 1996, *37* (9), 1481-4.

69. Pazynina, G., Nasonov, V., Belyanchikov, I., Brossmer, R., Maisel, M., Tuzikov, A., & Bovin, N. Koenigs-Knorr glycosylation with neuraminic acid derivatives. *Int. J. Carbohydr. Chem.*, 594247, 594248 pp.

70. Premathilake, H. D., & Demchenko, A. V. Superarmed and superdisarmed building blocks in expeditious oligosaccharide synthesis. *Top. Curr. Chem.*, *301*(Reactivity Tuning in Oligosaccharide Assembly), 189-221.

71. Premathilake, H. D., Mydock, L. K., & Demchenko, A. V. Super-arming Common Glycosyl Donors by Simple 2-O-Benzoyl-3,4,6-tri-O-benzyl Protection. *J. Org. Chem.*, *75*(4), 1095-1100.

72. Ramage, R.; Rose, G. W.; MacLeod, A. M., Chemistry of 5-ylidene-1,3-dioxolan-4-ones. Syntheses of 3-deoxy-D-arabino-2-heptulosonic acid (DAH) and 3-deoxy-D-manno-2-octulosonic acid (KDO). *Tetrahedron Lett.* 1988, *29* (38), 4877-80.

73. Rawal, G. K.; Rani, S.; Kumari, N.; Vankar, Y. D., Regio- and Stereocontrolled Selective Debenzylation and Substitution Reactions of C-2 Formyl Glycals. Application in the Synthesis of Constrained β-Sugar Amino Acids. *J. Org. Chem.* 2009, *74* (15), 5349-5355.

74. Rawalay, S. S.; Shechter, H., Oxidation of primary, secondary, and tertiary amines with neutral permanganate. Simple method for degrading amines to aldehydes and ketones. *J. Org. Chem.* 1967, *32* (10), 3129-31.

75. Sakurai, N., Ikegai, K., & Mukaiyama, T. (2007). Copper(II)-catalyzed O-phenylation of alcohols with organobismuth(V) reagents. *ARKIVOC (Gainesville, FL, U. S.)*(7), 254-264.

76. Saleem, A. M., Dhasan, P. B., & Rafiullah, M. R. M. (2006). Simple and rapid method for the isolation of forskolin from Coleus forskohlii by charcoal column chromatography. *J. Chromatogr., A, 1101*(1-2), 313-314.

77. Saman, D., Wimmerova, M., & Wimmer, Z. (2006). Synthesis and structure assignment of 2-(4-methoxybenzyl)cyclohexyl β-D-galactopyranoside stereoisomers. *Collect. Czech. Chem. Commun., 71*(8), 1186-1198.

78. Schmidt, R. R.; Michel, J., Simple syntheses of α- and β-O-glycosyl imidates; preparation of glycosides and disaccharides. *Angew. Chem.* 1980, *92* (9), 763-4.

79. Schulenberg, J. W.; Archer, S., The Chapman rearrangement. *Org. Reactions* 1965, *14*, 1-51.

80. Seeberger, P. H., Automated oligosaccharide synthesis. *Chem. Soc. Rev.* 2008, *37* (1), 19-28.

81. Shaikh, N., Russo, L., Cipolla, L., & Nicotra, F. Ultrasonic assisted Fischer glycosylation: generating diversity for glyco-chemistry. *Mol. Diversity, 15*(2), 341-345.

82. Shimizu, T.; Masuzawa, T.; Yanagihara, Y.; Itoh, H.; Nakamoto, S.; Achiwa, K., Biological activities of chemically synthesized derivatives of lipid A: tetraacetyl-monosaccharides linked to 2,3-acyloxyacylglucosamine-4-phosphate. *Chem. Pharm. Bull.* 1989, *37* (9), 2535-6.

83. Shirai, R.; Ogura, H., Studies on sialic acid. Part XX. Improved syntheses of two 3-deoxyald-2-ulosonic acids (KDN,KDO) by condensation of oxalacetic acid with aldoses followed by nickel(II)-catalyzed decarboxylation. *Tetrahedron Lett.* 1989, *30* (17), 2263-4.

84. Shohda, K.-I.; Wada, T.; Sekine, M., Detailed studies on trimethylsilyl triflate mediated glycosylation via a 3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-O-

methylribofuranos-1-yl trichloroacetimidate intermediate. *Nucleosides Nucleotides* 1998, *17* (12), 2199-2210.

85. Silipo, A., Molinaro, A., Sturiale, L., Dow, J. M., Erbs, G., Lanzetta, R., . . . Parrilli, M. (2005). The elicitation of plant innate immunity by lipooligosaccharide of Xanthomonas campestris. *J. Biol. Chem.*, *280*(39), 33660-33668.

86. Stick, R. V.; Stubbs, K. A., From glycoside hydrolases to thioglycoligases: the synthesis of thioglycosides. *Tetrahedron: Asymmetry* 2005, *16* (2), 321-335.

