LUNDS UNIVERSITET

Bachelor thesis

Synthesis of the sialic acid 9-C derivative with an amino group replacing the OH-group.

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Abstract

Almost everyone is aware of the big problem with increasing antibiotic resistance among bacteria and the importance and need of developing new antibiotics. This bachelor thesis is a part of one such project with the aim to find new possible antibiotics. A promising candidate is a derivative of the sialic acids. The sialic acids are important molecules in the human body where it from the cell membrane is responsible for a lot of the communication between cells. One of its many messages is between the human cells and the immune system where it signalizing that they are human and not harmful. This is something many bacteria has choose to take advantage of by developing a method to absorb this molecule and place it at their own cell membrane, and in that way hinder the immune system from recognize them as bacteria. Thus, this process is an interesting target for new antibiotics and by inhibiting the involved transport protein at the bacteria we can stop this process. Therefore this project has been part of a larger study where the aim is to find and synthesize a sialic acid derivative that can bind more strongly to the involved protein than the natural ligand. Previous studies have resulted in one promising candidate, the sialic acid derivative where the OH-group at carbon 9 has been replaced by an amino group. With the intention to study this molecule more my focus has been to synthesize this molecule again. The synthesis has been quite problematic and has not resulted in the intended amine. However, it has led to some improvements of the synthetic pathway as well as new insights in the reactions, for example that protecting groups might be needed.

Acknowledgements

I would not have been able to do this project without all support and help from all the members of the Ulf Ellervik's group, and I will really thank you all for a fun and worthwhile semester. I will especially like to thank….

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I also want to thank all the rest of the staff at the Centre for Analysis and Synthesis (CAS) at Lund University.

Sialinsyra-derivat som framtidens antibiotika

Sialinsyra är en viktig molekyl i den mänskliga kroppen. Den deltar bland annat i kommunikationen mellan cellerna eftersom den är placerad på cellmembranet hos de mänskliga cellerna. Av alla dessa meddelande som den kan sända ut är det ett speciellt som flera bakterier har valt att utnyttja. Detta meddelande talar nämligen om för kroppens immunförsvar att denna cell med sialinsyra är mänsklig och ofarlig, något som såklart ses som gynnsamt för bakterierna. Genom olika processer har bakterierna därför utvecklat strategier för att ta upp dessa molekyler och sätta dem på deras egna cellmembran och på så vis lura immunförsvaret att inte bryta ner dem. Och i dagens samhälle då stora problem råder med antibiotika resistens och nya antibiotika måste framställas, kan forskning inom detta område vara ett steg i rätt riktning. Genom att undersöka möjligheten att stänga av dessa processer som bakterierna använder för att överföra sialinsyran kan vi hindra bakterierna från att ”kamouflera sig” som mänskliga celler och därav kan immunförsvaret själv bryta ner dem. Detta är grunden till det projekt jag har arbetat med där jag utvecklat metoder för att framställa en modifierad sialinsyra molekyl som eventuellt kan binda till de protein som deltar vid transporten av sialinsyra molekyler hos flera bakterier och på så viss hindra denna process, och efter fortsatt forskning skulle detta i framtiden eventuellt kunna leda till en ny form av antibiotika.

Tidigare studier har visat att en viss modifikation på sialinsyran ger en molekyl som binder starkt till det involverade proteinet hos bakterierna och därför kan vara en lovande läkemedelskandidat för att stoppa denna process. Målet med mitt projekt har därför varit att syntetisera mer av denna molekyl för att kunna göra ytterligare tester. Tyvärr kom jag aldrig hela vägen. Sialinsyra molekylen har nämligen en del egenskaper som gör den lite svår att arbeta med och framställa. Därför har mitt fokus istället varit på att förbättra de olika stegen i syntesen, vilket har lett till en del nya insikter och idéer för att till slut kunna framställa den.

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1. Introduction

1.1 Sialic acids

Sialic acids are important molecules in the interaction between cells. They are located on the human cell membranes at the outer parts of the glycan. Among others they signalizing that the cells are human and not harmful, thereby regulating the immune response. This property is something that some bacteria has taken advantage of by developing a method to absorb the sialic acids, either free or from bounded to human cell membranes. The bacteria can then use them either as nutrition or place them on their own cell membrane and thereby protect themselves from the innate immune response of the human.[1]

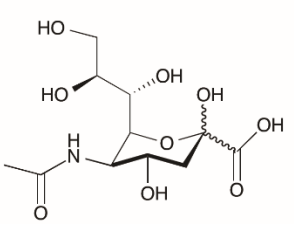


Figure 1: Neu5Ac

There are many different types of sialic acids but all are monosaccharides build up by a nine-carbon backbone.[1] They also has a carboxylic acid attached at the hemiacetal and a glycerol chain starting in position 6. The most common one is the *N*-acetylneuraminic acid (Neu5Ac) but other examples could be *N*-glycolylneuraminic acid (Neu5Gc) and 2-keto-3-deoxy-D-glycero-D-galactonononic acid (KDN). All this different types could be absorbed by bacteria.[1]

1.2 The sialic acid uptake

Although sialic acids can be used both as nutrition and to avoid the human immune response and is therefore a very important molecule for many different bacteria, many bacteria still rely on the ability to absorb this molecule from the environment. Bacteria cannot synthesize them themselves and this uptake is therefore regulated by different transport proteins at the bacteria. So far four different transport proteins for sialic acids has been identified. The tripartite ATP-independent periplasmic (TRAP) transporter, the ATP Binding Cassette (ABC), the Major Facilitator Superfamily (MFS), and the Sodium Solute Symporter (SSS), which this research is based on. The sodium solute symporter protein uses a sodium gradient to transport the sialic acid.[2] Despite all those different transporters, many bacteria only uses one of them and by inhibiting one of these transporters we might be able to block the sialic acid uptake and thus the protecting mechanism against the human immune response.

