Universität für Bodenkultur Wien

Department of Chemistry
Institute of Organic Chemistry

**Master thesis**

# Novel glycosyl donors of Kdo

**Target Degree:**
**Master of Science**

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## Contents

**Abbreviations**

| | |
|---|---|
| (HR)MS | (high resonance) mass spectroscopy |
| $Ac_2O$ | Acetic anhydride |
| $BF_3$ | Boron trifluoride etherate |
| COSY | Correlated Spectroscopy |
| CPS | Capsular polysaccharide |
| CSA | Camphorsulfonic acid |
| DABCO | 1,4-diazabicyclo[2.2.2]octane |
| DBTO | Dibutyl tin oxide |
| DCM | Dichloromethane |
| DMAP | 4-Dimethylaminopyridine |
| DMF | Dimethylformamide |
| $Et_3N$ | Triethylamine |
| EtOAc | Ethylacetate |
| HCl | Hydrochloric acid |
| HPTLC | High-performance thin-layer chromatography |
| HSQC | Heteronuclear Single Quantum Coherence |
| Kdo | 3-deoxy-D-manno-oct-2-ulosonic acid |
| LPS | Lipopolysaccharide |
| MeOH | Methanol |
| $MgSO_4$ | Magnesium sulfate |
| $Na_2SO_4$ | Sodium sulfate |
| $NaHCO_3$ | Sodium hydrogen carbonate |
| NaOH | Sodium hydroxide |
| NaOMe | Sodium methoxide |
| NIS | *N*-Iodosuccinimide |
| NMR | Nuclear magnetic resonance |
| OAc | O-acetyl group |
| OMBn | O-methoxybenzyl group |
| OMe | O-methyl group |
| $P_2O_5$ | Phosphorpentoxide |
| PhSH / OSPh | Thiophenol / O-thiophenyl group |
| PTSA | *p*-Toluenesulfonic acid |
| RT | Room temperature |
| TBAB | *tetra*-N-butylammonium bromide |
| tBDMSCl | tert-Butyl(chloro)dimethylsilane, |
| tBDPSCl | tert-Butyl(chloro)diphenylsilane, |
| TfOH | Trifluoromethanesulfonic acid |
| $TiBr_4$ | Titanium tetrabromide |
| TIPSCl | Triisopropylsilyl chloride |
| TLC | Thin layer chromatography |
| TMS | Tetramethylsilane |
| TMSOTf | Trifluoromethanesulfonic acid trimethylsilylester |
| Ytterbium-Triflate | Ytterbium - trifluoromethanesulfonic acid |

**Abstract**

Die Octulosonsäure Kdo ist ein zentraler Baustein der äußeren Zellmembran der Gram-negativen Bakterien. Im Rahmen der Masterarbeit wurde die Eignung neuer Derivate von Kdo zur Synthese von bakteriellen Kohlenhydratstrukturen untersucht. Im speziellen wurden Thioglycoside von Kdo hergestellt, die eine neuartige Silylbrücke im Molekül aufweisen. Dabei wurde eine mehrstufige Synthese entwickelt, die über Silylierungen und Alkylierung von Zinnacetalen zu neuen Kdo-Donatoren führte. In zwei Modellreaktionen zur Disaccharidbildung mit einem Glukosaminakzeptor konnte jedoch lediglich die Eliminierung des Kdo Donors beobachtet werden. Damit wurde die publizierten Ergebnisse ähnlicher Thiodonatoren bestätigt. Darüber hinaus kann das neu entwickelte Schutzgruppenmuster aber auch zum Aufbau verzweigter Kdo-Strukturen genutzt werden, die beispielweise als Kohlenhydratantigen in der Zellwand von *Chlamydien* vorkommen.

The octulosonic acid Kdo is a central component of the outer membrane of Gram-negative bacteria. In the context of this master thesis the suitability of novel derivatives of Kdo for the synthesis of bacterial carbohydrate structures was studied. In particular the formation of thioglycosides of Kdo which possess a new form of silyl clip was performed. Therefore a multi-level synthesis was developed *via* silylation reactions and alkylation of tin acetals which led to novel forms of Kdo donors. Applying the Kdo donor in two model reactions for the disaccharide formation using a glucosamine acceptor, however, gave only elimination products of the Kdo donor. Thus, the published results of similar thio-donors were confirmed. Nevertheless, the new protecting group scheme allows for assembly of branched Kdo structures, such those occurring as carbohydrate antigens in the cell wall of *Chlamydia*.

**Acknowledgments**

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# 1 Introduction

## 1.1 General

During the whole history of evolution, carbohydrates always played a very important role as basic structures for a great variety of organisms to enable evolutionary advantages for survival of the fittest.

One of their main biochemical functions is founded in recognition of biomolecules as well as signal transduction in organisms. Especially some microorganisms use different kinds of sugars and complex carbohydrate which are absent in vertebrates. As a consequence, these structures will be recognized as antigens for innate and adaptive immune system of higher organisms.

**Bacterial cell wall of gram-negative bacteria**

Bacteria can be classified by form and Gram stain as gram-negative and gram-positive cells. Gram-positive cells are well known to own a thick layer made up of peptidoglycan which is responsible for retention of the colour during gram staining experiment. Peptidoglycan is a heteroglycan containing long parallel polysaccharide-chains. N-Acetylglucosamine and N-Acetylmuraminic acid alternate and are connected by a β-1,4 glycosidic linkage. Polysaccharide chains are crosslinked by peptide bridges. Two different peptides (pentapeptide [$Gly_5$] and tetrapeptide) linked to each other are responsible for stability of this layer.

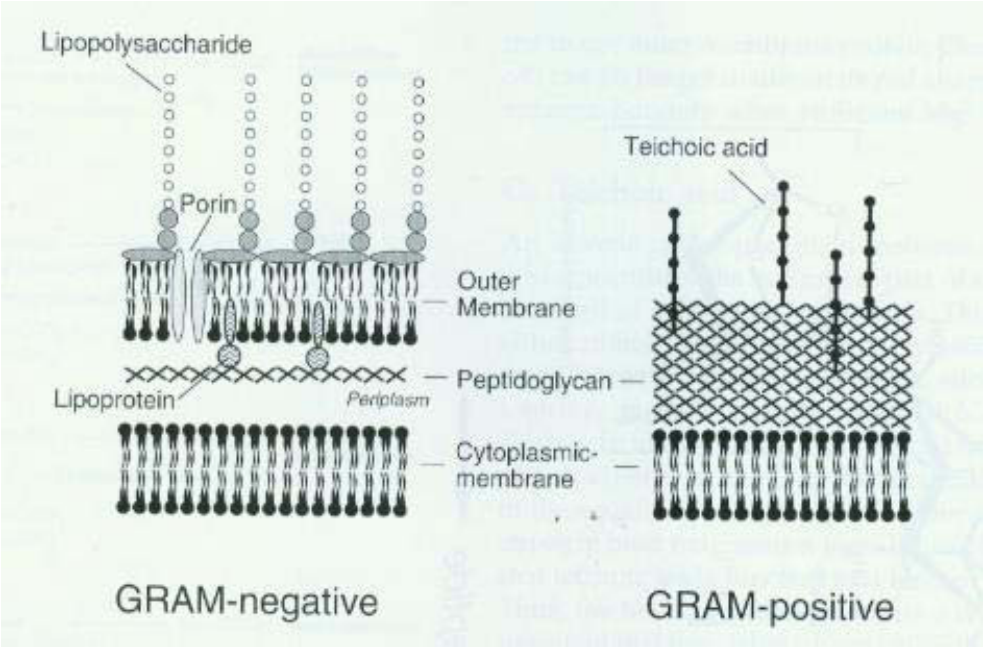



Figure 1: bacteria cell wall of gram-negative/gram-positive bacteria [1]

The bacterial cell wall of gram-negative species has two membranes which are structurally distinguishable. The inner membrane is made up of a phospholipid double layer which mainly forms an anchor for many transmembrane proteins and serves as physical barrier between inner cell and periplasmatic space between inner and outer membrane. The periplasmatic space seems to be a very important part of the cell in order to form and store inclusion bodies as it is known from *Escherichia coli*. Compared to gram-positive bacteria gram-negative bacteria only have a thin peptidoglycan layer. As part of the outer membrane it is located next to the periplasmatic space which is followed by another phospholipid layer. Outermost part of the outer membrane is one of the main characteristics of gram-negative bacteria which is also responsible for many pathogenic properties: lipopolysaccharide. Made up of different sugars it is scattered over the whole surface. For this reason and because of the thin layer of peptidoglycan no colour can be retained. Gram-negative bacteria remain colourless at gram-staining experiment [1,2].

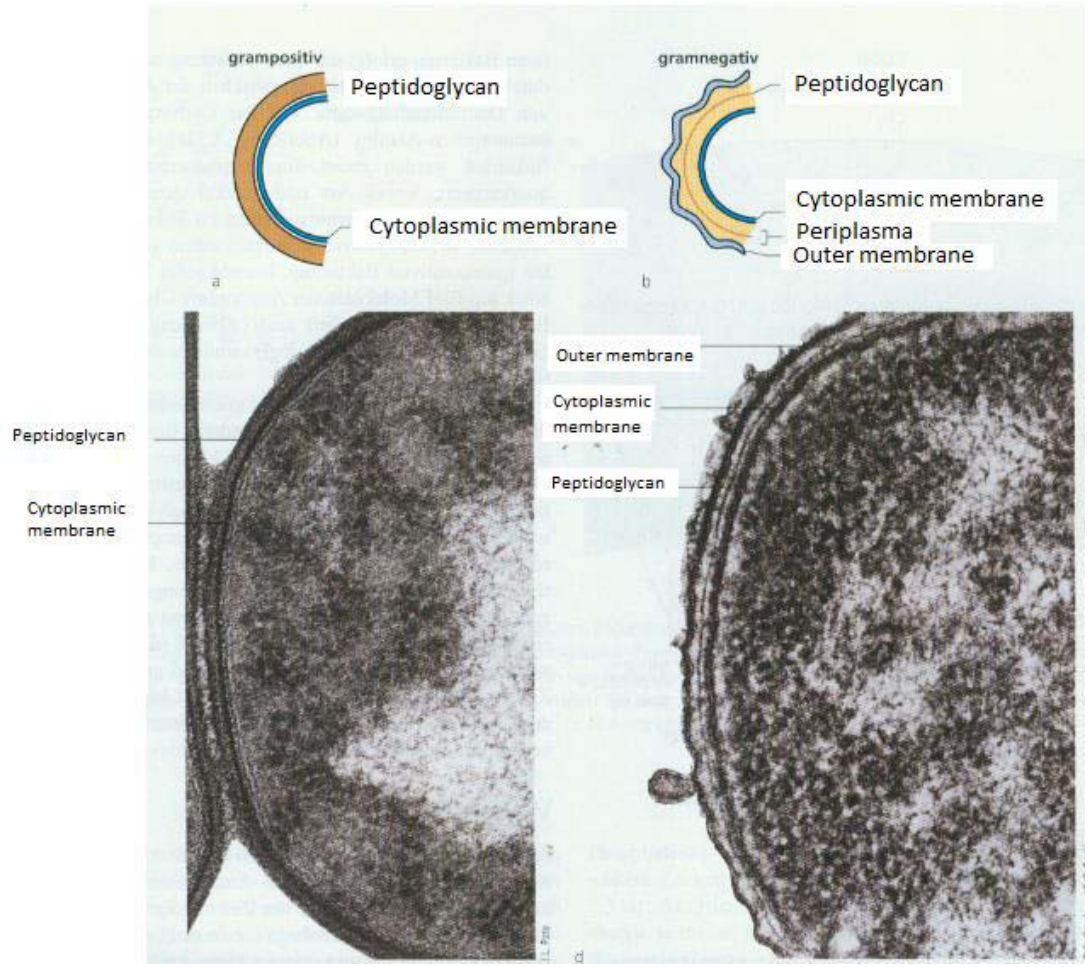



**Figure 2: Schemes of grampositive/gramnegative bacterial cell wall, electron microscopic picture of grampositive/gramnegative cell wall, adapted from [2]**

