Contents

Foreword

This report is based on laboratory work performed from January through May of 2011 in the bioorganic chemistry group of Institute of Chemistry at The University of Aarhus. The work has been done under supervision of assistant professor Henrik Helligsø Jensen and is equivalent to a 10 ECTS points work load. The entire report is written in English as this is most convenient for the future use by others and with future publication in mind.

I would like to thank everyone who has helped me with this project. Every member of Group 103 has been wonderful and always ready to answer any and all questions. Special thanks go to my supervisor Henrik Helligsø Jensen for always being ready to help solving an unexpected problem and never hesitating in putting everything down and throw on a lab coat when it was needed. I also appreciate the help coming from discussing the project with Michelle Ricafranca Rasmussen, who was involved in a similar project last year. Lastly I would like to thank Mikkel Troldtoft Jensen and Elsebeth Nielsen for revising my report.

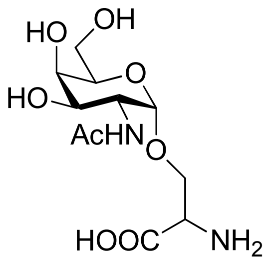
Rasmus Dahl-Lassen, juli 2011

Abreviations

Ac	Acetyl
aq.	aqueous
Ar	Aryl
Calcd	Calculated
COSY	Correlation spectroscopy
DCM	Dichloromethane
d	doublet
dd	double doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMAP	N,N-Dimethylaminopyridine
dt	double triplet
EtOAc	Ethyl acetate
GalNAc	N-acetyl-D-galactosamine
GlcNAc	N-acetyl-D-glucosamine
HMQC	Heteronuclear Multiple Quantum Coherence
HRMS	High Resolution Mass Spectrometry
J	Coupling constant
LRMS	Low Resolution Mass Spectrometry
M	Molar
m	multoplet
MHC	Major Histocompatability complex
Mp	Melting point
NIS	N-Iodosuccinimide
NMR	Nuclear Magnetic Resonance
Piv	Pivaloyl (trimethylacetate)
PMA	Phosphor Molybdic Acid
Ppm	parts per million
R_f	Retention factor
rt	room temperature
S_N1	1st order nucleophile substitution
S_N2	2nd order nucleophile substitution
t	triplet
TLC	Thin Layer Chromatogr

Abstract

It has been shown that the T_N-antigen can be used in treating a variety of cancer types, as part of a multi component vaccine. Cancer cells often over express this specific antigen and if the body is taught to see this as a foreign antigen cancers can be prevented.



The T_N-antigen

The synthesis of the T_N-antigen is long and difficult. In this project a new way of synthesizing it will be explored. This involves two key steps which have not previously been attempted. One is the catalytic direct glycosylation on an O-1 pivaloyl group. The other is the stereoselective α-glycosylation which usually is almost unobtainable due to the neighboring group participation of the N-acetyl group. The first key step was successfully attempted and the second step has yet to be attempted.

1. Introduction

1.1 Carbohydrates

Carbohydrates are of extreme importance in many biological systems. Not only are carbohydrates, and chief among them glucose, of the outmost importance in terms of biological energy sources and metabolism. Sugar derivatives are also very important in mediating information in mammalian cells. Three kinds of polymers are normally considered important in mediating information in mammalian cells. Polynucleotides are the components of the DNA, storing and reproducing genetic information. Polypeptides, along with carbohydrates, transfer signals throughout the entire organism.

Carbohydrates are the most diverse group of these three groups of polymers. Polypeptides and polynucleotides are always linear, whereas carbohydrates have the ability to branch out, due to the number of free hydroxyl groups. In addition to branching points, no more than one stereogenic center is present for each monomer in polynucleotides and polypeptides, whereas in carbohydrates five stereogenic centers are present along with different configurations of a single monomer.[1]

In figure 1, four of the most common carbohydrate monomers are shown.

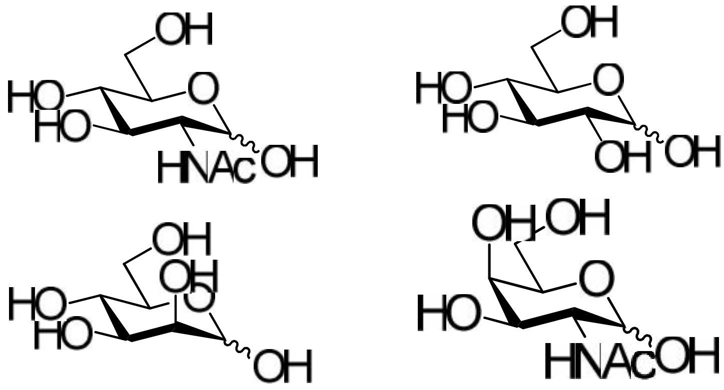


Figure 1: From left to right top to bottom: D-Glucose, N-Acetyl-D-Glucosamine (GlcNAc), D-Mannose, N-Acetyl-D-Galactosamine (GalNAc). All of the monomers are depicted as α/β-mixtures.

[1] Werz, D. B., Ranzinger, R., Herget, S., Adibekian, A., von der Lieth, C.-W., Seeberger P., *ACS Chem. Biol.*, 2007, **2**, 685-691

Carbohydrates are central in a wide variety of functions in the mammalian body. Everything from cell, pathogen and toxin recognition to structural features and protein regulation, involve carbohydrates.[2]

Typically the carbohydrate chain (oligosaccharide chain) is linked to either a protein or a lipid, depending on the function of the specific oligosaccharide chain. The oligosaccharide can be either α or β-linked, both internally between each monomer, and terminally to the polypeptide chain.[3]

Oligosaccharides linked to proteins are divided into two categories, N-linked and O-linked. N-linked oligosaccharides are linked to the nitrogen atom of an aspargine residue, whereas O-linked oligosaccharides are linked to the oxygen atom of either a serine or a threonine residue.

1.1.1 Biological significance of oligosaccharides

A number of different cell surface proteins have carbohydrates attached to the surface. The primary function of these protein linked carbohydrates is cell recognition, this being both in cell to cell interactions and signaling as well as binding sites for foreign microorganisms, toxins and antigens.[4] Along with the before mentioned recognition, surface protein linked carbohydrates are important in autoimmune responses. An example is the T_N-antigen (see figure 2), which has shown potential as an anticancer vaccine.[5]

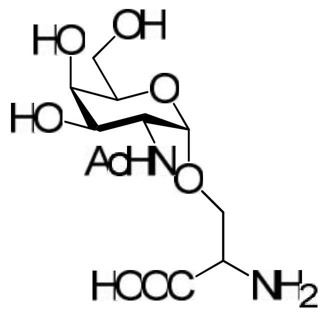


Figure 2: T_N-antigen, which is an α-linked Ser-GalNAc unit

1.2 Antibodies and antigens

Antigens are substances, foreign or from the host organism, that the body recognizes, which trigger a response from antibodies. For the host organism to be able to make an appropriate response to an antigen, the host organism must be able to distinguish foreign antigens from self-antigens.[6]

[2] Varki, A.; *Glycobiology*, 1993, **3**, 97-130
[3] Werz, D. B., Ranzinger, R., Herget, S., Adibekian, A., von der Lieth, C.-W., Seeberger P., *ACS Chem. Biol.*, 2007, **2**, 685-691
[4] Varki, A.; *Glycobiology*, 1993, **3**, 97-130
[5] Wilkinson, B. L.; Day, S.; Malins, L. R.; Apostolopoulos, V.; Payne, R. J.; *Angew. Chem. Int. Ed.*, 2011, **50**, 1635-1639
[6] Berg, J. M.; Tymoczko, J. L.; Stryer, L.; *Biochemistry*, W. H. Freeman and Company, **2007**, s. 945-956

Antibodies reacting with self-antigens are used in preventing cancer.[7] Unhealthy cells are not necessarily easily recognized and so therefore, a mechanism must be present for the T-cells to recognize unhealthy cells, such as cancer cells. All cells carry small peptide fragments on the cell surface, showing the proteins synthesized inside the cell. These fragments are bound to the major histocompatibility complex (MHC), where the T-cells also bind and check all the peptide fragments. If the peptide fragments do not fit the expected, the cell will undergo apoptosis.[8]
A specific example of this is the T_N-antigen. This specific antigen is expressed on the surface of most human cells, but the body does not respond to it. It has been discovered that this antigen is over expressed in cancer cells.[9] This can be used to trigger the immune system to kill cancer cells by recognition of the T_N-antigen.