The crystal structure of the SSS transport protein has recently been discovered and has contributed to the study of new sialic acid derivatives as possible ligands. Many different projects are aiming towards new sialic acid derivatives that can bind more strongly to its active site and in that way hinder the transport. In the most recent study, by Tiago Bozzola, derivatives modified at position 9 and 3 had been considered and

resulted in one promising candidate at position 9. The OH-group at position 9 binds to the active site by an H-bond to the glutamine 82 but is mostly surrounded by many hydrophobic amino acids, in particular valine 281, proline 285, leucine 59 and phenylalanine 55, which indicates that only small groups would be tolerated at this position.[3]

1.3 The replacement of the OH-group at position 9 with an amino group

The previous studies have resulted in one compound, which was able to bind to the SSS-membrane protein. This compound was synthesized and tested by Tiago Bozzola[3] and preliminary data confirmed that this compound had higher affinity than the natural ligands. This compound is the 5-acetamido-9-amino-3,5,9-trideoxy-D-glycero-D-galacto-nonulosonic acid(**7**), were the OH-group at position 9 had been replaced by an amino group. Its K_D-value was determined to 60 x 10^{-6} M compared to the sialic acids at 91.3 x 10^{-6} M.[3] The high affinity might be explained by its ability to maintain the hydrogen bond from the OH-group to the active site and at the same time it might introduce new interactions because of its ionic state. Thus, this compound is a promising candidate as a future drug and therefore this project has been focusing on re-synthesizing this molecule and to improve the synthetic methods.

1.4 Synthetic strategies

The sialic acid, Neu5Ac, used as starting point in the reaction pathway, contains five hydroxyl groups at position C-2, C-4, C-7, C-8, and C-9. This makes it quite hard to work with and protecting groups might be considered. It also has a carboxylic functionality at C-1 with a pKa value of 2.6,[4] which is much lower compared to other carboxylic acids. This is a result of its many neighboring electron withdrawing groups and therefore the first reaction step will be to methylate the Neu5Ac to the methyl ester, to avoid this problematic properties.

According to Ercegovic,[5] the hydroxyl group at position C-9 has the highest nucleophilicity followed by OH-4, OH-8, and OH-7. Since this research is based on modifying the C-9 position the first attempts will be to not use any protecting groups. However in Ercegovic research[5] the hydroxyl group at the C-2 position was already protected and therefore its nucleophilicity was never determined and might lead to some problems in this synthetic pathway.

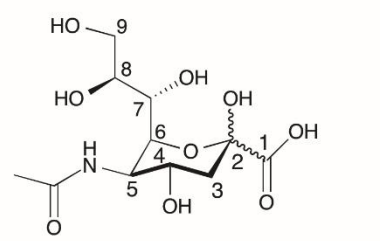


Figure 2: Neu5Ac with numbered carbons

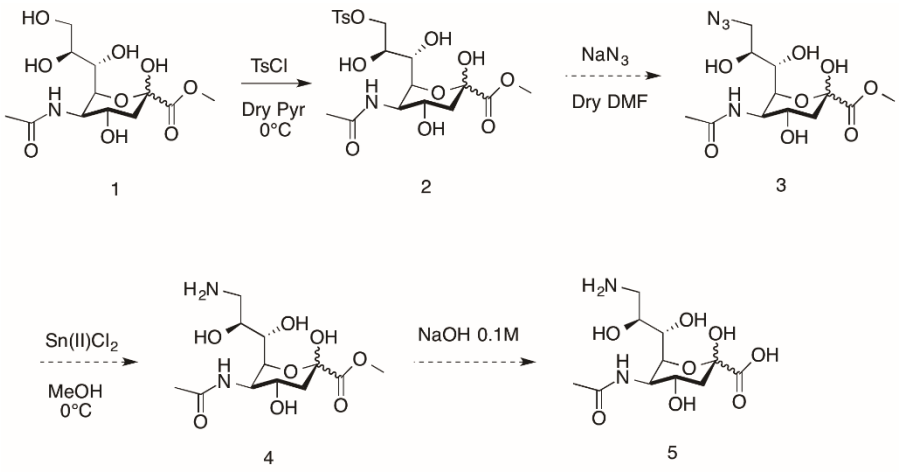
Another problem with the sialic acid, and its many hydroxyl groups, is its high polarity and water-solubility, which is problematic for example in the work-up where aqueous work-up cannot be used.