## LPS

Lipopolysaccharide (LPS) is an amphipatic structurally complex microheterogeneous glycolipid and part of the outer membrane of gram-negative bacteria. It accounts for 10% of the dry weight of gram-negative bacteria cells. This tripartite structure is made up of:

- Lipid A: A biphosphorylated and acylated β(1→6) linked glucosamine disaccharide. The backbone contains ester linked fatty acids as well as amide linked fatty acids.
- Core
    - The inner core of LPS consists of 2 linked monosaccharides of 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo).
    - The outer core consists of defined structure out of e.g. heptoses, galactose, glucose and N-acetylglucosamine which are linked to the Kdo-region
- O-specific polysaccharide-chain: This part of the LPS shows a great variety in structure and length. It consists of mainly 4-5 sugars including manifold branches, which are repeated. Number of repeats differs from kind of bacteria [3,4].

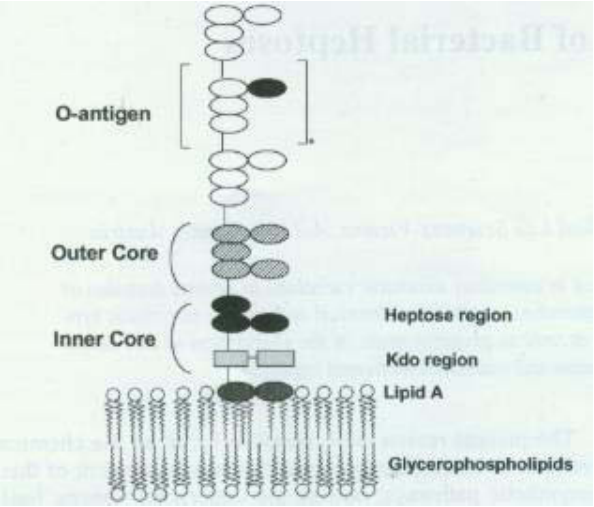


Figure 3: common scheme of lipopolysaccharide [3]

Kdo, O-Antigen and the phosphate moieties of Lipid A are responsible for the hydrophilic character of the LPS. Negative charges of the LPS are compensated by incorporating $Ca^{2+}$ and $Mg^{2+}$ in the outer membrane of bacteria in order to stabilize the structure. As a consequence there is a natural resistance against lipophilic compounds as for instance mammalian bile acid and many kinds of antibiotics.

## 3-Deoxy-D-*manno*-oct-2-ulosonic acid - Kdo

The monosaccharide 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) is one of the key molecules for the core region of enterobacterial LPS as well as an important part of several capsular polysaccharides (CPS). In LPS Kdo occurs as α-anomer, in CPS both forms (α-/β-anomer) are present. It is known to influence inflammatory activity of lipid A [5]. Kdo can be synthesized by many different chemical and chemoenzymatic ways. The most popular synthesis strategies are summarized by Kosma and Zamyatina [6]. There are mainly two different organic-chemical ways for the synthesis. Firstly [6+2] C atom incorporation with starting material *D*-mannose is one option. Second one would be a [5+3] elongation strategy

starting with α-oxocarboxylic acid moiety. The Conforth reaction should be mentioned which is remarkable because of its simplicity and inexpensiveness. High amounts of ammonium salt of Kdo can be prepared. Because of the acid-labile properties of Kdo, acidic reactions are done under mild conditions. However there is no generally accepted high yield procedure in order to obtain Kdo stereoselectively. Chemoenzymatic syntheses use Aldolases and Synthases as catalysts for aldol condensation of pyruvate or phosphoenolpyruvate onto D-arabinose 5-phosphate. As an important part of LPS, Kdo is involved in eliciting antigenic properties. It serves as diagnostic marker and antigen for future development of antibodies and other vaccines. Kdo also serves as a substrate for different enzymes for elongation of the macromolecule.

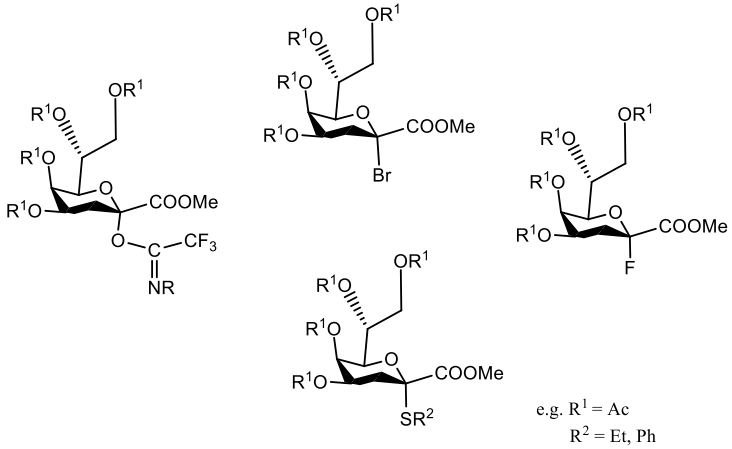
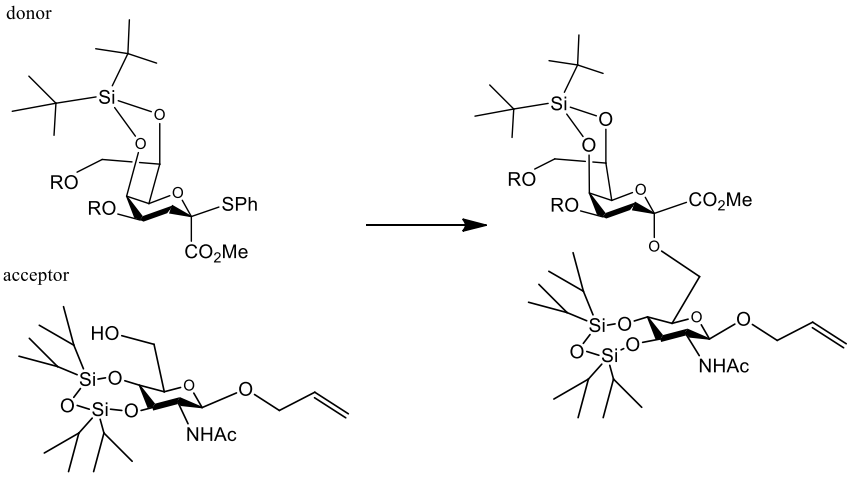



**Figure 4: examples of Kdo donors**

The chemistry of Kdo plays an important role for the development of glycosyl donors. During the past years most Kdo donors in use are halide derivatives of Kdo as for instance bromide, fluoride or chloride Kdo because of their high stability, and some advantage for glycosylation in contrast to other ones (figure 4). Also development of trihaloacetimidate Kdo donors is very important. A. Shimoyama and his working group [5] described the usage of N-Phenyltrifluoroacetimidate Kdo donors for α-selective glycosylation. It does not need large excess of Lewis-acids which often cause cleavage of other protecting groups and lead to decreased stereoselectivity. On the other hand yield and resulting stereoselectivity are acceptable but should be improved. As an alternative, the use of methyl 2-thio Kdo as donor for iodinium ion –promoted glycosylations were explored by working group of Boons [7] and Oscarson [8]. Usage of thiophilic promotor system NIS/TfOH resulted in better knowledge about advantages of thio-Kdo donors, such as being stable and readily accessible. The main problems of these donors are the additional formation of unsaturated ester through elimination reaction, low reactivity through glycosylation reaction and low stereoselectivity because of the 3-deoxy function.

## 1.2 Definition of work



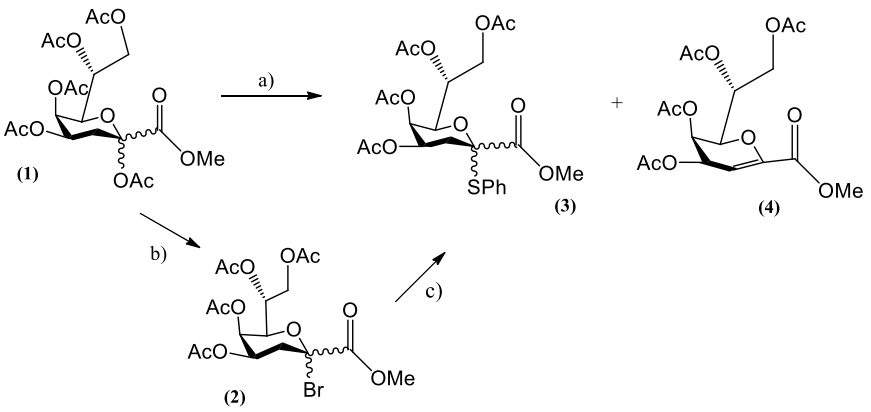

**Scheme 1: Planned glycosylation reaction. Target thioglycoside donor and selected N-acetylglucosamine-acceptor should be coupled to form disaccharide**

This work aims to develop new forms of Kdo donors using thioglycosides as a basis for glycosylation experiments.