[7] Wilkinson, B. L.; Day, S.; Malins, L. R.; Apostolopoulos, V.; Payne, R. J.; *Angew. Chem. Int. Ed.*, 2011, **50**, 1635-1639
[8] Berg, J. M.; Tymoczko, J. L.; Stryer, L.; *Biochemistry*, W. H. Freeman and Company, **2007**, s. 970-971
[9] Wilkinson, B. L.; Day, S.; Malins, L. R.; Apostolopoulos, V.; Payne, R. J.; *Angew. Chem. Int. Ed.*, 2011, **50**, 1635-1639

1.3 Synthesis

A problem posed by the synthesis of the T_N-antigen is the markedly slower rate of reaction of the aminosugars in glycosylations, compared to their regular sugar analogues. Wong demonstrated that the reaction rate of pthalimido protected aminosugars had reaction rate constants about half of the corresponding O-acetyl sugars. He also implies that the same tendencies can be expected of the N-acetyl aminosugars. [10]

A recently conducted study shows that using rare earth metal triflates as a catalyst is a very efficient way of glycosylation with ester protected donors. Christensen *et al*. (2008) shows that the synthesis of a β-GlcNAc linkage can be done with reasonable reaction times, using rare earth metal triflates, like Lewis acids, as catalysts, with scandium- and cupper-triflates standing out as the most potent. A peracetylated donor was used (see figure 3). The system was also tested using the 4-epimer GalNAc with promising results. [11]

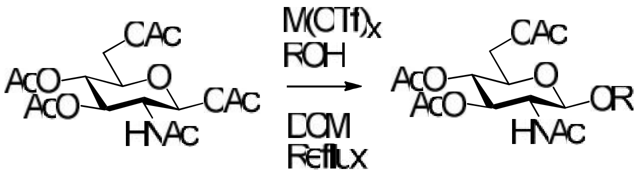


Figure 3: Glycosylation of peracetylated donor with rare earth metal triflates

Commonly a thioglycoside is used as glycosyl donor, due to the high stability of the thionyl group toward many protection group manipulating conditions such as Zemplin.[12]

As demonstrated by Krag *et al*.,[13] (2010) the use of rare earth metal triflates can also be expanded to include a pentenyl donor, making direct glycosylations of donors, independently of their protecting groups, possible. This means that multiple protecting group manipulations can be avoided, using a single glycosylation step instead, and thus saving large amounts of time and giving higher yields.

In comparison to the before mentioned thionyl donor, the pentenyl donor is more reactive, giving higher yields and a faster reaction.

[10] Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, R.; Wong, C.-H.; *J. Am. Chem. Soc.*, 1999, **121**, 734-753
[11] Christensen, H.; Christiansen, M. S.; Petersen, J.; Jensen H. H.;, *Org. Biomol. Chem.,* 2008, **6**, 3276-3283
[12] Krag, J.; Christiansen, M. S.; Petersen, J. G.; Jensen, H. H.; Carb. Res., 2010, **345**, 872-879
[13] Krag, J.; Christiansen, M. S.; Petersen, J. G.; Jensen, H. H.; Carb. Res., 2010, **345**, 872-879

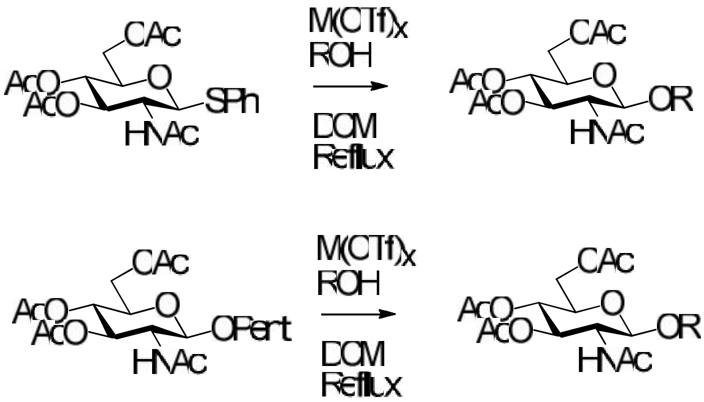


Figure 4: Two different glycosylation reactions used by Krag et al.

Another significant challenge in working with GalNAc is that it is very expensive. Whereas GlcNAc is easily acquired from the hydrolysis of chitin, GalNAc is not readily available from commercial sources. A new and rather simple synthetic strategy has been proposed by Feng and Ling, using a simple selective pivaloyl protection of the oxygen O-1, O-3 and O-6. When the GlcNAc is protected, the free O-4 is turned into a very good leaving group by addition of a triflate group. This triflate is hydrolysed by addition of water and followed by migration of the 3-O pivaloyl group. [14]

[14] Feng, J.; Ling, C.-C.; *Carb. Res.*, 2010, **345**, 2450-2457

1.4. Project idea

1.4.1 Aim of the project

The aim of this project was twofold. Firstly, we wanted to show that direct catalytic glycosylation of a perpivaloylated GalNAc is possible and analogous to the previous study of peracetylated GalNAc and GlcNAc using rare earth metal triflates.

Secondly, we aimed to be able to show that it is possible to make exclusively α-linked GalNAc, using a 4,6-O-di-*tert*-butylsilylene protecting group.

We used N-acetyl-D-glucosamine as starting material, due to the large difference in price between GlcNAc and GalNAc. GlcNAc is available from Sigma Aldrich for 583 DKK per 5 grams, whereas the same amount of GalNAc was 7487 DKK.[15] In other words GalNAc is more than 12 times as expensive as GlcNAc.