2. Aim of the project

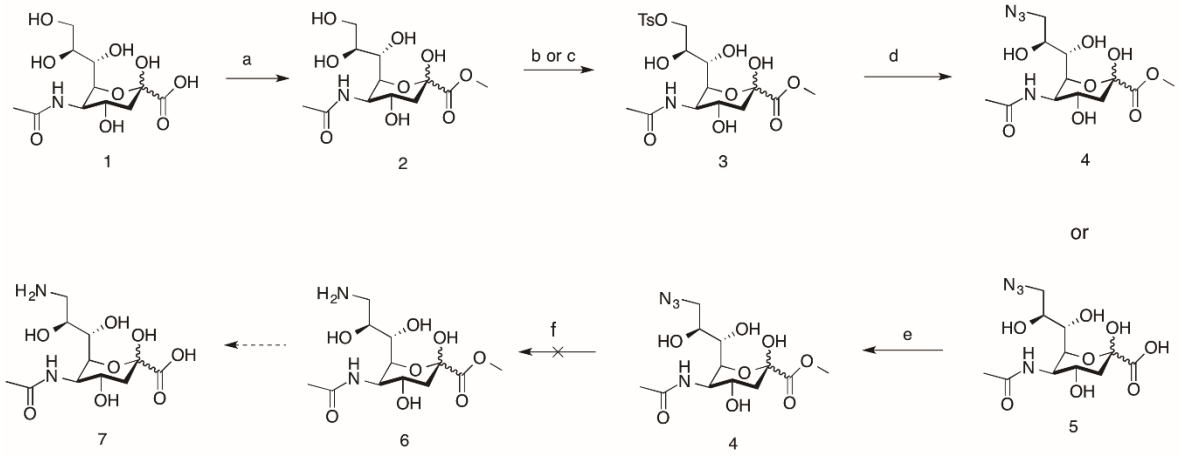
In this project the focus has been to synthesize new sialic acid derivatives in priority the 5-acetamido-9-amino-3,5,9-trideoxy-D-glycero-D-galacto-nonulosonic acid(**7**), which has been synthesized before by Tiago Bozzola[3] and showed good inhibitory effect on the SSS-protein. To synthesis this molecule, the Bozzola´s reaction pathway has been considered and tested as well as other possible methods to increase reaction rate and yield. Because of some problems during this synthesis, no time was left to study other compounds or this compounds inhibitory affect and the aim of this project was therefore changed to improve the synthetic pathway of the derivative were the hydroxyl group on carbon 9 was changed to an amino group.

3. Reaction pathway

Scheme 1 shows the reaction pathway that I first planned to use, the way Tiago Bozzola had synthesized the amine. But due to some problems the reaction pathway more or less resulted in the way illustrated in Scheme 2, all though I never succeed to reach the amine.



Scheme 1: Bozzola´s synthetic pathway



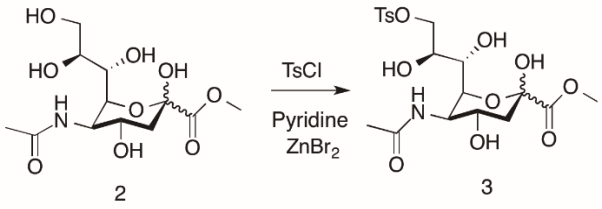
Scheme 2: a) MeOH, amberlite-H^+120, o.n. 84%, b) p-TsCl, pyridine, 0 ℃, 46%, c) TsCl, $ZnBr_2$, pyridine, -40 ℃→ r.t., 87%, d) NaN_3, dry DMF or water/acetone, 8% vs. 64% e) MeOH, amberlite-H^+120, o.n., 74% f) Sn(II)Cl_2, MeOH, 0 ℃, o.n.

4. Results and discussion

4.1 Methylation of sialic acid

The first step in this reaction pathway was to make the methyl ester (**2**) of sialic acid (Neu5Ac, **1**). The method used was quite simple and only involved dissolution in methanol with Amberlite-H^+120 and was left under stirring overnight (see scheme 2).

4.2 Tosylation reaction



Scheme 3: Tosylation reaction

The second step was to convert the hydroxyl group at carbon 9 to a better leaving group, the tosyl group (see scheme 3). This did not work as well as the first step and different methods were tested. The first method was based on Tiago Bozzola´s methods,[2] which was done by leaving the reaction overnight. This worked however with low yield; it was difficult to get fully conversion of the substrate and it resulted in some overtosylation. To improve this method the *p*-TsCl was recrystallized and higher concentration, less solvent, was needed for the reaction to work. The *p*-TsCl was recrystallized to get rid of *p*-toluenesulfonic acid and HCl which could have formed if the *p*-TsCl was too old and had decomposed. But still the best yield was only 46%.

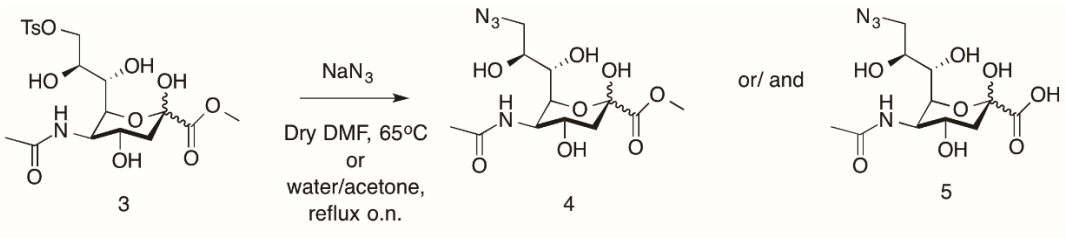
In the second method, $ZnBr_2$ was used as a catalyst, which resulted in higher reaction rate, and when the reaction mixture was placed at room temperature almost full

conversion was observed. The role of the $ZnBr_2$ as catalyst has been explained by previous research by Yamamura et al.[6] They argued that sulfonyl chloride is converted into the more reactive sulfonyl bromide and that it also might coordinate the oxygen atom at the hydroxyl group and in that way bring the reactants closer together and thus facilitate the reaction. This method was repeated several times to improve the yield and the reaction time by adding different equivalents of $ZnBr_2$ and *p*-TsCl, and at different temperatures (see Table 1). Room temperature was necessary for full conversion of the substrate but could led to some over-tosylation. Therefore adding the $ZnBr_2$ and the *p*-TsCl in portions improved the result, which can be seen in the last attempt where the yield was 78% and could have been better if it wasn´t for some purification problems and lack of time. In that attempt the *p*-TsCl was added in nine portions of 0.5 equivalents, which resulted in less over-tosylation, but on the other hand this method requires longer time, although it might not have been needed to leave the reaction overnight.