Main objectives of this work:

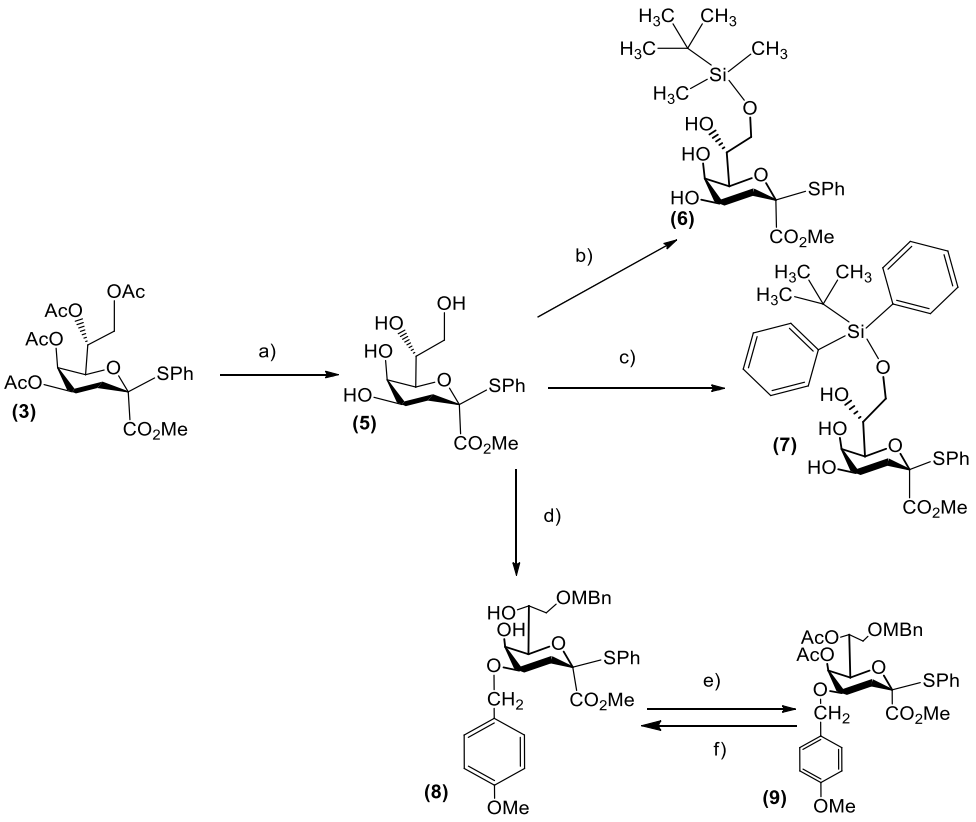
1) The 5,7-*O*-silylene group should fix the C5/C7 conformation, but also allow the modification of the 4,8-positions, which is a frequently present substitution pattern of the Kdo region. In addition, introduction of electron-donating protecting groups at these positions should also increase the reactivity of the Kdo donor.
2) Could thioglycoside of Kdo be new attractive donor for glycosyl acceptors?
3) Would it be possible to raise selectivity?
4) Study about formation of elimination product
5) Improvement of yield
6) Optimization of reaction conditions

# 2 Synthesis schemes




**Scheme 2: Reagents and conditions: a) Thiophenol, $BF_3.Et_2O$, DCM; b) DCM, $TiBr_4$; c) DCM, Thiophenol, $Et_3N$**

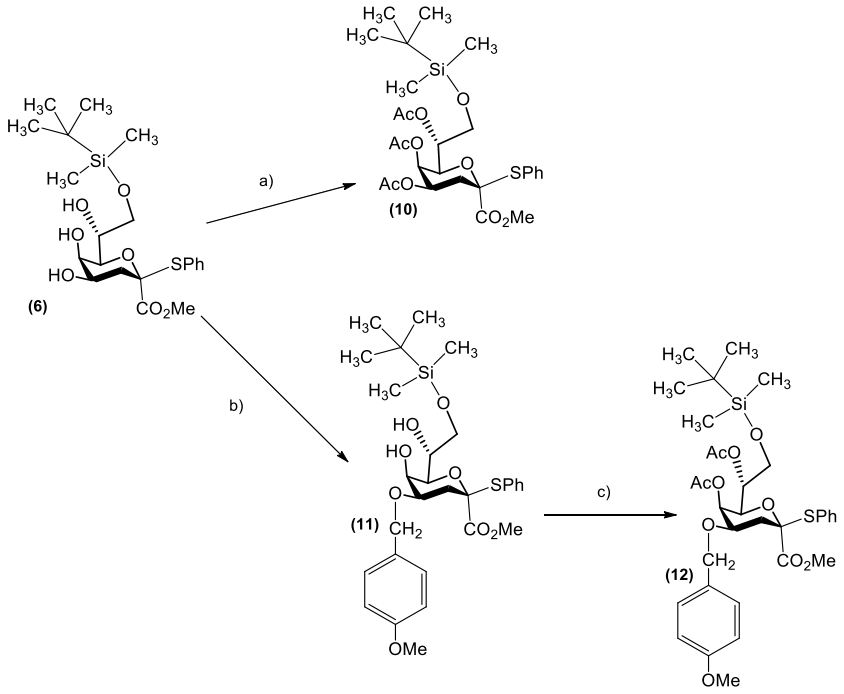
Synthesis of a Kdo thioglycoside was the first aim of the synthesis (scheme 2). Two ways were compared to each other. First a direct, one step conversion of the peracetate **(1)** should be assessed. As second attempt, glycosidation of bromide ***(2)*** should be explored.




**Scheme 3: Reagents and conditions: a) NaOMe, MeOH; b) TBDMSCl, DABCO, acetonitrile; c) TBDPSCl, DABCO, acetonitrile; d) 1. DBTO, toluene; 2. TBAB, p-methoxybenzyl chloride, MeOH; e) DMAP, $Ac_2O$, pyridine; f) NaOMe, MeOH**

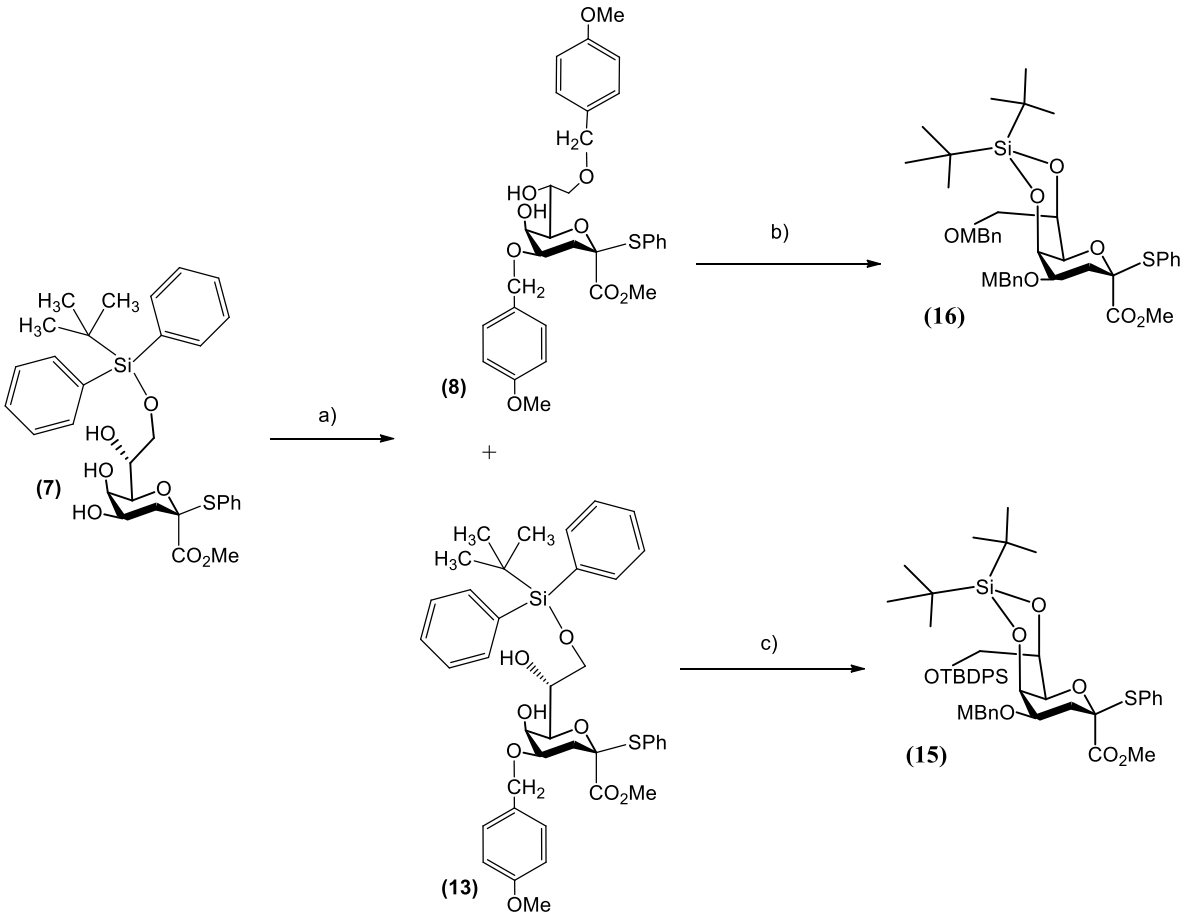
Acetylgroups of the 4th, 5th, 7th, and 8th position were to be removed in order to introduce selective protective groups. Because of its higher reactivity, the 8th position was protected

first. Therefore three different protective groups (TBDMS-, TBDPS-, methoxybenzyl-) should be compared to each other (scheme 3,4).



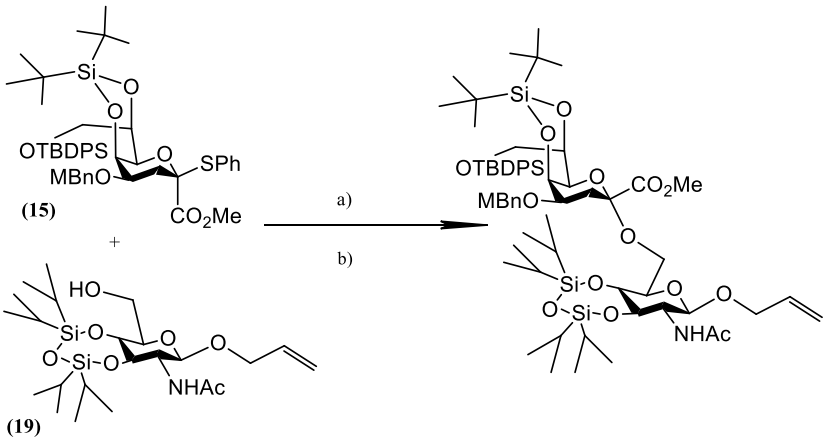

**Scheme 4: Reagents and conditions: a) DMAP, $Ac_2O$, pyridine; b) 1.DBTO, toluene; 2. TBAB, p-methoxybenzyl chloride, dry MeOH; c) DMAP, $Ac_2O$, pyridine**

In order to install the 5,7-silylene bridge, selective protection of the primary hydroxyl group at C-8 should be accomplished. The 8-*O*-substituted derivatives should then be submitted to regioselective alkylation at O-4 *via* intermediate formation of stannylidene acetals. [9] In addition, a direct bisalkylation of the precursor ***(5)*** should be studied (scheme 5).