Another reason for choosing GlcNAc over GalNAc was the fact that the proposed synthetic strategy used to invert the stereochemistry on C-4 gave us the desired pivaloyl protection groups.[16]

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[15] Prices retrieved from www.sigmaaldrich.com at 15:00 on the 23rd of June.
[16] Feng, J.; Ling, C.-C.; *Carb. Res.*, 2010, **345**, 2450-2457

1.4.2 Synthetic strategy

The T_N-antigen is the target compound of this synthesis, using N-acetyl-D-glucosamine as the starting material. First N-acetyl-D-glucosamine (**1**) was selectively pivaloylated in the 1, 3 and 6 position, leaving free the 4-hydroxyl group needed for the inversion (**2**). After the pivaloylation, the 4-hydroxyl was reacted with trifluormethyl sulfonic anhydride to create a better leaving group, and thus making the inversion possible. Water was then added to yield the inverted product (**3**). The GalNAc is then perpivaloylated to give fully protected GalNAc (**4**). Direct catalytic glycosylation is attempted on the perpivaloylated compound using a catalytic amount of bismuth triflate and pent-4-en-1-ol, yielding 1-pentenyl-GalNAc (**5**). This glycoside is then deprotected using standard Zemplin conditions, followed by the introduction of the DTBS group on 4- and 6-hydroxyl groups by $DTBS(OTf)_2$ and acetylation of the 3-hydroxyl group. The resulting glycoside (**6**) is the desired compound for attempting a stereoselective α-glycosylation directed by the DTBS group. This is done using an activator, NIS, and a catalytic amount of bismuth triflate along with the acceptor amino acid, giving the desired product (**7**). (For complete reaction overview see figure X).

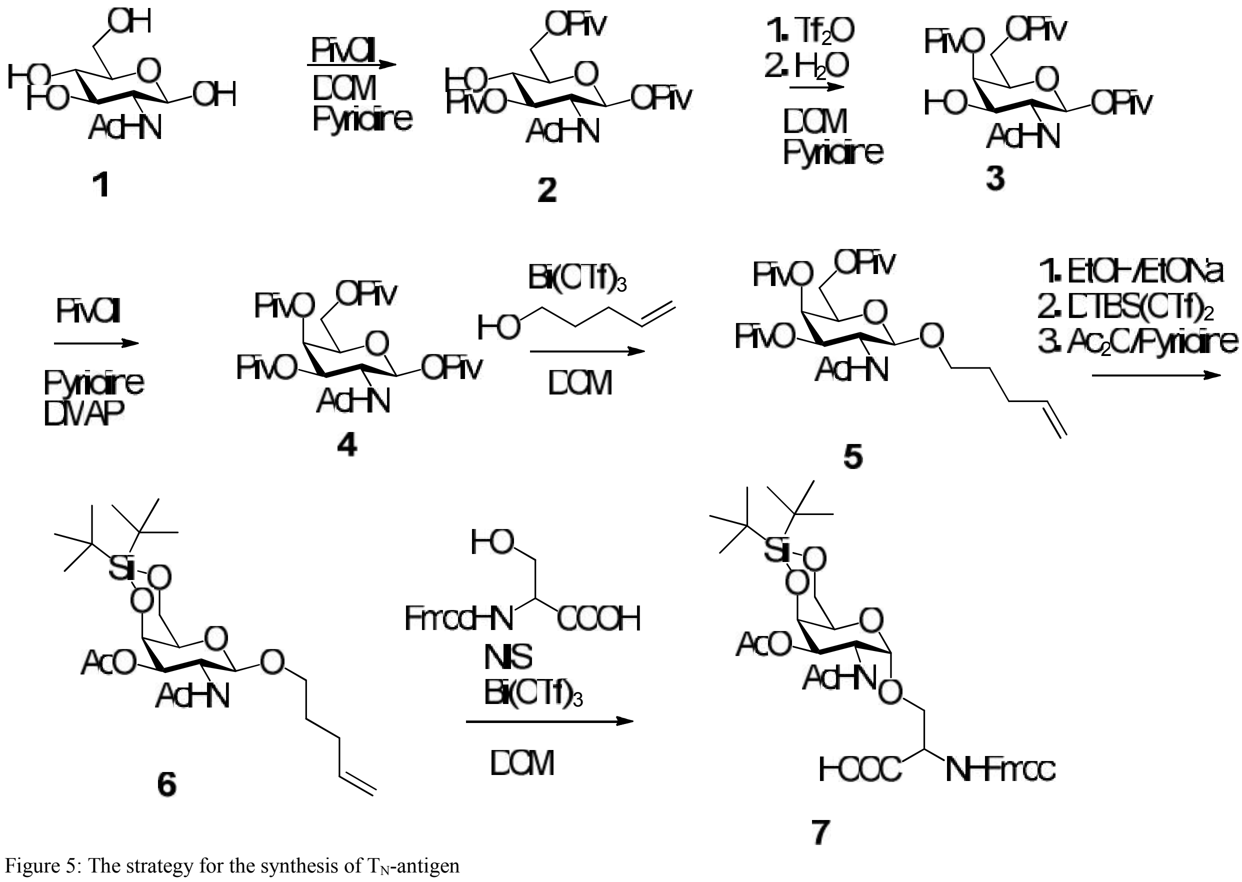

Figure 5: The strategy for the synthesis of T_N-antigen

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2. Results

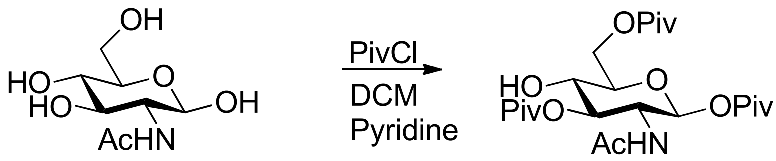
The aim of this project is to synthesize the T_N-antigen (α-linked serine GalNAc).

2.1 Synthesis of N-acetyl-1,3,6-tripivaloyl glucosamine

The starting material for the synthesis of pivaloyl protected glucosamine (**2**) is the commercially available N-acetyl-D-glucosamine (**1**).

The reaction is done by dissolving the starting material glucosamine in predistilled dichloromethane (DCM) and pyridine dried over molsieves. The solvents are left stirring under nitrogen atmosphere overnight, in order to dissolve the glucosamine properly.

When the glucosamine is dissolved, pivaloyl chloride is added at -10 °C. After a few minutes the reaction mixtures is heated to 0 °C and left for 2 days.

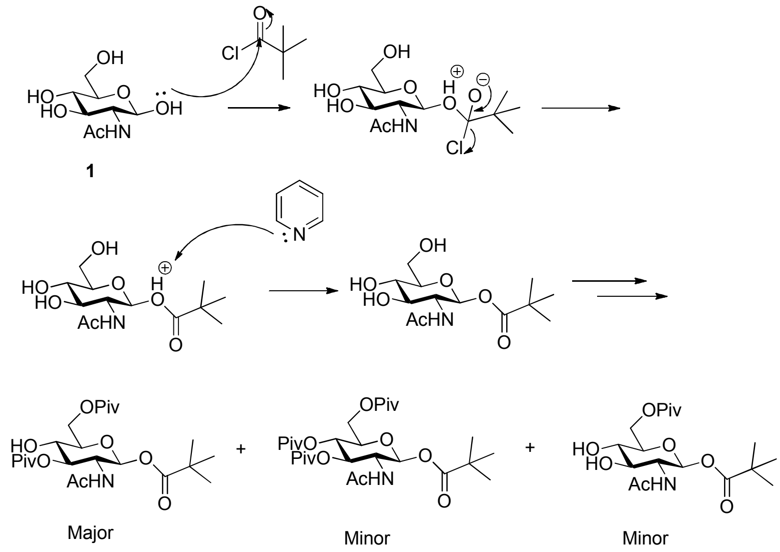


1 **2**

Figur 6: Reaction scheme for the synthesis of tripivaloyl GlcNAc (**2**)

The reaction is a simple nucleophilic substitution on a carbonyl group. In this case the more reactive acid chloride[17] is turned into an ester. The reaction mechanism is shown in the scheme 1 (see below).