Table 1: The optimization process of the tosylation reaction

Nr.	Concentration	TsCl	$ZnBr_2$	Temperature	Reaction time	Results	Yield	Lab
1	100mg/5 ml	1.75 eq.	-	0℃ - r.t	o.n. x 2	not finished, still a lot of substrate	-	HM-A008
2	100 mg/2 ml	recrystallized 1.5 + 0.25 + 0.25 eq.	-	0℃ - r.t.	o. n. - not finished during day 2 - left over weekend	not finished, still a lot of substrate	-	HM-A010
3	100 mg/ 0.5 ml	recrystallized 2.0 + 0.25 eq.	-	0℃ - r.t.	o. n. + 7 h	finished, but still some substrate + overtosylation	30%	HM-A012
4	100 mg/ 10 ml	recrystallized 2.0 eq.	2 eq.	(-)40℃	3 h + o. n.	too diluted, hard to follow reaction, not finished when quenched, still a lot of substrate	-	HM-A014
5	700 mg/ 3.4 ml	recrystallized 2.0 + 0.25 eq.	-	0℃ - r.t.	o.n. + 5h	finished, but still some substrate + overtosylation	46%	HM-A016
6	100 mg/ 3 ml	recrystallized 2.0 eq.	2 eq.	(-)40℃ - r.t.	30 min	finished, but quenched too early so still substrate left	-	HM-A022
7	100 mg/ 2 ml	recrystallized 2.0 + 0.25 eq.	2 eq.	(-)40℃ - r.t.	2.5 h	finished, but still some substrate + not much of overtosylation	45%	HM- A026
8	100 mg/ 2 ml	recrystallized 2.0 + 0.25 + 1.0 eq.	4 + 1 eq.	(-)40℃ - r.t.	4 h	finished, some overtosylation but very little substrate	55%	HM-A028
9	100 mg/ 2 ml	recrystallized 5.0 eq.	5 eq.	(-)40℃ - r.t.	1 h	finished, but a lot of overtosylation	-	HM-A030
10	100 mg/ 2 ml	recrystallized 1.5 + 1.5 eq.	5 eq.	(-)40 - 0℃	2 h	finished, but mostly overtosylated + some substrate	-	HM-A032
11	100 mg/ 2 ml	recrystallized 2.0 + 1.0 eq.	4 + 1 eq.	(-)40 - 0℃ - r.t.	3 h	finished, but some overtosylation + some substrate	47%	HM-A034
12	200mg/ 4 ml	recrystallized 9 x 0.5 eq.	3.0 + 4 x 1 eq.	(-)40℃ - r.t.	6.5 h + o.n. + 2 h	finished, very little substrate and overtosylation	78%	HM-A070

4.3 Azide reaction



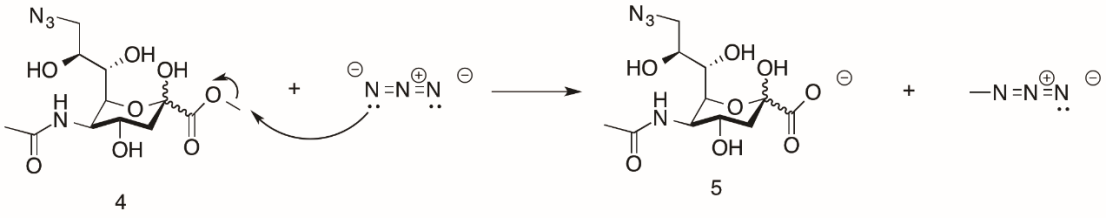
Scheme 4: Azide reaction

In the third step (see Scheme 4) some major problems were observed. The first time this reaction was tested, the product was not visible on TLC after the work-up although it was visible before. The work-up was done by adding 20% MeOH in dichloromethane to form a precipitate of the free azide followed by filtration and concentration. However, no precipitate was formed, apparently due to the low concentration, and when filtered the product might have got stuck in the filter. This was improved during the second time by no addition of MeOH and instead filtered through silica and washed with a large volume of CH_2Cl_2:MeOH (8:2) to rinse out the product. Later some problem appeared with the purification, when column chromatography was used, due to bad separation between the product and the rest of the tosyl group. This was improved with a larger column and change of eluent, although it was difficult to see when the product had come out because of the low concentration. Even though the product was now visible and purified, the yield was very low, only 8%, and new methods and improvements had to be considered. First, the solvent was changed to acetonitrile, which did not result in any improvement. No product was formed and some degradation occurred. Secondly the solvent was changed to acetone and water and the reaction mixture was refluxed overnight. This didn´t look much better on TLC because most of the reaction mixture get stuck on the baseline when CH_2Cl_2:MeOH 8:2 was used as eluent although no substrate was left either. The explanation for this can be that the methyl ester has been removed and the free carboxylic acid has formed (**5**), which may explain the spot on the baseline, as described in previous research by Mbua et al.[7] This molecule was therefore instead tried to be purified, and resulted in a much better yield at 64%. It was also successfully methylated at the same way as the sialic acid to the desired compound **4**, in a 74% yield.