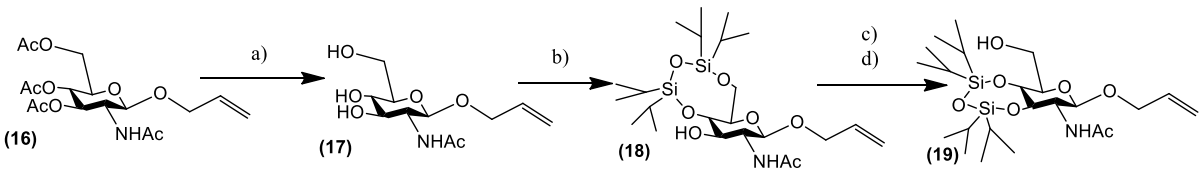

**Scheme 5: Reagents and conditions: a) 1.DBTO, toluene; 2.TBAB, p-methoxybenzyl chloride, dry MeOH; b) di-tert-butylsilyl bis(trifluoromethanesulphonate), dry pyridine,**

After successful protection of positions 4,5,7,8 the new thioglycoside donors should be reacted with model acceptor in glycosylation reactions (scheme 6).




**Scheme 6: Reagents and conditions: a) TfOH, NIS, dry $CH_2Cl_2$; b) Ytterbium-Triflate, NIS, dry $CH_2Cl_2$**

Therefore acceptor N-Acetylglucosamine was synthesized (scheme 7).




**Scheme 7: Reagents and conditions: a) NaOMe, MeOH; b) TIPSCl, dry pyridine, c) PTSA, dry DMF, d) CSA, dry DMF**

# 3 Results and discussion

## 3.1 Synthesis of the GlcNAc acceptor

**Allyl 2-acetamido-2-deoxy-*β*-*D*-glucopyranoside (17)**

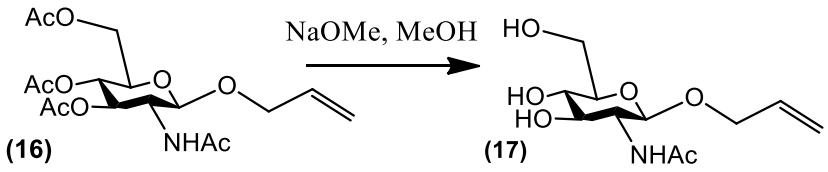



Figure 5 Transesterification of N-glucosamine acceptor

In this work N-glucosamine acceptor was chosen as model for glycoside acceptor derivatives corresponding to a part structure of the lipid A backbone. First the acetylated N-glucosamine was deacetylated by transesterification (Zemplén-de-O-acetylation). Position 3, 4 and 6 are unprotected in order to enable introduction of a selective protective group on position 3 and 4. High yields of product were obtained (~99%).

**Allyl 2-acetamido-2-deoxy-4,6-*O*-(1,1,3,3-*tetra*isopropyldisiloxane-1,3-diyl)-*β*-*D*-glucopyranoside (18)**

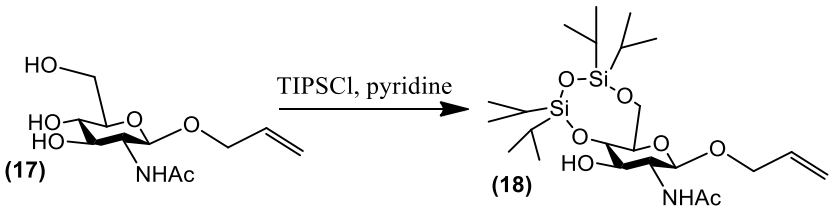



Figure 6: Introduction of 4,6-O-TIPS group on N-glucosamine acceptor

The TIPDSi-group is well known as a protective group with high stability and compatibility and being unaffected by tertiary amines and pyridine [17]. Therefore it was chosen as to form a O-silyl-1,3-diyl group. Because of the higher reactivity of the 6 OH-group subsequent conversion of the 4,6-O-silyl-diyl group to a 3,4-protected one had to be performed.

**Allyl 2-acetamido-2-deoxy-3,4-*O*-(1,1,3,3-*tetra*isopropyldisiloxane-1,3-diyl)-*β*-*D*-glucopyranoside (19)**

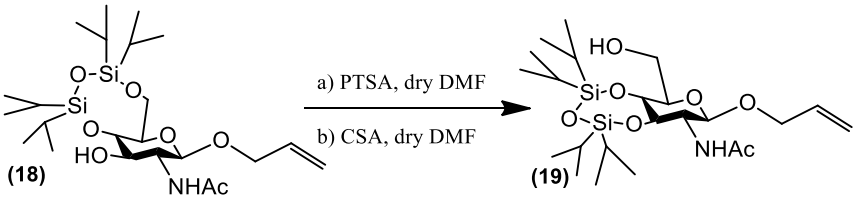



Figure 7: Conversion of 4,6-O-TIPS group to 3,4-O-TIPS group

Migration of TIPDSi-group from O-6 was induced by a strong acid. Protective group attacks O-3 as next favourable reactive position and consequently O-6 is free for glycosylation. At first try with *p*-Toluenesulfonic acid (PTSA) as catalyst did not work well, although of previous experiments confirmed success of the acid. Second approach using camphorsulfonic acid (CSA) resulted in yield up to 83% after purification via silica column chromatography. Product is invisible on TLC under UV-light.

## 3.2 Synthesis of Kdo thioglycosides

***Methyl* (phenyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-2-thio-*D*-*manno*-oct-2-ulopyranosid)*onate* (3)**

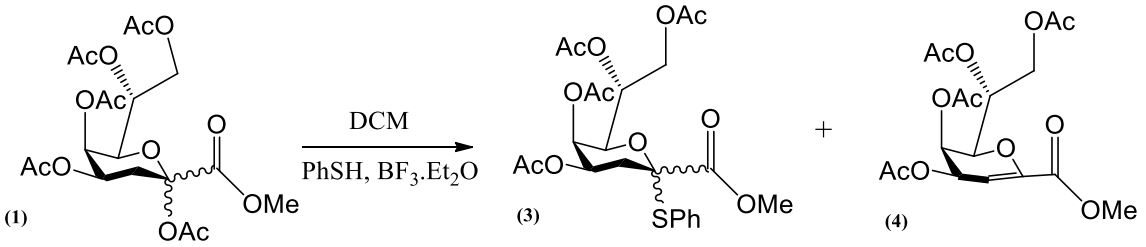



Figure 8: Reaction of acetylated Kdo-methylester to phenyl thioglycoside

Boron trifluoride etherate functions as trigonal planar Lewis acid since it does not possess a complete electron octet. Other Lewis-acids ($SnCl_4$) could also be used, but could also give the anomeric halide [8,10].

Figure 8 shows the expected course of the reaction. As illustrated both anomeric forms are possible [9].

Furthermore formation of the α-β-unsaturated ester product **(4)** is to be expected. In this case second position undergoes an elimination reaction with removal of a proton from C-3 and formation of a double bond between position 2 and 3 **(4)** (fig.8) [10]

The main advantage of this reaction is the possibility to get the desired product in a simple one-step reaction mechanism. Yield of 33.7% for both anomers **(3)** seems to be very low as a consequence of formation of elimination product (18.2%) **(4)**. On the other hand reaction showed a good conversion of educt **(1)** to products.

On TLC both anomeric forms as well as intermediate product were formed right from the beginning. Formation of intermediate product seemed to be slow and did not change its formation rate during reaction time. The reaction was stopped when educt spot was not observable on TLC. All reaction products which contain thiophenol-group are detectable under UV-light.

First proton spectrum was pure enough to detect and observe both α-/β-anomers. Some signals fall together which could not be fully characterized. The ratio of the anomers seemed to be 50:50. As it is known from empirical studies H-4 of acetylated Kdo glycosides in α-anomers are shifted downfield compared to the β-anomer. Based on these knowledge differentiation and classification between the two anomers is possible. Assignment of the H-4 protons was supported by COSY NMR spectrum. As expected typical singlet peak of the methyl group was found at 3.6 ppm. Aromatic groups of thiophenol are found between 7.57-7.28 ppm. Acetylgroups are located at high field. Typical peaks of solvent EtOAc are detectable. In comparison to spectrum of synthesis β-product **(3)** showed a shift to the high field. One of the main reasons could be the presence of boron trifluoride etherate which is a reason for other signals to be shifted.

$^{13}$C-NMR and HSQC spectra confirmed the analysis of proton spectra. $CH_2$ groups of C-3 and C-8 were detected. As expected methyl groups of the two anomers were found around 52 ppm.

The reaction was also monitored by In-Situ NMR. Therefore it was performed in a NMR tube, using deuterated DCM as solvent. After adding $BF_3.Et_2O$ the reaction was monitored after 10, 13 and 16 min. Experiment showed a constant increase of the determined signals of the products. On the other hand decrease of typical signals of the educt was observed (figure 9).

There were no observable changes in the formation of the two anomers. Formation of elimination product **(4)** did not seem to influence the main reaction products over time.

As a consequence of this experiment formation of several reaction products are not dependent on a certain time of the reaction. All reaction products were formed from the beginning as it was also observed on TLC.