[17] Clayden, J.; Greeves, N.; Warren, S.; Wothers, P.; *Organic Chemistry,* Oxford University Press, **2001**, s. 287



Scheme 1: The reaction mechanism for the synthesis of **2**

This reaction is initiated by the attack of the lone pair of the oxygen in the free hydroxyl group. The initial attack comes from the hemiacetal and the primary hydroxyl group, since they are the more reactive nucleophiles. A tetrahedral intermediate is created upon attack. This intermediate collapses and leads to recreation of the carbonyl group using the chloride as a good leaving group. The base and solvent pyridine is used to remove the proton on the positively charged oxygen atom of the ester functionality. It is worth noticing that although nitrogen atoms are often considered better nucleophiles than oxygen atoms, in this case the pyridine does not act as a nucleophile because pyridine is considered a stable imine.[18]

3.5 equivalents of the pivaloyl chloride were added, ensuring that only three of the four free hydroxyl groups were protected. Due to the low temperatures, the more reactive hydroxyl groups will be much more likely to react with the acid chloride, thus reducing the formation of by products. This will result in the reaction of the 3-hydroxyl group selectively over the 4-hydroxyl. It should, however, be noted that the reaction is only partially selective. Some of the starting material will be converted to tetrapivaloylated GlcNAc and a smaller fraction will only be pivaloylated on the O-1 and O-6 positions.

Excess acid chloride is hydrolysed and removed by aqueous work-up. Pyridine is removed by washing with aqueous hydrochloric acid. The resulting brown oil was purified using column chromatography.

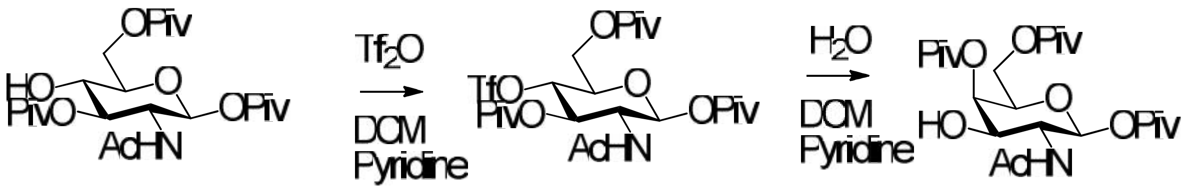
A thin layer chromatography (TLC) confirmed the presence of three different compounds in the brown oil. The first compound to be eluated from the column is the tetrapivaloylated GlcNAc. This

[18] Clayden, J.; Greeves, N.; Warren, S.; Wothers, P.; *Organic Chemistry,* Oxford University Press, **2001**, s. 485

compound largely overlaps the tripivaloylated compound, leading to large fractions of a mixture between the two compounds. The third compound is much more polar than the first two and, thus, is left on the column. The compound was obtained in 31 % yield, with another 41 % mixed with the tetrapivaloyl impurity, both as white crystals.

2.2 The synthesis of N-acetyl-1,4,6-tripivaloyl-D-galactosamine

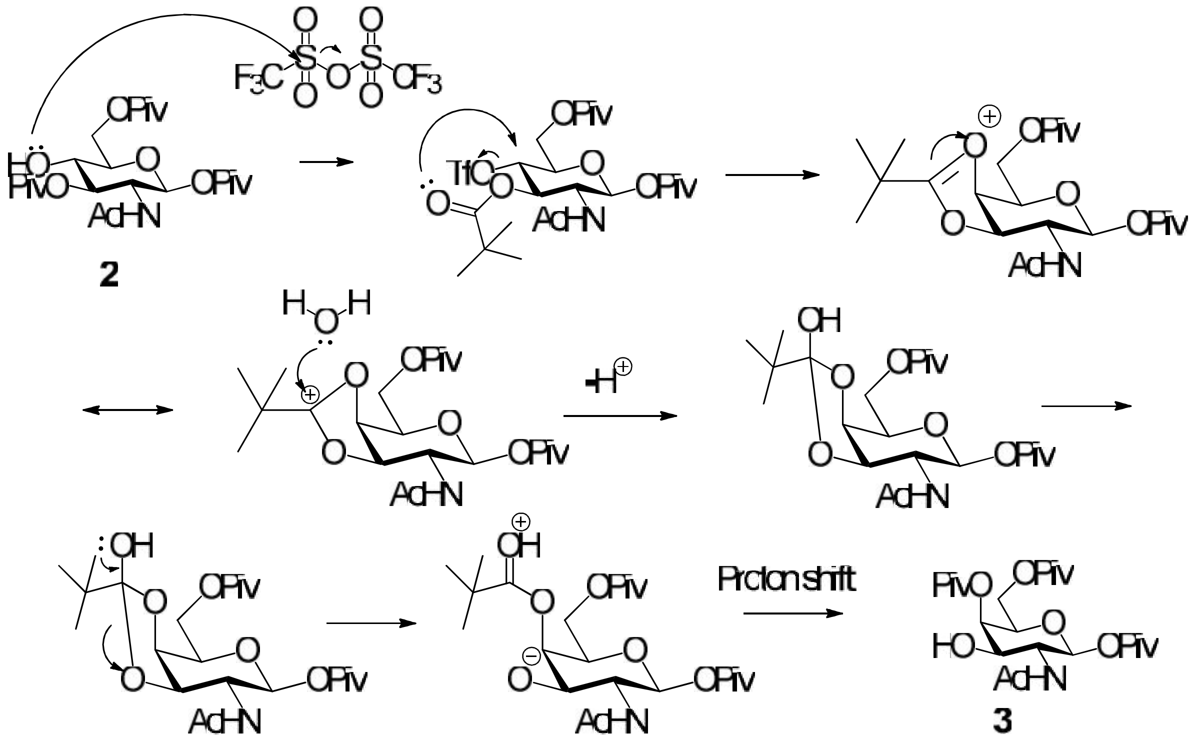
The second step of this synthesis is the inversion of the stereochemistry at C-4. This step is a two step one pot synthesis. Firstly, the free hydroxyl group of the C-4 is activated by addition of triflic anhydride, resulting in a triflate on the C-4. Triflate is an excellent leaving group, and upon addition of water, the triflate is pushed out by the neighboring pivaloyl group in an S_N2 like manner. This causes the inversion of C-4 stereocenter. Also it is important to notice that the pivaloyl group migrates from O-3 to O-4.



2 3

Figure 7: Reaction scheme for the second step

The mechanism of the reaction can be seen in the following scheme.



Scheme 2: The reaction mechanism for the synthesis of **3**

The reaction is initiated by attack of the free 4-hydroxyl group on the electrophilic sulphur, which acts as a carbonyl analogue. As a result the acid anhydride sulphur analogue is cleaved. Since this is a sulphur analogue no tetrahedral intermediate is present.

When the triflate is added to the hydroxyl group, two reactions are possible. Either the added water or the pivaloyl group on O-3 can react on C-4 to invert the stereocenter. Since reaction with the pivaloyl is an intramolecular reaction, this reaction is much faster than the reaction with water.

A lone pair of the carbonyl oxygen attacks at the C-4 to create a positively charged five-membered ring intermediate. The positive charge is delocalized between the oxygen and the carbonyl carbon, which leads to the above shown resonance structures (Scheme 2).

Water attacks at the most electrophilic center, the carbon. This creates a tetrahedral intermediate with a positive charge, which the solvent pyridine removes in the form of a proton from the added water. The tetrahedral intermediate collapses which results in the breakage of the five-membered ring. Either oxygen act as leaving group, but most commonly the O-3 acts as leaving group, resulting in the migration of the pivaloyl group from C-3 to C-4.[19]

Following a proton shift, the desired compound was obtained.