This reaction step is an S_N2-reaction were it is preferable to use an aprotic solvent, like DMF, because it only dissolves the cations, like Na^+, and leaves the anion, the azide, free for reaction and makes it a better nucleophile. The question is therefore why would it be better to change to a solvent with water and acetone where water is a protic solvent that dissolves both cations and anions. The reason for this might be that by adding water, the solubility of the starting material increases which might favor the reaction. However, the aprotic solvent will still be in large excess. As mention before acetonitrile, which is an aprotic solvent, was also tested but did not result in any improvement.

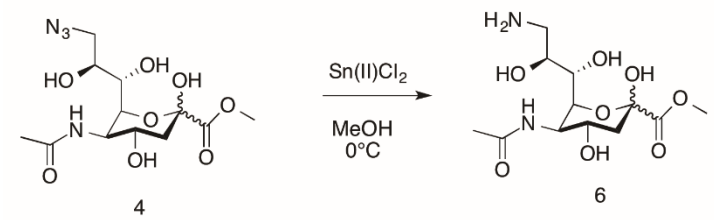
As previously mentioned the methyl ester was cleaved during this reaction conditions and resulted in the free carboxylic acid with the azide. A proposed mechanism for this

removal can be seen in Scheme 5 were the azide in addition to replace the tosyl group also attacks the methyl ester and leaves the free carboxylic acid as a good leaving group.



Scheme 5: Proposed mechanism for the removal of the methyl ester

4.4 Reduction of the azide

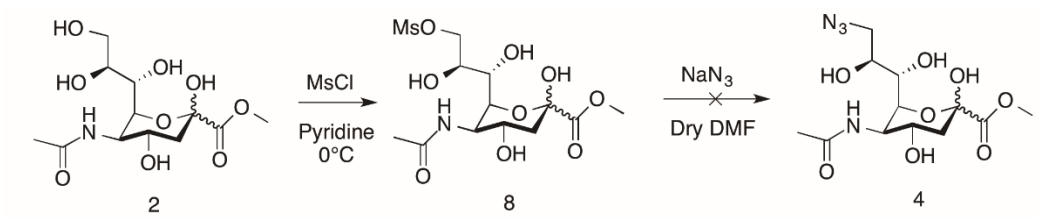


Scheme 6: Reduction of the azide.

To reduce azide (**4**) to the amine (**6**) tin(II)chloride was used as reducing agent (Scheme 6). According to Fitz et al.[8] other methods have been tested, such as catalytic hydrogenation and different catalyst (Pd/C, Lindlar, Pt) and solvents (MeOH, water), resulted in additional reduction of the α-ketoacid functionality. The α-ketoacid functionality appears in the open form of the sialic acid molecule, which is in equilibrium with the closed form. If this gets reduced it drives the reaction in the open form direction and in that way decreases the amount of the closed form with the amino group.

During the reaction everything looked good and the product was purified. But when this product was analyzed by H^1-NMR the result didn´t correspond to the reference for the amine therefore further analysis was done using mass spectrometry, which resulted in a mass at 256.1 instead of the expected at 345.1. This result was hard to find any explanation for.

4.5 Mesylation



Scheme 7: Reaction steps with the mesyl group as leaving group.

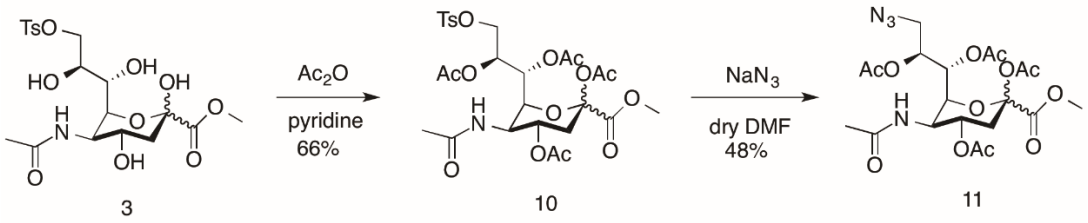
To improve the synthetic method other leaving groups were considered, among them the mesyl group (Scheme 7). The mesylation reaction worked well to form compound **8**

although the yield (40%) could have been better if the reaction was not quenched too early. Unfortunately, the following reaction where the mesyl group was to be replaced by the azide did not work that well. The reaction was started at room temperature and when nothing happened the heat was turned on to 30 ℃, it was hard to follow the reaction by TLC, because of similar R_f-value, so mass spectrometry was used and showed no product but still a lot of the starting material. The heat was therefore increased to 40 ℃ and left over night. This resulted in some product, determined by MS, but still a lot of the starting material and a by-product in form of the epoxide between carbon 8-9. To further study the temperatures impact on the reaction the reaction was left over night at 65 ℃, the temperature used when the same reaction was made with the tosylate. This resulted in complete fragmentation and no known compounds could be determined by the mass spectrometry. Although this reaction did not work that well new insights was given. The formation of the epoxide could be something that also have happened when this reaction was made with the tosylate, but was not discovered by TLC, and thereby explain the bad yield. The conclusion was also that the mesyl group might be a too good leaving group and thereby explain the formation of the epoxide.

4.6 Protecting groups

Because of the non-promising results from the previous reactions, especially from the reduction of the azide, the use of protecting groups was considered. The silicon based protecting groups TBDMS and TIPS was tested as protecting groups at position 9, however with low yields. To do a fully evaluation other methods might need to be tested as well as the rest of the steps in the synthetic pathway.