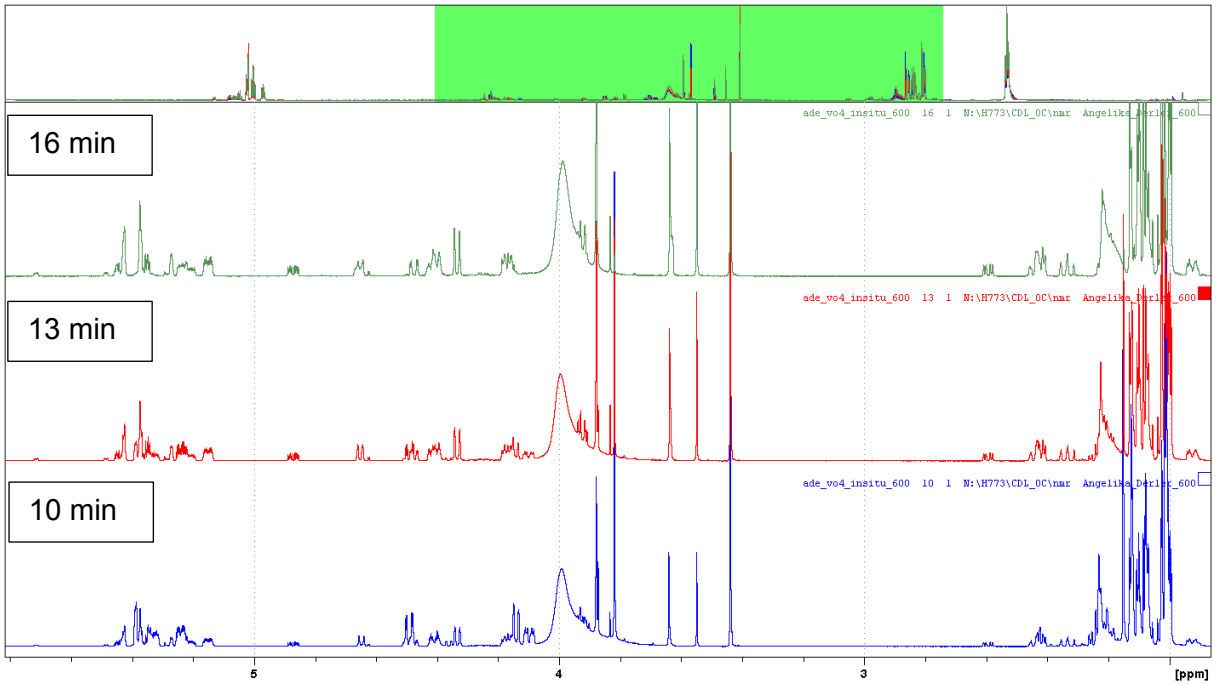



Figure 9: In-situ NMR of reaction

***Methyl* (phenyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-2-thio-*β*-*D*-*manno*-oct-2-ulopyranosid)*onate via Methyl* (phenyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-*α*-*D*-*manno*-oct-2 ulopyranosyl bromide)*onate***

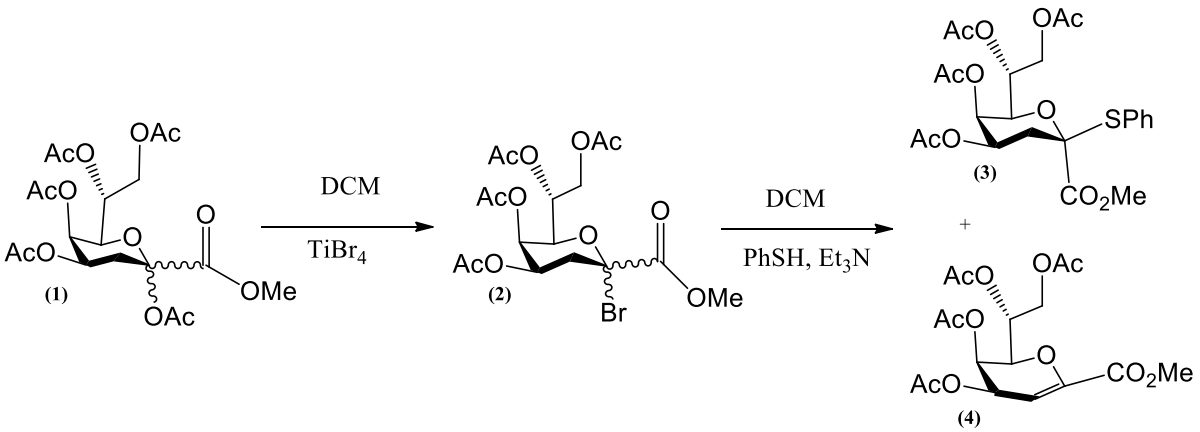



Figure 10: Reaction of acetylated Kdo-methyl ester to thioglycoside via 2-step-reaction

In the first reaction step $TiBr_4$ acts as a catalyst and reactant as well. Other possible catalysts for synthesis of thioglycosides could be TMSOTf, tin (IV)chloride, iron(III)chloride or p-toluenesulfonic acid [20]. Because of its function as Lewis-acid $TiBr_4$ eliminates the 2-O-acetylgroup to form the bromide **(2)**.

The second reaction with thiophenol works in the same way as it was described before [9]. Triethylamine is a strong Lewis-base and activates thiophenol group by deprotonation. Fig.10 shows both steps of reaction. The final desired product was obtained as β-anomer. Elimination product **(4)** also occurs as a by-product of this reaction mechanism.

In comparison to the direct synthesis, the reaction did not give a total conversion of educt. In spite of the disadvantage of its two-step reaction system, yield increased up to 58%. Formation of pure β-anomer is a very important advantage. For this reason and because of the higher yield, the two-step synthesis was chosen for further experiments.

Contrary to expectations the β-anomer has a very high Rf-value.

The proton spectrum was measured at 300 MHz NMR. The following signals confirmed the β–anomeric configuration and no impurities were detectable. Aromatic and acetyl groups remained on same position. Comparison to the spectrum of the first experiment proves that the product is the pure β-anomer. There is a strong coupling between H4 and both deoxy protons. Coupling constants of H5 and H4 also show a small coupling. There is also a strong coupling between H7 and both protons of position 8 as well as H6.

***Methyl* (phenyl 3-deoxy-2-thio-*β*-*D*-*manno*-oct-*2*-ulopyranosid)*onate* (5)**

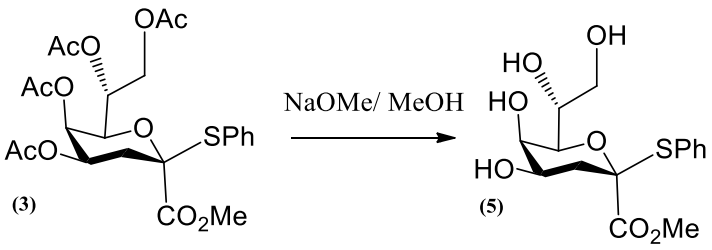



**Figure 11: Zemplén Reaction of acetylated thioglycoside to (5); NaOMe: Sodium methoxide, MeOH: Methanol**

Zemplén-de-O-acetylation reaction is one of the most popular reaction mechanisms in carbohydrate chemistry in order to remove acyl groups by transesterification. Deacetylation reaction starts under alkaline conditions in dry methanol. The O-acetyl-protected carbohydrates are transformed into unprotected ones by transsaponification.

Fig.11 shows reaction mechanism. Zemplén reaction achieves high yield up to 99% of desired product and the product does not normally need to be purified from any side products.

After removal of acetyl groups, free form of Kdo does not migrate on TLC by using standard eluent toluene:ethylacetate 1:1. As a consequence differentiation between educt which has a high Rf-value and product is very simple. Intermediate products from last reaction with lower polarity might also be separated easier after using gradient chromatography. Spots are visible under ultraviolet light.

## 3.3 Synthesis of silyl-protected Kdo-derivatives

***Methyl* (phenyl 8-*O*-*tert*butyldimethylsilyl-3-deoxy-2-thio-*β*-*D*-manno-oct-2-ulopyranosid)*onate* (6)**

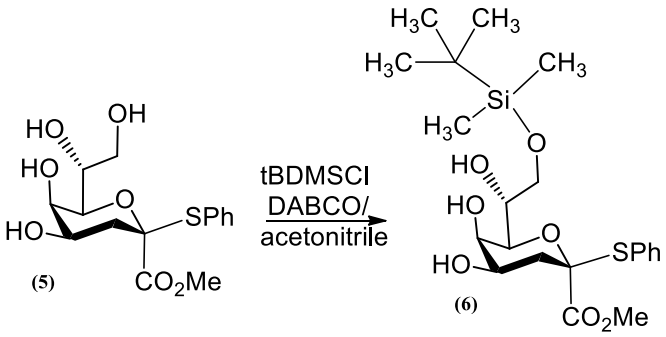



**Figure 12: Reaction of free thioglycoside to 8-*O*-silylated Kdo derivative (6)**

As expected, the 8-position should be the most reactive of the educt molecule. Silyl groups are very often used as protecting groups in organic chemistry. They are known to be very stable and not very reactive derivatives. With sterical hindrance at the silicium atom the stability of the whole protecting group increases. On the one hand stability can be a great advantage for specific synthesis but on the other hand removal of silyl groups could be very difficult. Silyl groups are the only protecting groups which can be cleaved selectively by fluoride ions [11].

In is silylation reaction chloride-Ion of the reagent reacts with the proton of the –OH group on 8 position to give HCl and introduce the silyl group (fig.12). 1,4-Diazabicyclo[2.2.2]octane (DABCO) acts as a very strong base which detaches the proton mainly of 8-OH thereby providing regioselectivity. For this reason it is very important to work under dry conditions.

In order to test acidic stability of the product on chromatography column, mini work-up of the probe was made. The reaction mixture and ethylacetate was mixed with a small amount of silica gel and tested on HPTLC plate. No change between mixtures with and without silica gel was detectable during a test over 1 day. As a consequence stability of **(6)** seems to be good for further experiments. Product yield of ~45% could be compared to results of working of Kosma [12]. Similar experimental conditions gave 70% yield of *Methyl* (allyl 8-*O*-*tert*-butyldimethylsilyl-3-deoxy-*α*-*D*-*manno* 2-octulopyranosid)*onate.* Non anhydrous experimental conditions, the formation of oversilylated and other intermediate products and regiochemical differences could be reasons for the lower yield. On HPTLC a continuous formation of higher and lower spots beneath product spot were detectable. Besides, the educt could not be fully

converted. In order to raise the yield of the 8–substituted similar experimental conditions with tBDPS-protective group were tested for product **(7)**.

$^{1}$H-NMR spectra protective group of **(6)** resulted in a high-field shift of signals of several protons. Especially H4 is strongly shifted upfield between signals of H8a and H8b. There is no change of signals of 3$^{rd}$ axial proton and the methyl group. In order to confirm the correct position of the protective group acetylation of free positions of Kdo was made.

Combustion analysis resulted in a good accordance with theoretical data and were confirmed by MS data which showed a small deviation form theory (Δ0.22 ppm).

***Methyl* (phenyl 4,5,7-tri-*O*-acetyl-8-*O*-tertbutyldimethylsilyl-3-deoxy-2-thio-*β*-*D*-*manno*-oct-2-ulopyranosid)*onate* (10)**

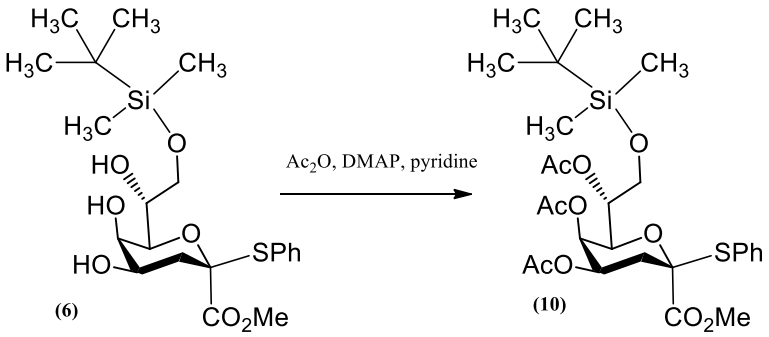



**Figure 13: Acetylation of position 4,5 and 7**

Acetylation of **(6)** should confirm the success of the previous reaction with tBDMS group and give **(10)** (fig.13). Therefore conventional reaction to protect free-OH groups with acetyl-groups using 4-*Di*methylaminopyridine (DMAP) as catalyst was chosen.