[19] Feng, J.; Ling, C.-C.; *Carb. Res.*, 2010, **345**, 2450-2457

The reaction mixture was again washed with water to hydrolyze any leftover triflic anhydride along with removing any charged molecules. Furthermore, the mixture was washed with aqueous hydrochloric acid to remove any unused pyridine.
The impure product was obtained as white crystals. The product was purified using column chromatography followed by a recrystallization in heptane and ethyl acetate and gave a yield of 42 %.

2.3 Synthesis of N-acetyl-1,3,4,6-tetrapivaloyl-D-galactosamine

The third step is a simple addition of a pivaloyl group to a free hydroxyl group, much like the first step of this synthesis, only on a very weakly reactive hydroxyl group. This makes a catalyst important for the reaction. For this purpose dimethylaminopyridine (DMAP) was used. Also instead of leaving the reaction at room temperature, the reaction was heated to 43 °C to speed up the reaction. Pyridine was used as solvent and base for the reaction. (Figure 8)

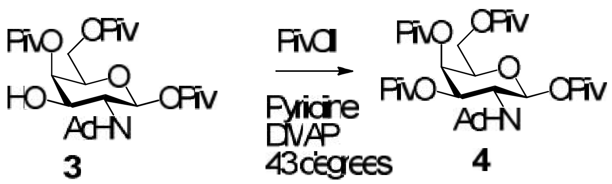
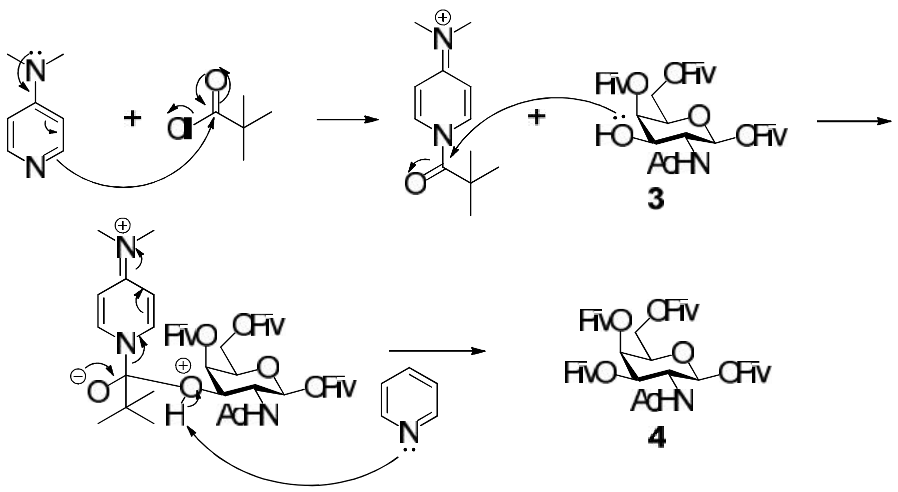


Figure 8: Reaction scheme for the third step

The mechanism is similar to that of the first reaction, only this time a catalyst is used. The mechanism for this reaction is shown in scheme 3.



Scheme 3: The reaction mechanism for the synthesis of **4**

The catalyst of this reaction is dimethylaminopyridine (DMAP) which activates the reagent pivaloyl chloride. Even though an amide is normally considered very unreactive, this activated acid chloride is very reactive, which is necessary for this very weakly reactive free hydroxyl group.[20]
The activation step is a nucleophilic substitution of the pivaloyl carbonyl group. The added DMAP-group will act as an excellent leaving group and be recovered to be used in the next catalytic cycle. The solvent pyridine will act as a base to remove the proton from the oxygen on C-3.
The reaction mixture is washed with water and brine to remove any charged molecules and hydrolyze any remaining pivaloyl chloride. This was followed by washing with aqueous hydrochloric acid to remove unused pyridine. Sodium sulphate was used as a drying agent in place of the more commonly used magnesium sulphate as a precaution due to a slight acid sensitivity of the product.
The product was isolated as a clear oil in 62 % yield.

2.4 Synthesis of N-acetyl-1-(pent-4-enyl)-3,4,6-tripivaloyl-D-galactosamine

This fourth step is one of two key steps in this project. This is the first attempt to perform a direct catalytic glycosylation. It is done using bismuth triflate as the catalyst and pent-4-en-1-ol as the acceptor. The reaction was done in dichloromethane at 43 °C.

[20] Clayden, J.; Greeves, N.; Warren, S.; Wothers, P.; *Organic Chemistry,* Oxford University Press, **2001**, s. 1153

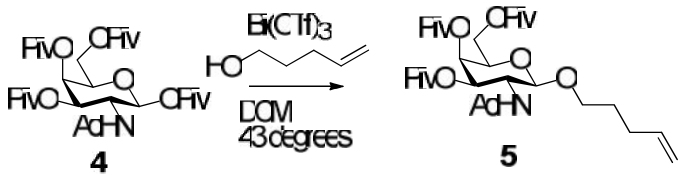


Figure 9: The reaction scheme for step 4

This attempt was the first ever to do this reaction and therefore the reaction was monitored closely with TLC analysis.

This analysis, however, showed that the product and the donor are co-polar, meaning that the reaction could not be followed by TLC analysis. To avoid this problem, a test system was set up using 1-octanol as acceptor.

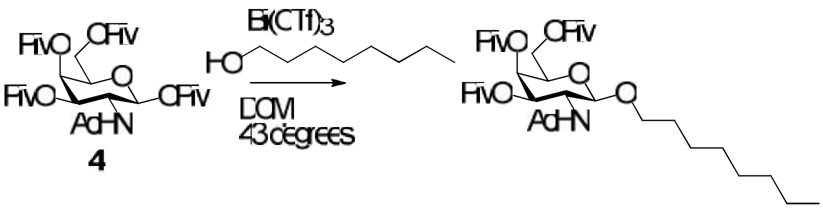


Figure 10: Test system with octanol

Again, the reaction was followed by TLC analysis, and again co-polarity was observed. This time the octanol and the donor were inseparable in TLC analysis. Another test system was made, using allyl alcohol as acceptor.

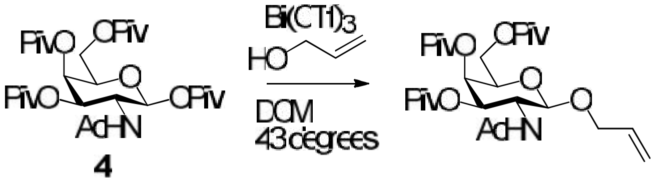
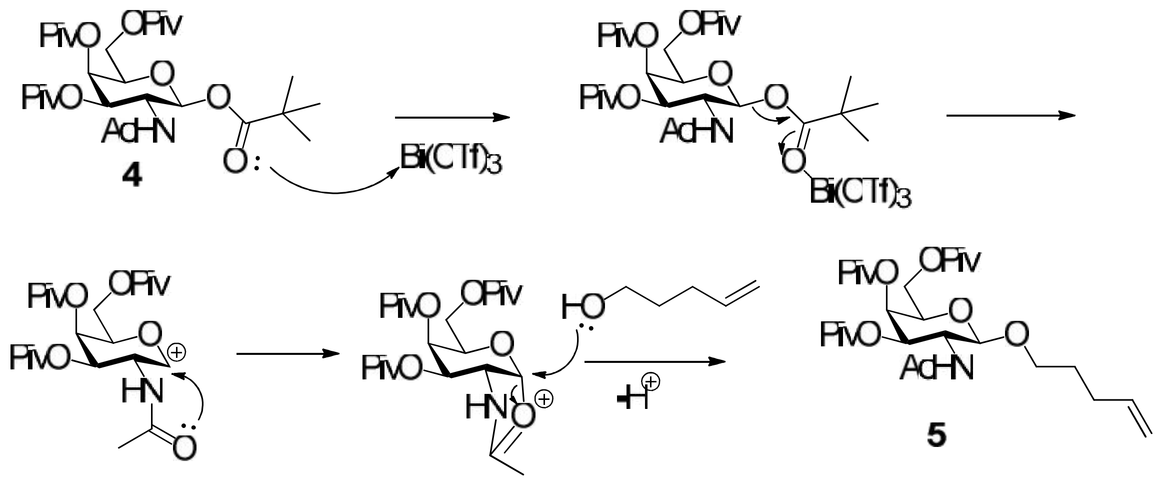