Another protecting group that was tested was the acetate group. The tosylate was acetylated before the azide reaction to protect the other hydroxyl groups as well as make the compound less polar, and in that way more easy to work with (Scheme 8). This worked quite well with good yield and this product (**10**) was then used to make the azide (**11**). The azide reaction also worked much better when the hydroxyl groups was acetylated and resulted in a yield at 48%, which indicates a need of protecting groups.



Scheme 8: The acetylation step and the azide reaction.

5. Conclusion and future aspects

Unfortunately the final product with the amino modification was never reached due to the synthetic difficulties with sialic acids. But instead the synthetic pathway has been well studied and resulted in some improvements and new insights. Especially in the tosylation reaction where the optimization process led to an increase in yield, and due to some purification problems it is possible that the yield could have been even better.

But still many problems remains and maybe to really improve the synthetic method the use of protecting groups might be necessary. A method which can be considered are to use protection of position 9 by silicon based protecting groups like for example TBDMS and TIPS before the acetylation step and then after removal of this group do the tosylation and the rest of the steps. This was only done by one primary test because of lack of time, but will be something worth tested in the future due to the problematic properties of the free sialic acid. One reason for the low yields and problems with some of the synthetic steps might for example be that the sialic acid can coordinate with a water molecule which in that case would disturb the tosylation and azide reaction.

One thing that also was tested and resulted in some real improvements was the protection of the hydroxyl groups in the tosylate by acetylation before the azide reaction. This resulted in the intended azide product with quite good yield, which could have been improved by following the reaction by mass spectrometry and not with TLC to get fully conversion. And if it wasn´t for the lack of time I would definitely have tried to reduce this compound to the amine. This leads to the conclusion that the use of protecting groups might be something worth considering in future synthesis of this sialic acid derivative.

6. Experimental

General

Column chromatography was performed either by using silica gel (40-60 μm, 60Å) columns or on a Biotage Isolera One flash purification system by using Biotage SNAP KP-Sil silica cartridges.

Methyl 5-Acetamido-3,5-dideoxy-D-glycero-D-galacto-non-2-ulopyranosonate (2)

Sialic acid, Neu5Ac (**1**) (9.99 g, 0.032 mol) was dissolved in dry MeOH (500 mL) and Amberlite-H^+120 (30 g), pre-washed with MeOH, was added. The mixture was then stirred overnight. When the reaction was finished the Amberlite-H^+120 was filtered of and rinsed by MeOH and the filtrate was concentrated to get compound **2** (8.76 g, 0.027 mol, 84%).

^{1}H-NMR (400 MHz, Methanol-d_4): δ 4.07-3.99 (m, 2 H), 3.85-3.80 (m, 2H), 3.78 (s, 3H, COOCH_3), 3.72-3.60 (m, 2H), 3.47 (dd, J = 1.2 Hz, J = 9.2 Hz, 1H, H-7), 2.22 (dd, J = 5.2 Hz, J = 13.2 Hz, 1H, H-3e), 2.02 (s, 3H, NHCOCH_3), 1.89 (dd, J = 11.2 Hz, J = 12.4 Hz, 1H, H-3a)

Methyl 5-acetamido-9-*O*-tosyl-3,5-dideoxy-D-glycero-D-galactononulopyranosylonate (3)

Method 1: Compund **2** (700 mg, 2.17 mmol) was dissolved in pyridine (3.4 mL) at 0℃ under N_2-flow. *p*-Toluenesulfonyl chloride (825 mg, 4.33 mmol, 2 eq.) was added over 1 h. The reaction mixture was then stirred and allowed to reach r.t. over night. Additional *p*-toluenesulfonyl chloride (104 mg, 0.545 mmol, 0.25 eq.) was added and 4 hours later the reaction was finished and the residue was concentrated. The residue was then purified by column chromatography (CH_2Cl_2:MeOH 10:1) and resulted in compound **3** (474 mg, 0.993 mmol, 46%).

Method 2: Compund **2** (200 mg, 0.619 mmol) was dissolved in pyridine (4 ml) at -40℃. And $ZnBr_2$ (415 mg, 1.84 mmol, 3 eq.) and *p*-TsCl (60.0 mg, 0.315 mmol, 0.5 eq.) was added. The reaction mixture was then allowed to reach r.t. After 30 min more *p*-TsCl (58.8 mg, 0.308 mmol, 0.5 eq.) was added. For 5 h more $ZnBr_2$ (414 mg, 1.84 mmol, 3 x 1 eq.) and *p*-TsCl (293 mg, 1.54 mmol, 5 x 0.5 eq.) was added in portions. When the reaction wasn´t finished late in the afternoon one last addition of *p*-TsCl (59.2 mg, 0.311 mmol, 0.5 eq.) were added and the reaction was left under stirring overnight. The morning after was the reaction still not finished and additional $ZnBr_2$ (137 mg, 0.609 mmol, 1 eq.) and *p*-TsCl (59.0 mg, 0.309 mmol, 0.5 eq.) were added and after 1 h the reaction was quenched by addition of MeOH (2 mL) and concentrated. The residue was then purified by column chromatography (CH_2Cl_2:MeOH 100:0-88:12) but due to bad separation it was repeated (CH_2Cl_2:MeOH 100:0-81:19) but still did not reach complete separation, the pure fractions was combined and resulted in compound **3** (231 mg, 0.484 mmol, 78%).