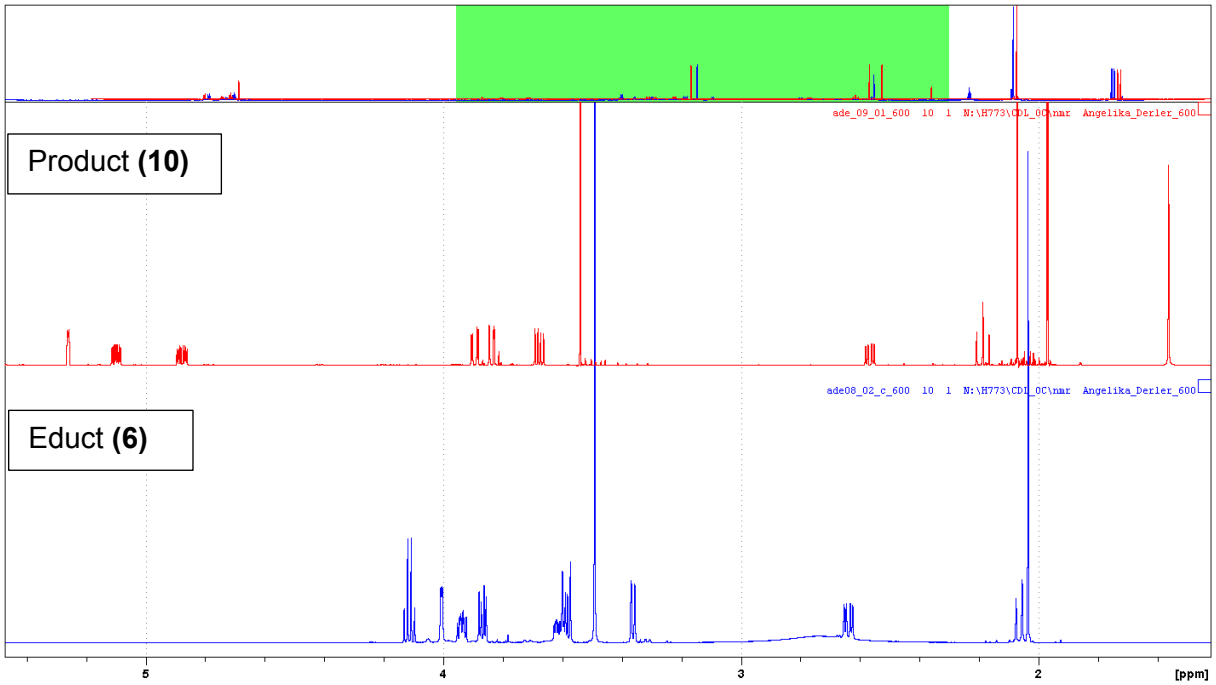



Figure 14: Comparison of NMR of educt (6) and product (10)

$^{1}$H-NMR spectra (fig.14) showed a strong downfield shift of H5, H7, and H4 (~Δ1.25 ppm). The shift of these 3 protons is an indication of successful acetylation whereas both signals of 8-protons nearly remained unchanged. H6 shifted downfield between proton 8a and 8b. Both signals of H3(eq.) and H3(ax.) remained on their position as in **(6)**.

As a conclusion it was proved that the tBDMS group is located at position 8.

***Methyl* (phenyl 8-*O*-*tert*butyldiphenylsilyl-3-deoxy-2-thio-*β*-*D*-*manno*-oct-2-ulopyranosid)*onate* (7)**

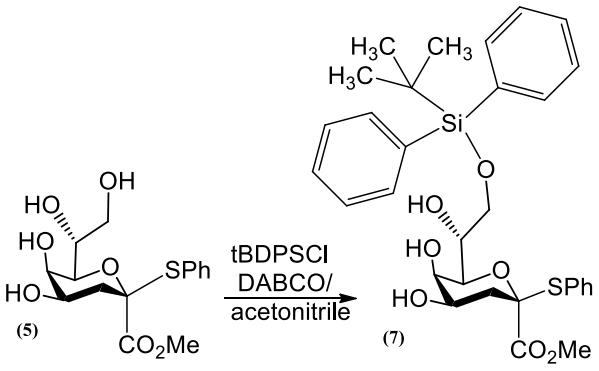



Figure 15: Silylation of 8th position with tBDPS-group

Because of its two phenyl groups, tBDPS has a higher sterical hindrance than the tBDMS group (fig.15). For substitution reaction this means an advantage for its properties as protective group. Weak interactions like Van-der-waals force between other atoms and groups of the molecule could reinforce linkage.

On the other side sterical hindrance and the size of the group could also be a problem by subsequent substitution reactions. Furthermore the higher binding affinity could be a problem for the deprotection. As it was shown for **(7)**, acidic stability of **(10)** was proved.

Fortunately, synthesis of **(10)** was successful. The tBDPS-product **(10)** was isolated in higher yield (70%) than tBDMS (45%) derivative **(7)**, comparable to the yield of Kosma [12]. Reaction times were very similar (scale-up batch for 67 h). Based on these results and its higher chemical stability, tBDPS was chosen as protective group for 8-position for further experiments.

Because of the increased polarity of **(7)** Rf-values are a little bit higher than **(6)** on TLC. Intermediate products which have more than one silyl group can be detected by their higher Rf-values.

In comparison to proton spectra of **(6)** the most interesting difference is the changing position of the H4 proton. In contrast to **(6)**, H4 is shifted upfield from both H8 protons.

Combustion analysis did not show a sufficient agreement of theoretical and practical percentage of carbon and sulphur. Mass spectrometry showed an increased deviation of $\Delta$0.61 ppm. Nevertheless the data provide agreement to theoretical calculation of exact mass.

## 3.4 Alkylation reactions

**M*ethyl* (phenyl 8-*O*-*tert*butyldimethylsilyl-3-deoxy-4-*O*-*p*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-2-ulopyranosid)*onate* (12)**

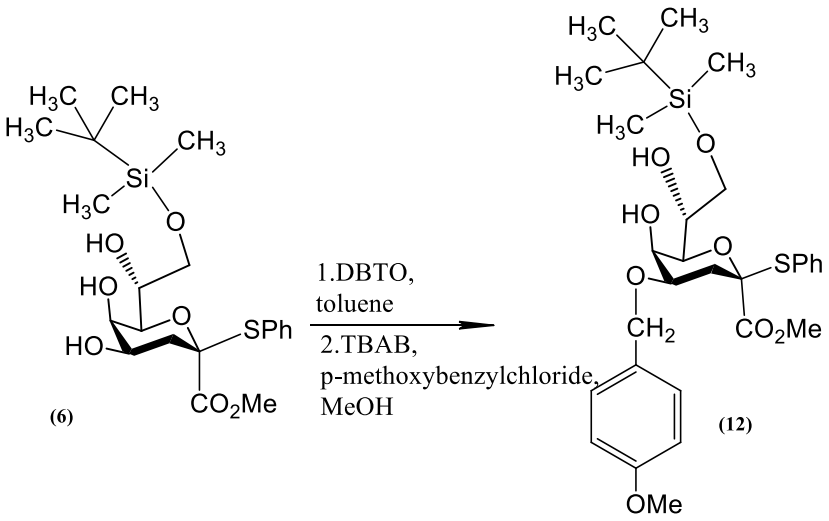



Figure 16: Protecting of the 4th position with methoxy benzyl group

Protection of position 4 of **(6)** was done by a two-step reaction mechanism (fig.16). First, reaction with dibutyl tin oxide and toluene was carried out with azeotropic removal of water using conventional Dean-Stark apparatus. As an intermediate product stannylene acetals were formed on 5th and 4th position in order to activate for the next step. Second reaction was catalysed by *tetra*butylammonium bromide using *p*-methoxybenzyl chloride as reagent. As described by Sekljic [14] this reaction could also result in formation of multiple methoxybenzylated products.

In this case TLC showed more than one spot. Because of the low yield of ~14%, the product was not chosen for any further experiments. Intermediate spots were not analysed. Based on later experiments it could be assumed that this intermediate was a dimethoxybenzylated form of thioglycoside.

Proton spectrum of product **(12)** was compared to spectrum of **(6)**. There was only a slight downfield shift of protons H5, H7 and H8a,b ($\Delta$ ~ 0.20 ppm). H4 had a stronger shift to high field.

***Methyl* (phenyl 8-*O*-*tert*butyldiphenylsilyl-3-deoxy-4-*p*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-*2*-ulopyranosid)*onate* (13) and *Methyl* (phenyl 3-deoxy-4,8–di-*O*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-*2*-ulopyranosid)*onate* (8)**