Figure 11: Test system with allyl alcohol

The mechanism is the same independently of the acceptor used. The mechanism is shown in the following scheme.



Scheme 4: The reaction mechanism for the synthesis of **5**

In this key synthetic step, the metal starts out coordinating to the carbonyl oxygen of the 1-pivaloyl group. This promotes leaving of the pivaloyl group, by attracting the electrons of the other oxygen, which along with the stability of the carbocation is responsible for that this reaction is has a mixture of S_N1 and S_N2 type character. This stability is due to a resonance structure, which is not shown above. It is the lone pair on the ring oxygen, which is responsible for the resonance stabilisation. Upon expelling the pivaloyl group, an intramolecular reaction takes place. The lone pair of the carbonyl oxygen of the amide functionality attacks the carbocation and thus creating the oxazoline intermediate. This intermediate reacts with the acceptor alcohol in a somewhat sluggish way. This reaction has primarily S_N2 character, thus accounting for the inversion of the stereochemistry for this reaction and the overall conservation of the stereochemistry for the entire step.
The pentenyl acceptor is chosen because it is resistant to the following Zemplin deprotection, and at the same time it is an excellent leaving group for glycosylations when using NIS as an activator.[21]
When the reaction is done, the product mixture is washed with water and brine to remove the catalyst followed by the aqueous sodium bicarbonate to remove any trimethylacetic acid created when the pivaoyl group leaves.
The product mixture was then purified using column chromatography. The product was isolated as a clear oil in 59 % yield.

[21] Krag, J.; Christiansen, M. S.; Petersen, J. G.; Jensen, H. H.; Carb. Res., 2010, **345**, 872-879

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3. Future work

Only one part of the projects aim was achieved. The other part, that is to determine whether α-stereoselective glycosylation can be achieved using 1-pentenyl-GalNAc as donor and a 4,6-O-silylene group as the directing group, is yet to be determined. For this to be determined further synthetic steps are required.

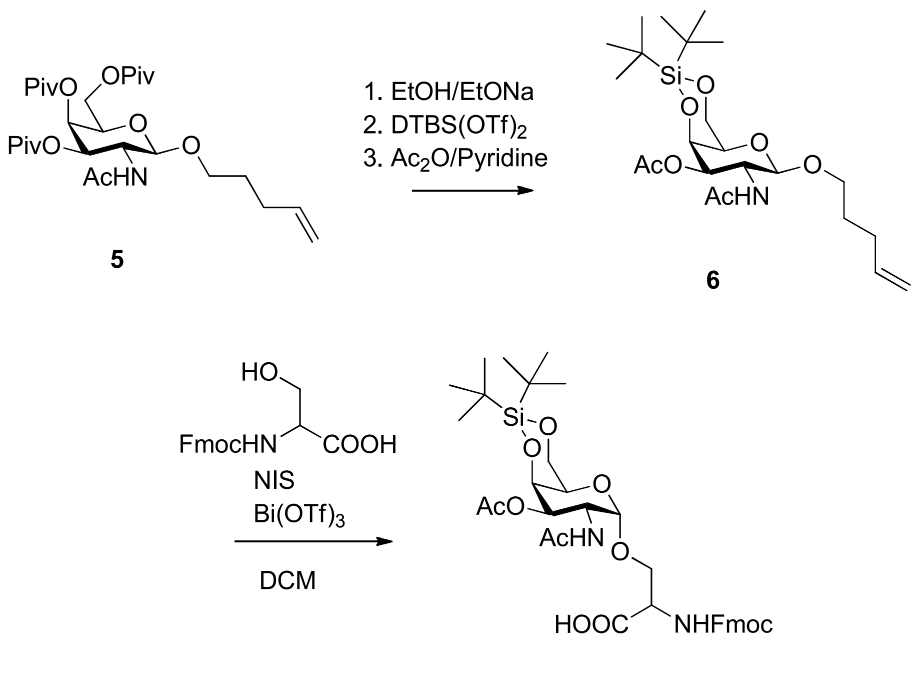


Figure 12: Proposed final steps for the synthesis of the T_N-antigen

Firstly a simple Zemplin deprotection of all the ester functionalities is performed using sodium dissolved in ethanol. This is followed by addition of the suspected α-directing di-*tert*-butylsilylene group and acetylation of the last free hydroxyl group to avoid unwanted side reactions.

Then the actual test of this project will be performed. The stereoselective glycosylation will be done using a protected serine as acceptor and NIS as activator along with bismuth triflate as catalyst, in the test. Hopefully this will provide an easier way of synthesizing the T_N-antigen.

4. Conclusion

One part of the project aim has been achieved. It has proven possible to use pivaloyl protecting groups on C-1 in direct catalytic glycosylation, analogous to the acetyl group used by Christensen and co-workers. However, as expected the pivaloyl group slowed down the reaction markedly. The more reactive GalNAc made up for some of the lost rate of reaction, but it is still slower than an acetyl protected GlcNAc. We found that a test system was necessary in order to follow the reaction, due to the co-polarity of the pivaloyl protected starting material and the pentenyl protected product.

A few steps still remain in obtaining the desired product, with the last step, which is the stereoselective catalytic direct glycosylation, being the one of most interest. Here it will be clear if a new and easier way of synthesizing the T_N-antigen has been discovered.

5. Experimentals

5.1 General

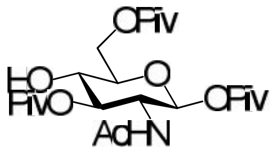
All reactions with moisture sensitive compounds were conducted under nitrogen atmosphere and all solvents were dried prior to use. All evaporation of solvent was done using reduced pressure and at 40 °C. TLC analysis was carried out on aluminum foil coated with silica gel (Merck Kieselgel 60 F_{254}). TLC plates were stained using either PMA (phosphor molybdic acid) or potassium permanganate ($KMnO_4$) followed by heating.