^{1}H-NMR (500MHz, Methanol-d_4): δ 7.80-7.78 (m, 2H, Ts-Ho), 7.45-7.43 (m, 2H, Ts-Hm), 4.28 (dd, J = 7.8 Hz, J = 2.3 Hz, 1H, H-9), 4.07-3.99 (m, 2H, H-9´, H-4), 3.93 (dd, J = 1.3 Hz, J = *10*.5 Hz, 1 H, H-6), 3.85 (ddd, J = 2.3 Hz, J = 6.0 Hz, J = 9.0 Hz, 1H, H-8), 3.77 (m, 4H,

COO*CH*$_3$, H-5), 3.43 (dd, *J* = 1.5 Hz, *J* = 9.1 Hz, 1H, H-7), 2.46 (s, 3H, Ts*CH*$_3$), 2.20 (dd, *J* = 5.0 Hz, *J* = 12.9 Hz, 1H, H-3e), 2.01 (s, 3H, NHCO*CH*$_3$), 1.86 (dd, *J* = 11.5 Hz, *J* = 12.9 Hz, 1H, H-3a)

Methyl 5-acetamido-9-azido-3,5,9-trideoxy-D-glycero-D-galactononulopyranosonate (4)

From compound 3: Compound **3** (101 mg, 0.211 mmol) was dissolved in dry DMF (1 mL) and NaN_3 (110 mg, 1.69 mmol, 8 eq.) was added. The reaction mixture was stirred at 65℃ for 5.5 h and was then filtered through silica and washed with CH_2Cl_2: MeOH 8:2, to remove the excess of NaN_3. The filtrate was then concentrated and purified by column chromatography (CH_2Cl_2:MeOH 95:5) to gain compound **4** (6 mg, 0.017 mmol, 8%).

From compound 5: Compund **5** (35.6 mg, 0.106 mmol) was dissolved in dry MeOH (5 mL) and Amberlite-H^+120, pre-washed with MeOH, (106 mg) was added. The reaction mixture was stirred for 24 h and the Amberlite-H^+120 was filtered of. The filtrate was then concentrated to gain compound **4** (27.5 mg, 0.0790 mmol, 74%).

^{1}H-NMR (400 MHz, D_2O): δ4.08-4.02 (m, 2H), 3.93 (m, 1H), 3.90-3.85 (m, 1H), 3.83 (s, 3H), 3.73(ddd, *J* = 2.6 Hz, *J* = 6.3 Hz, *J* = 9.1 Hz, 0.6H), 3.65-3.58 (m, 1H), 3.54 (dd, *J* = 5.2 Hz, *J* = 9.3 Hz, 1H), 3.47 (dd, *J* = 6.0 Hz, *J* = 13.2 Hz, 0.6H), 2.31 (dd, *J* = 4.8 Hz, *J* = 13.2 Hz, 1H), 2.05 (s, 3H), 1.91 (t, *J* = 12.4 Hz, 1H)

5-acetamido-9-azido-3,5,9-trideoxy-D-glycero-D-galactononulopyranoside (5)

Compund **3** (101 mg, 0.211 mmol) was dissolved in water (0.45 mL) and acetone (1.34 mL), and NaN_3 (54.7 mg, 0.841 mmol, 4 eq.) was added. The reaction mixture was refluxed at 65℃ for 22 h. The heat was then turned off and the reaction mixture was concentrated when it had reach r.t. Purification by column chromatography (CH_2Cl_2:MeOH 2:1 – 1:1) resulted in compound **5** (44.8 mg, 0.134 mmol, 64 %) confirmed by mass spectrometry.

^{1}H- NMR (400 MHz, D_2O): δ 4.03-3.86 (m, 4H), 3.59 (dd, *J* = 2.8 Hz, *J* = 13.2 Hz, 1H), 3.53-3.42 (m, 2H), 2.16 (dd, *J* = 4.8 Hz, *J* = 12.8 Hz, 1H), 2.04 (s, 3H), 1.80 (t, *J* = 12 Hz, 1H)

^{13}C- NMR (400 MHz, D_2O): δ 176.65, 174.70, 96.37, 70.00, 69.04, 68.96, 67.27, 53.88, 52.26, 39.36, 22.07

IR: ν 2102 cm^{-1} (N_3)

Methyl 5-acetamido-9-amino-3,5,9-trideoxy-D-glycero-D-galactononulopyranosonate (6)

Compound **4** (27.5 mg, 0.079 mmol) was dissolved in MeOH (0.5 mL) and Sn(II)Cl_2 (58.9 mg, 0.311 mmol, 4 eq.) dissolved in MeOH (0.5 mL) was added dropwise at 0℃. The reaction mixture was stirred for 24 h and was allowed to reach r.t. It was then concentrated and purified by column chromatography (CH_2Cl_2:MeOH 4:1) but it turned out not to be the right product.

^{1}H-NMR (400 Hz, MeOH-d_4): not the right product

MS: 256.1 not the expected 345.1268 (Na^+)

Methyl 5-acetamido-9-*O*-mesyl-3,5-dideoxy-D-glycero-D-galactononulopyranosylonate (8)

Compound **2** (100 mg, 0.309 mmol) was dissolved in pyridine (5 mL) at 0°C, and MsCl (24 μL, 0.31 mmol, 1 eq.) was added. The reaction mixture was stirred at 0°C and after 3 h more MsCl (6 μL, 0.08 mmol, 0.25 eq.) was added. 40 minutes later the reaction was quenched by addition of MeOH (3 ml) and the reaction mixture was allowed to reach r.t. before it was concentrated. Purification by column chromatography (CH_2Cl_2:MeOH 9:1) resulted in compound **8** (48.3 mg, 0.125 mmol, 40%).