87. Sun, D.-Q.; Busson, R.; Herdewijn, P., Synthesis of deoxygenated disaccharide precursors for modified lipid II synthesis. *Eur. J. Org. Chem.* 2006, (22), 5158-5166.

88. Tanaka, H.; Iwata, Y.; Takahashi, D.; Adachi, M.; Takahashi, T., Efficient Stereoselective Synthesis of β-N-Glycosyl Asparagines by N-Glycosylation of Primary Amide Groups. *J. Am. Chem. Soc.* 2005, *127* (6), 1630-1631.

89. Tatai, J.; Fuegedi, P., A New, Powerful Glycosylation Method: Activation of Thioglycosides with Dimethyl Disulfide-Triflic Anhydride. *Org. Lett.* 2007, *9* (22), 4647-4650.

90. Tamura, K.; Mizukami, H.; Maeda, K.; Watanabe, H.; Uneyama, K., One-pot synthesis of trifluoroacetimidoyl halides. *J. Org. Chem.* 1993, *58* (1), 32-5.

91. Tully, S. E.; Mabon, R.; Gama, C. I.; Tsai, S. M.; Liu, X.; Hsieh-Wilson, L. C., Chondroitin sulfate small molecules that stimulate neuronal outgrowth. *Abstracts of Papers, 228th ACS National Meeting, Philadelphia, PA, United States, August 22-26, 2004* 2004, BIOL-173.

92. Valerio, S.; Iadonisi, A.; Adinolfi, M.; Ravida, A., Novel Approaches for the Synthesis and Activation of Thio- and Selenoglycoside Donors. *J. Org. Chem.* 2007, *72* (16), 6097-6106.

93. van den Bos, L. J.; Boltje, T. J.; Provoost, T.; Mazurek, J.; Overkleeft, H. S.; van der Marel, G. A., A synthetic study towards the PSA1 tetrasaccharide repeating unit. *Tetrahedron Lett.* 2007, *48* (15), 2697-2700.

94. Waaglund, T.; Claesson, A., Stereoselective synthesis of the α-allyl C-glycoside of 3-deoxy-D-manno-2-octulosonic acid (KDO) by use of radical chemistry. *Acta Chem. Scand.* 1992, *46* (1), 73-6.

95. Wang, J.; Hsung, R. P.; Ghosh, S. K., Stereoselective Ketal-Tethered Intramolecular Diels-Alder Cycloadditions. An Approach to the 2-Oxadecalin Spiroketal Core of Antifungal Agent Fusidilactone C. *Org. Lett.* 2004, *6* (12), 1939-1942.

96. Warren, L., Thiobarbituric acid assay of sialic acids. *Methods Enzymol.* 1963, *6*, 463-465.

97. Weck, S.; Opatz, T., β-selective C-mannosylation of electron-rich phenols. *Synthesis* (14), 2393-2398.

98. Wheeler, O. H.; Roman, F.; Rosado, O., Intramolecular nature of the rearrangement of benzimidates. *J. Org. Chem.* 1969, *34* (4), 966-8.

99. White, J. D.; Tiller, T.; Ohba, Y.; Porter, W. J.; Jackson, R. W.; Wang, S.; Hanselmann, R., Total synthesis of rutamycin B via Suzuki macrocyclization. *Chem. Commun. (Cambridge)* 1998, (1), 79-80.

100. Winzar, R.; Philips, J.; Kiefel, M. J., A simple synthesis of C-8 modified 2-keto-3-deoxy-D-manno-octulosonic acid (KDO) derivatives. *Synlett* (4), 583-586.

101. Yan, L., & Kahne, D. (1996). Generalizing Glycosylation: Synthesis of the Blood Group Antigens Lea, Leb, and Lex Using a Standard Set of Reaction Conditions. *J. Am. Chem. Soc.*, *118*(39), 9239-9248.

102. Yu, B.; Tao, H., Glycosyl trifluoroacetimidates. Part 1: Preparation and application as new glycosyl donors. *Tetrahedron Lett.* 2001, *42* (12), 2405-2407.

103. Yu, H. N.; Ling, C.-C.; Bundle, D. R., Synthesis of three Salmonella epitopes for biosensor studies of carbohydrate-antibody interactions. *Can. J. Chem.* 2002, *80* (8), 1131-1140.

104. Zheng, Q.-H.; Liu, X.; Fei, X.; Wang, J.-Q.; Ohannesian, D. W.; Erickson, L. C.; Stone, K. L.; Martinez, T. D.; Miller, K. D.; Hutchins, G. D., Synthesis of radiolabeled O6-benzylguanine derivatives as new potential PET tumor imaging

agents for the DNA repair protein O-6-alkylguanine-DNA alkyltransferase. *J. Labelled Compd. Radiopharm.* 2002, *45* (14), 1239-1252.

105. Zhu, X.; Schmidt, R. R., New principles for glycoside-bond formation. *Angew. Chem., Int. Ed.* 2009, *48* (11), 1900-1934.