5.2 Apparatus

NMR spectra were recorded on a Varian Mercury 400 spectrometer. ^{1}H-, gCOSY-, DEPT-135-, and gHMQC-NMR spectra were recorded at 400 MHz whereas ^{13}C-NMR spectra were recorded at 100 MHz. The chemical shifts (δ) are stated in ppm using the residual solvent signal as secondary reference. All spectra are recorded using $CDCl_3$ as solvent, giving 7.27 ppm and 77.16 ppm as reference for H-NMR and C-NMR respectively. Assignment of NMR spectra is done by using gCOSY, DEPT-135 and gHMQC techniques. Mass spectra were recorded on a Micromass LC-TOF spectrometer with positive electronspray ionization. In high resolution mass spectras, BzGlyPheOMe: $C_{19}H_{20}N_2O_4Na$: 363.1321 m/z or BocSer(OBn)SerLeuOMe: $C_{25}H_{39}N_3O_8Na$: 532.2635 m/z were used as internal standard. Melting points were measured using a Büchi B-540 instrument. Optical rotations were measured on a Perkin-Elmer 241 polarimeter and concentrations are given in g/100mL.

5.3 Synthesis

N-acetyl-1,3,6-tri-O-pivaloyl-β-D-glucosamine (2)



2

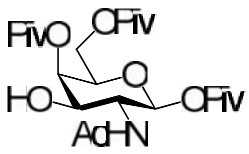
N-acetyl-D-glucosamine (1 g, 4.52 mmol) was mixed with dry dichloromethane (5 mL) and dry pyridine (2.5 mL). The mixture was stirred vigorously overnight under nitrogen atmosphere. Trimethyl acetyl chloride (1.7 mL 13.82 mmol) was added to the mixture at -10 °C and after addition was removed from the cooling. The mixture was left at room temperature for 48 hours. After 48 hours the mixture was diluted with dichloromethane and washed twice with water and brine. Hydrochloric acid was added until the aqueous phase was acidic to remove pyridine. The organic phase was dried with sodium sulphate and concentrated under reduced pressure. The resulting yellow oil was purified using column chromatography. N-acetyl-1,3,6-O-tri(trimethylacetyl)-β-D-glucosamine was isolated as a clear oil, in 72 % yield, which slowly crystallizes (1,54 g 3,32 mmol,).

R_f: (EtOAc:pentane 2:3) 0.40

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.00 (1H, d, J = 9.6, N**H**Ac), 5.60 (1H, d, J = 8.8, H-1), 5.06 (1H, dd, J = 10.8, 9.2, H-3) 4.43 (1H, dd, J = 12.0, 4.8, H-6α), 4.33 (1H, dd, J = 12.0, 2.4, H-6β), 4.24 (1H, dt, J = 11.2, 10.0, H-2), 3.68-3.64 (1H, m, H-5), 3.55 (1H, t, J = 9.2, H-4), 1.86 (3H, s, Ac), 1.21 (9H, s, Piv), 1.18 (9H, s, Piv), 1.16 (9H, s, Piv).

^{13}C NMR (100 MHz, $CDCl_3$): δ 179.8, 179.3, 177.2 (O**C**OC(CH_3)$_3$), 169.8 (NH**C**OCH_3) 92.7 (C-1), 75.0 (C-5), 74.8 (C-3), 69.0 (C-4), 62.7 (C-6) 52.6 (C-2), 39.0, 39.0, 38.8 (**C**(CH_3)$_3$), 27.2, 27.0, 26.7 (C(**C**H_3)$_3$), 23.0 (NHCO**C**H_3).
Mass spectrum is not available because the spectrometer was out of order at the time.

N-acetyl-1,4,6-tri-O-pivaloyl-β-D-galactosamine (3)



3

N-acetyl-1,3,6-O-pivaloyl-β-D-glucosamine (**2**) (1 g, 2.16 mmol) was dissolved in dry dichloromethane (2 mL) and dry pyridine (2 mL). The mixture was cooled to -10 °C before addition of trifluormethanesuphonic anhydride (0.40 mL, 3.24 mmol). The mixture was stirred at 0 °C for one hour. After one hour water (0.40 mL) was added and the mixture was heated to 43 °C overnight. The mixture was diluted with dichloromethane and washed twice with water, once with brine and once with hydrochloric acid. The solution was then dried over sodium sulphate and concentrated under reduced pressure. The product was obtained by column chromatography using pure ethyl acetate as eluent. The product was isolated as white crystals in 62.5 % yield (625 mg, 1.35 mmol).

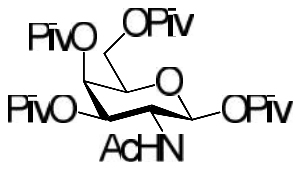
R_f: (EtOAc) 0.40
Mp: 143.9-147.8 °C

^{1}H NMR 400 (MHz, $CDCl_3$): δ 5.68-5.60 (2H, m, NH + H-1), 5.32 (1H, s, H-4), 4.18-4.05 (3H, m, H-2 + H-6α + H-6β), 3.98 (1H, t, J = 7.2, H-5), 3.88 (1H, dd, J = 11.2, 3.6, H-3), 3.42 (1H, s weak broad, 3-OH), 1.99 (3H, s, Ac), 1.29 (9H, s, Piv), 1.23 (9H, s, Piv), 1.18 (9H, s, Piv).

^{13}C NMR (100 MHz, $CDCl_3$): δ 178.1, 178.0, 177.5 (Piv), 172.4 (NHAc), 92.5 (C-1), 71.9 (C-5 + C-3), 68.2 (C-4), 61.4 (C-6), 52.9 (C-2), 39.2, 38.8, 38.7 (**C**$(CH_3)_3$), 27.2, 27.0, 26.8 (C(**C**$H_3)_3$), 23.0 (NHAc)
Both spectra show traces of ethyl acetate, but are otherwise in fine accordance with known spectra.[22]
Mass spectrum is not available because the spectrometer was out of order at the time.

[22] Inversionsprep

N-acetyl-1,3,4,6-tetra-O-pivaloyl-β-D-galactosamine (4)



4

N-acetyl-1,3,6-O-tripivaloyl-β-D-galactosamine (**3**) (540 mg, 1.14 mmol) was dissolved in dry dichloromethane (4 mL) and dry pyridine (4 mL). Trimethylacetyl chloride (0.49 mL, 4.48 mmol) and dimethylaminopyridine (19.2 mg, 0.17 mmol) was added and the mixture was heated to 40 °C under reflux for 48 hours. The mixture was diluted with dichloromethane and washed twice with water and once with brine and hydrochloric acid. The solution was then dried over sodium sulphate and concentrated under reduced pressure. The product was obtained by column chromatography using (EtOAc:pentane) 2:3 as eluent. The product was isolated as a clear oil in 62 % yield (394 mg, 0.71 mmol).

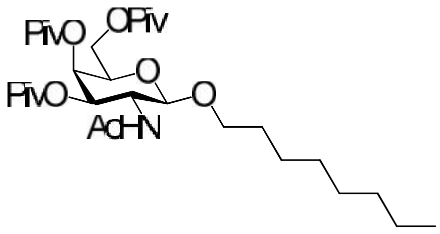
R_f: (EtOAc:Pentane) 0.40

^{1}H NMR 400 (MHz, $CDCl_3$): δ 5.64 (1H, d, J = 9.2, NH), 5.54 (1H, d, J = 10, H-1), 5.38 (1H, d, J = 3.0, H-4), 5.12 (1H, dd, J = 10.8, 3.6, H-3), 4.49 (1H, dt, J = 11.6, 9.2, H-2), 4.18-3.98 (3H, m, H-6α + H-6β + H-5), 1.84 (3H, s, Ac), 1.28 (9H, s, Piv), 1.19 (9H, s, Piv), 1.15 (9H, s, Piv), 1.11 (9H, s, Piv).