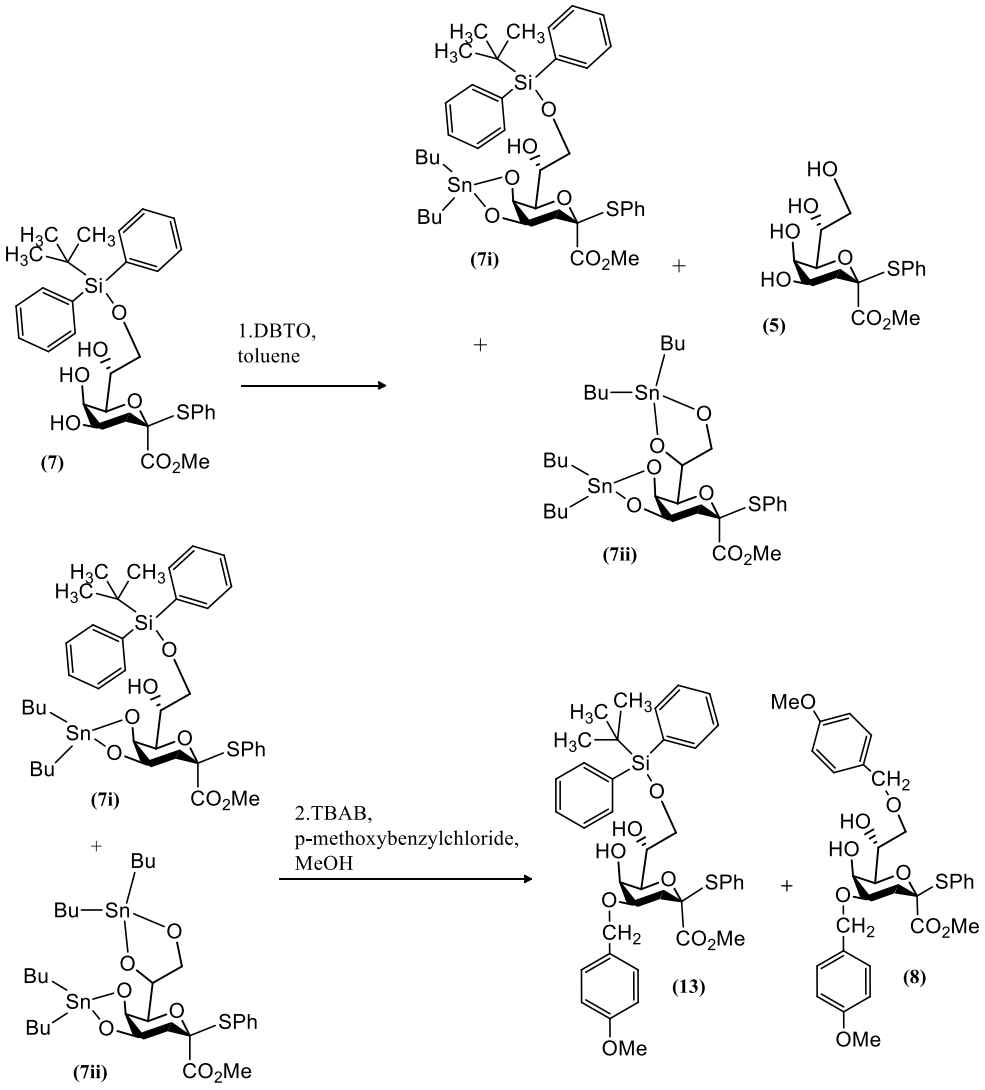



**Figure 17: Formation of stannylidene acetals and protection of the 4th (and 8th position) with methoxybenzyl-groups**

In comparison the synthesis of **(12)**, TLC showed two major product spots besides spots of reagents and educt which were constantly formed right from the start. The higher running spot could be separated into minor intermediate product spots and compound **(13)**. Lower running spot could be identified as product **(8)**.

Yield of both products and ratio varied with each batch. In comparison to results of Sekljic [14] (with product ratio around 65% and 9%) the resulted yield was very low. Product formation is dependent on activation of the right position via stannylene acetal in first reaction step.

As described by Grindley [9] regioselectivity of stannylene acetals are not given. For this reason and because of the addition of 2.7 equivalents of DBTO it is likely that more than one specific activated center was formed (fig.17). Various forms of activation and alkylation reactions are possible. As a consequence, tBDPS group on 8$^{th}$ position could dissociate and formation of a second activated center on 7$^{th}$ and 8$^{th}$ position is possible. Because of the double addition of reagent p-methoxybenzyl chloride, formation of dimethoxybenzylated product **(8)** can occur. On the other hand premature hydrolysis of activated group results in non-reactive glycoside **(5)**. Additionally, the reaction did not give full conversion of educt. Therefore the recovery of educt **(7)** was 14%.

In comparison to synthesis of product **(12)** there was a much better yield. Identification of both substances was made by NMR analysis. The higher running product on TLC was compound **(13)** with *tert*-butyldimethylsilyl-group on position 8 and methoxybenzylation on position 4. The lower running spot could be identified as the dimethoxybenzylated form of thioglycoside **(8)**. In this case the tBDPS group had been hydrolysed and methoxybenzyl chloride reacted with the –OH group of both position 4 and 8.

$^{1}$H-NMR spectrum of compound **(13)** was compared to educt **(7)**. As a proof of protection of the 4$^{th}$ position, H4 is highfield shifted from 3.60 ppm to 3.41 ppm. There are no further significant differences of spectra.

The purity check by combustion analysis showed deviation for experimental values for carbon and sulphur. Mass spectrometry gave a higher deviation of -0.76 ppm to the exact theoretical mass.

Proton spectra of compound **(8)** confirmed 4,8-*di*-*O*-methoxybenzylated product. Some residues of methyl group of tBDPS are detectable. In comparison to educt, in compound **(7)** there was a highfield shift of H8a from 3.97 to 3.73, H8b from 3.64 to 3.49 and H4 from 3.60 to 3.40. Furthermore the integral of the signal of the OMe group at 3.80 ppm increased to 6H.

Similar reaction was also tried with starting material **(5)** and resulted first in low yield (11%). There was no full conversion of educt. TLC showed formation of a spot with similar Rf-values as compound **(8)**. Because of the strong interaction of dibutyl tin oxide on NMR spectra, identity of product was checked by acetylation of compound **(8)** and resulted in compound **(9)**.

***Methyl* (phenyl 5,7-di-*O*-acetyl-4,8–di-*O*-*p*-methoxybenzyl-3-deoxy-2-thio-*β*-*D*-*manno*-oct-*2*-ulopyranosid)*onate* (9)**

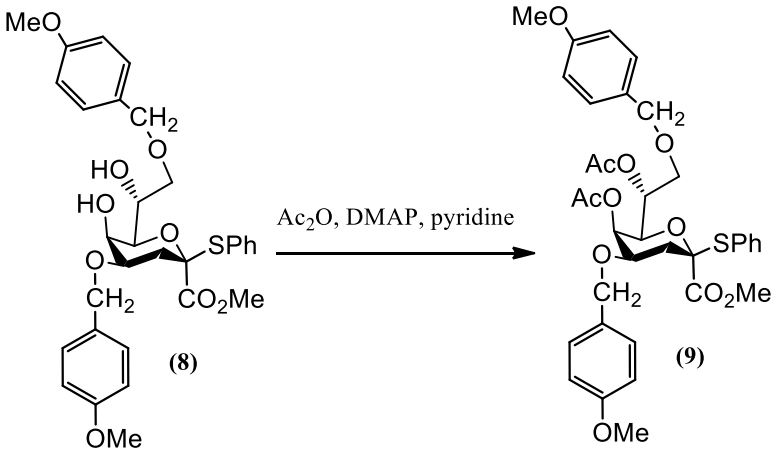



**Figure 18: Acetylation of the 5th and 7th position**

Compound **(9)** served as a proof for the successful reaction to compound **(8)** and served as intermediate step for an easier purification from the strong contamination with tin oxide. In table 1 shifts of starting material **(5)**, product **(9)** and **(8)** *Methyl* (phenyl 3-deoxy-4,8–di-*O*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-*2*-ulopyranosid)*onate* were compared to each other. H-5 and H-7 in **(8)** are downfield-shifted and similar to the ppm values of compound **(5)** (fig.19). Thus, acetylation occurred on these two positions. In contrast, shift-values of H4, H8a,b are similar to product **(8)**.

**Table 1: comparison of chemical shifts**

| Chemical shifts δ | | | |
|---|---|---|---|
| | **(5)** | **(8)** | **(9)** |
| H-3 (eq) | 2.60 | 2.65 | 2.61 |
| H-3 (ax.) | 2.22 | 2.13 | 2.11 |
| H-4 | 4.88 | *3.40* | *3.47* |
| H-5 | *5.28* | 4.12 | *5.42* |
| H-6 | *3.93* | 3.33 | *3.86* |
| H-7 | *5.22* | 4.16 | *5.18* |
| H-8 a | 4.48 | *3.73* | *3.71* |
| H- 8 b | 4.14 | *3.49* | *3.58* |

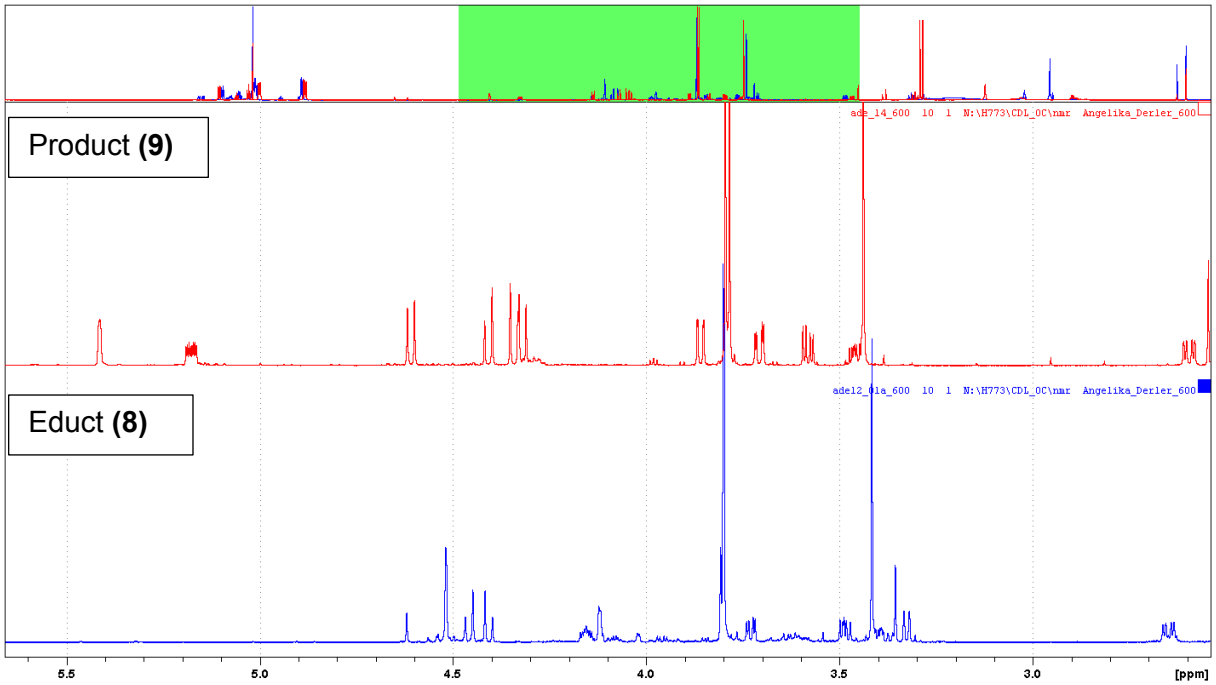



Figure 19: Comparison of NMR of educt (8) and product (9)

***Methyl* (phenyl 3-deoxy-4,8–di-*O*-*p*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-2-ulopyranosid)*onate* (8) from *Methyl* (phenyl 5,7-di-*O*-acetyl-3-deoxy-4,8–di-*O*-*p*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-2-ulopyranosid)*onate* (9)**