^{1}H- NMR (400 MHz, MeOH-*d*$_4$): δ 4.48 (dd, *J* = 2.0 Hz, *J* = 10.4 Hz, 1H, H-9), 4.32 (dd, *J* = 5.6 Hz, *J* = 10.4 Hz, 1H, H-9´), 4.08-3.91 (m, 3H, H-4, H-6, H-8), 3.79 (m, 4H, COO*CH*$_3$, H-5), 3.52 (d, *J* = 9.2 Hz, 1H, H-7), 3.08 (s, 3H, Ms*CH*$_3$), 2.23 (dd, *J* = 4.8 Hz, *J* = 13.2 Hz, 1H, H-3e), 2.02 (s, 3H, NHCO*CH*$_3$), 1.88 (dd, *J* = 11.6 Hz, 1H, H-3a)

^{13}C- NMR (400 MHz, MeOH-*d*$_4$): δ 171.88 (C-1 + NH*C*OCH$_3$), 73.59 (C-9), 71.79 (C-6), 70.00 (C-7), 69.50 (C-8), 67.68 (C-4), 54.05 (COO*CH*$_3$ or H-5), 53.35 (COO*CH*$_3$ or H-5), 40.71(Ms*CH*$_3$), 37.16 (C-3), 22.79 (NHCO*CH*$_3$), C-2 is not possible to see on ^{13}C NMR[3].

HRMS (ESI): *m/z* [M + H] $^+$ calcd for $C_{13}H_{23}NO_{11}S$: 402.1070; found: 402.1068.

$[\alpha]_D^{20}$ – 7 (*c* 0.5, MeOH)

Methyl 5-acetamido-2,4,7,8-tetra-*O*-acetyl-9-*O*-tosyl-3,5-dideoxy-D-glycero-D-galactononulopyranosylonate (10)

Compound **3** (83.9 mg, 0.176 mmol) was dissolved in pyridine (1 mL) and Ac_2O (0.5 mL) was added. The reaction mixture was stirred at r.t. for 24 h and was then concentrated and purified by column chromatography (Heptane: ethyl acetate 1:2) to gain compound **10** (75.0 mg, 0.116 mmol, 66%).

^{1}H- NMR (400 MHz, chloroform-*d*): δ 7.79-7.77 (m, 2H, Ts-Ho), 7.34-7.32 (m, 2H, Ts-Hm), 5.38 (dd, *J* = 2 Hz, *J* = 4.8 Hz, 1H, H-9), 5.32-5.21 (m, 2H, H-9´, H-4), 5.04-4.49 (m, 1H, H-8), 4.50 (dd, *J* = 2.8 Hz, *J* = 11.2 Hz, 1H, H-7), 4.13-4.04 (m, 2H, H-5, H-6), 3.76 (s, 3H, COO*CH*$_3$), 2.53 (dd, *J* = 5.0 Hz, *J* = 13.4 Hz, 1H, H-3e), 2.44 (s, 3H, Ts*CH*$_3$), 2.14-1.88 (m, 16H, H-3a, 4 x OAc, NHCO*CH*$_3$)

^{13}C- NMR (400 MHz, chloroform-*d*): δ 171.06, 170.50, 170.33, 170.20, 168.35, 166.39 (6 carbonyl carbons), 144.88, 133.17, 129.93 (Ts-Cm), 128.12 (Ts-Cp), 97.49, 72.90, 70.99 (C-8), 68.26, 67.84, 67.55, 53.32 (COO*CH*$_3$), 36.08 (C-3), 23.36 (NHCO*CH*$_3$), 21.79 (Ts-CH_3), 20.98, 20.91, 20.89, 20.85 (4 x AcO-CH_3), might not be possible to see C-2.

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{27}H_{35}NO_{15}S$: 646.18066, found: 646.1799, $[M+Na]^+$ calcd for $C_{27}H_{35}NO_{15}S$: 668.1625, found: 668.1619

Methyl 5-acetamido-2,4,7,8-tetra-*O*-acetyl-9-azido-3,5,9-trideoxy-D-glycero-D-galactononulopyranosonate (11)

Compound **10** (64.5 mg, 0.100 mmol) was dissolved in dry DMF (1 mL) and NaN_3 (25.9 mg, 0.398 mmol) was added. The reaction mixture was stirred first at r.t, then after 1.5 h at 30℃, and after additional 2 h the temperature was changed again to 40℃, and 2 h later to 50℃. 30 minutes later more NaN_3 (13.1 mg, 0.202 mmol, 2 eq.) was added and the reaction was then left over night. The morning after the reaction was allowed to reach r.t. and was concentrated. It was hard to follow the reaction by TLC so the concentrate was then analyzed by mass spectrometry and showed that the product was formed, but some starting material was left. The residue was then purified by column chromatography (Heptane: ethyl acetate 50:50-0:100) and resulted in compound **11** (24.8 mg, 0.048 mmol, 48%), confirmed by mass spectrometry.

^{1}H- NMR (400 MHz, chloroform-*d*): δ 5.53 (d, J = 9.6 Hz, 1H), 5.38 (dd, J = 2.4 Hz, J = 3.6 Hz, 1H), 5.26-5.19 (m, 1H, H-4), 4.89-4.86 (m, 1H), 4.19-4.02 (m, 2H), 3.82-3.74 (m, 3.6H, COOCH_3 + impurities), 3.36 (dd, J = 8 Hz, J = 13.6 Hz, 1H), 2.50 (dd, J = 5.2 Hz, 13.6 Hz, 1H, H-3e), 2.14-1.87 (m, 16H, H-3a, 4 x OAc, NHCOCH_3)

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