^{13}C NMR (100 MHz, $CDCl_3$): δ 178.1 (Piv), 177.8 (Piv), 177.2 (Piv), 176.8 (Piv), 169.5 (NHAc), 92.8 (C-1), 72.0 (C-5), 70.4(C-3), 66.0 (C-4), 60.9 (C-6), 49.9 (C-2), 39.1, 38.8, 38.7, 38.6 (**C**$(CH_3)_3$), 27.2, 27.0, 26.9, 26.7 (C(**C**$H_3)_3$)

Mass spectrum is not available because the spectrometer was out of order at the time.

N-acetyl-1-O-octanyl-3,4,6-tri-O-pivaloyl-β-D-galactosamine



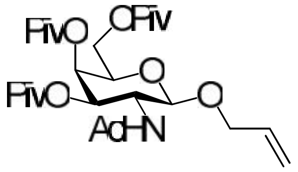
N-acetyl-1,3,4,6-tetra-O-pivaloyl-β-D-galactosamine (**4**) (100 mg, 0.18 mmol) was dissolved in dry dichloromethane (2 mL). Bismuth trifluoromethanesulfonate (17.72 mg, 0.027 mmol) and octanol (55.0 µL, 0.54 mmol) was added to the mixture and the mixture was heated to 43 °C for 48 hours. The solution was washed with water brine and saturated sodium bicarbonate and dried over sodium sulfate. The resulting mixture was concentrated under reduced pressure and was purified using column chromatography using (EtOAc:DCM) 1:19. N-acetyl-1-O-octanyl-3,4,6-tri-O-pivaloyl-β-D-galactosamine was isolated as a clear oil. No yield was measured because this reaction was only used as a test system.

R_f: (EtOAc:Pentane 1:4) 0.30, HRMS (ES): Calcd for $C_{31}H_{55}NO_9Na$ m/z: 608.3775. Found m/z 608.3777

^{1}H NMR 400 (MHz, $CDCl_3$): δ 5.42 (1H, d, J = 9.6, NH), 5.36 (1H, dd, J = 3.2, 1.2, H-1) 5.25 (1H, dd, J = 9.6, 3.2, H-4), 4.62 (1H, d, J = 8.4, H-3), 4.16 (1H, dd, J = 10.8, 6.8, H-2) 4.10-3.93 (3H, m, H-6α + OC$\mathbf{H_2}$), 3.84 (1H, dt, J = 10.4, 6.4, H-6β), 3.45 (1H, dt, J = 10.0, 7.2, H-5), 1.90 (3H, s, Ac), 1.30-1.23 (15H, m, octanol chain H2-H8), 1.25 (9H, s, Piv), 1.17 (9H, s, Piv), 1.12 (9H, s, Piv)

No carbon NMR was taken because the reaction was a test system.

N-acetyl-1-O-allyl-3,4,6-tri-O-pivaloyl-β-D-galactosamine



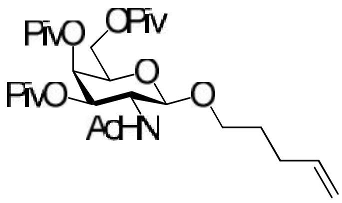
N-acetyl-1,3,4,6-tetra-O-pivaloyl-β-D-galactosamine (**4**) (100 mg, 0.18 mmol) was dissolved in dry dichloromethane (2 mL). Bismuth trifluoromethanesulfonate (17.72 mg, 0.027 mmol) and allyl alcohol (24.8 μL, 0.54 mmol) was added to the mixture and the mixture was heated to 43 °C for 2.5 hours. The solution was washed with water brine and saturated sodium bicarbonate and dried over sodium sulfate. The resulting mixture was concentrated under reduced pressure and was purified using column chromatography using (EtOAc:DCM) 1:9. N-acetyl-1-O-allyl-3,4,6-tri-O-pivaloyl-β-D-galactosamine was isolated as a clear oil. No yield was measured because this reaction was only used as a test system.

R_f: (EtOAc:Pentane 2:3) 0.35, HRMS (ES): Calcd for $C_{26}H_{43}NO_9Na$ m/z: 536.2836. Found m/z 536.2840

^{1}H NMR 400 (MHz, $CDCl_3$): δ 5.87 (1H, m, $CH_2C\mathbf{H}CH_2$), 5.38-5.18 (5H, m, term allyl + NH + H-1 + H-4), 4.64 (1H, d, J = 8.4, H-3), 4.35 (1H, dd, J = 13.2, 5.2, H-2), 4.20-3.92 (5H, m, $OC\mathbf{H_2}$ + H-6 + H-5), 1.92 (3H, s, Ac), 1.26 (9H, s, Piv), 1.18 (9H, s, Piv), 1.13(9H, s, Piv)

No carbon NMR was taken because the reaction was a test system.

N-acetyl-1-O-pentenyl-3,4,6-tri-O-pivaloyl-β-D-galactosamine



5

N-acetyl-1,3,4,6-tetra-O-pivaloyl-β-D-galactosamine (**4**) (300 mg, 0.54 mmol) was dissolved in dry dichloromethane (6 mL). Bismuth trifluoromethanesulfonate (49.0 mg, 0.081 mmol) and 4-penten-1-ol (112.2 μL, 1.62 mmol) was added to the mixture and the mixture was heated to 43 °C for 5 hours. The solution was washed with water brine and saturated sodium bicarbonate and dried over sodium sulfate. The resulting mixture was concentrated under reduced pressure and was purified using column chromatography using (EtOAc:DCM) 1:9. N-acetyl-1-O-allyl-3,4,6-tri-O-pivaloyl-β-D-galactosamine (171.6 mg, 0.32 mmol) was isolated as a clear oil.

R_f: (EtOAc:Pentane 2:3) 0.45, LRMS (ES): Calcd for $C_{26}H_{43}NO_9Na$ m/z: 564.31. Found m/z 563.8

^{1}H NMR 400 (MHz, $CDCl_3$): δ 5.83-5.71 (1H, m, $CH_2C\mathbf{H}CH_2$), 5.35 (1H, d, J = 3.2, NH), 5.24 (1H, dd, J = 11.2, 3.2, H-1), 4.97 (1H, t, J = 16.0, H-4), 4.61 (1H, d, J = 8.8, H-3), 4.18-3.94 (6H, m, term pent protons + $OC\mathbf{H_2}$ + H-6α+H-2), 3.86 (1H, dt, J = 13.2, 7.2, H-6β), 3.47 (1H, dt, J = 10.0, 7.2, H-5), 1.89 (3H, s, Ac), 1.25 (9H, s, Piv), 1.16 (9H, s, Piv), 1.11 (9H, s, Piv)

^{13}C NMR (100 MHz, $CDCl_3$): δ 178.1, 177.9, 176.9 (Piv), 169.8 (NHAc), 138.3, 137.9 (Aryl), 114.9 (Aryl), 101.2 (C-1), 71.0 (C-5), 70.2 (C-3), 68.8 (C-4), 66.4 (C-6), 62.4 (C-2), 61.3 ($O\mathbf{C}H_2$), 39.1, 38.8, 38.7, ($\mathbf{C}(CH_3)_3$), 27.2, 27.1, 27.0 ($C(\mathbf{C}H_3)_3$), 31.8, 30.1, 29.9, 28.6 (alkyl + $OOC\mathbf{C}H_3$)

Traces of ethyl acetate are present in both spectra.