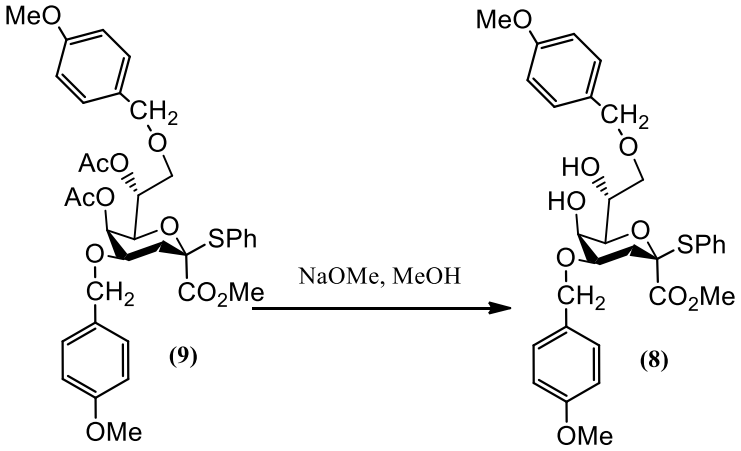



Figure 20: Transesterification of 5th and 7th position

Removing of acyl groups by transesterification was done by Zemplén-de-O-acetylation reaction, which also allowed an easier purification of product **(8)** (fig.20). Proton NMR of **(8)** confirmed data of previous measurements. Only H5 showed a more downfield shifted signal, but in the spectrum of **(9)** there is a highfield shift. Optical rotation showed a good agreement (26.4 and 25.8°C) between the two batches. Because of the precipitation of the sample it can be assumed that actual product was purer than compound **(8)** from previous experiments.

***Methyl* (phenyl 8-*O*-*tert*butyldiphenylsilyl-5,7-O-tert-butyl-silylene-1,1-diyl-3-deoxy-4-*O*-*p*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-2-ulopyranosid)*onate* (15)**

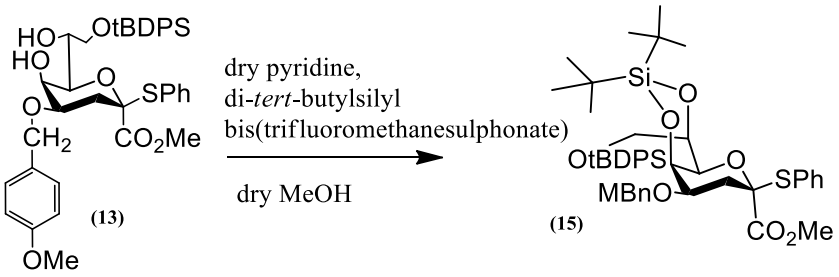



**Figure 21: Protection of the 5th and 7th position via silylene diyl group**

Reagent *di-tert*-butylsilyl bis(trifluoromethanesulphonate) is often used as promotor for glycoside formation in combination with silylenediyl protective groups (Kumagai [15]). In this case *di-tert*-butylsilyl bis(trifluoromethanesulphonate) should form the desired silylclip between position 5 and 7.

The reaction was fast (1 h) at room temperature and provided a good yield of 80%. A total conversion to product was observed and no additional spots were detectable. NMR confirmed the structure of the product.

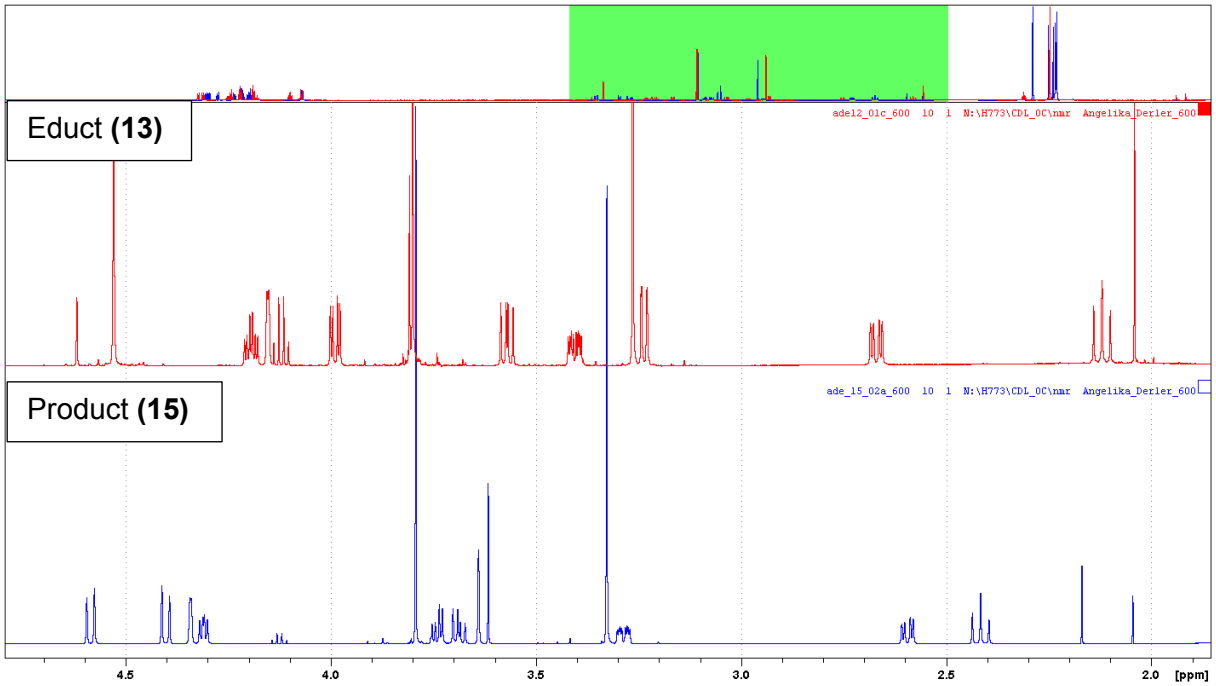



**Figure 22: Comparison of educt (13) and product (15)**

Figure 22 and table 2 shows a comparison of proton spectra of educt **(13)** and product **(15)**. H5 and H7 are shifted downfield, there is a significant shift difference to other signals which is an indication for the formation of the silylclip on the correct position. H8a and H8b are

close to each other whereas H4 shifts highfield to H6. Additionally there is a strong downfield shift of the axial H3. The confirmation is based on the significant change in the coupling constant $J_{H6,H7}$ which changes from 7.8 Hz for educt **(13)** and was smaller than 1 Hz in spectrum of product **(15)**.

Table 2: chemical shifts of (15)

| Chemical shifts δ | | |
|---|---|---|
| | **Educt (13)** | **Product (15)** |
| H-3 (eq) | 2.68 | 2.60 |
| H-3 (ax.) | 2.12 | 2.42 |
| H-4 | 3.41 | 3.29 |
| H-5 | 4.16 | 4.34 |
| H-6 | 3.24 | 3.64 |
| H-7 | 4.20 | 4.31 |
| H-8 a | 3.99 | 3.74 |
| H- 8 b | 3.58 | 3.69 |

## 3.5 Glycosylation experiments

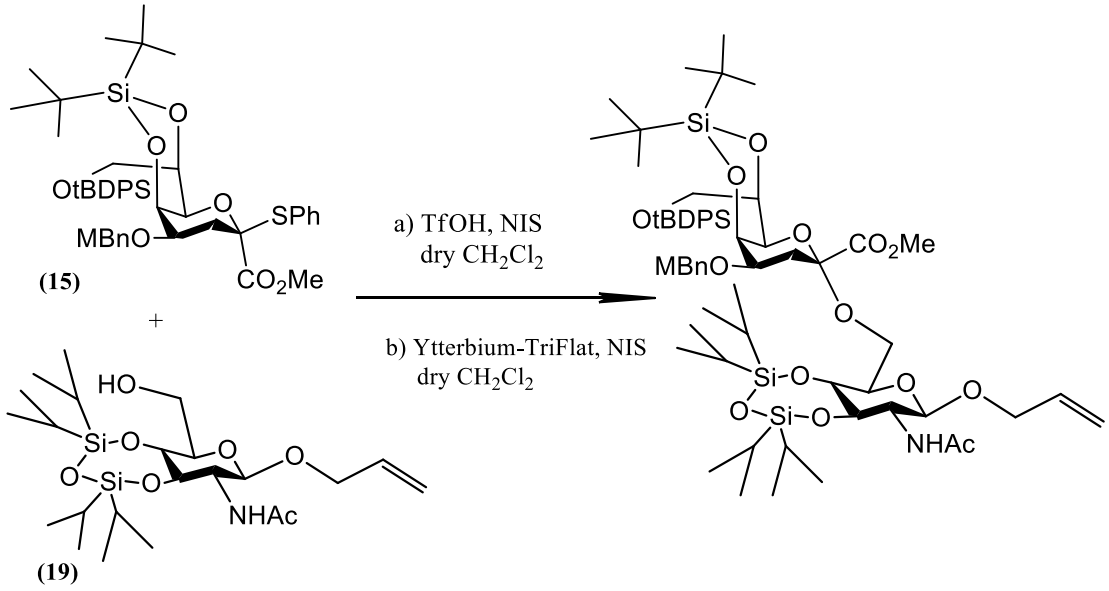



Figure 23: Glycosylation of model acceptor (20) and thioglycoside Kdo donor (15)

After synthesis of new thioglycoside donor **(15)**, glycosylation with model acceptor N-acetyl-glucosamine **(19)** was tested (fig.23). Glycosylation reactions are dependent on several conditions. Thioglycosides are generally known to perform better under mild activating conditions [11]. Influence of chosen temperature and solvent are important. Stereodirecting effect of neighbouring protective groups can dominate the outcome of the reaction.

For this glycosylation strong thiophilic promotor system NIS/TfOH and NIS/ Ytterbium(III)trifluoromethanesulfonate was chosen. As mentioned by Demchenko [11] bulkiness of anomeric thiogroup influences the reactivity of the donor. Bulky leaving groups like thiophenyl are expected to raise the reactivity. On the other hand the leaving group is also controlled by surrounding protective groups as mentioned before.

Both approaches of glycosylation did not result in product formation. On TLC formation of many minor spots was detectable. Donor and acceptor spot were not fully converted. Investigations under UV-light showed that several spots were derived from the donor **(15)**. Isolation of products and analysis by NMR confirmed degradation of donor and acceptor. A characteristic signal of the elimination product **(4)** in one of the samples was detected. This leads to the conclusion that glycosylation reactions provided dissociation of the thio-leaving group, as described by Oscarson [8] but caused increasing formation of unfavoured compound **(4)**. Further trials to isolate elimination product failed. Purification ended in recovery of several by-products.

## 3.6 Conclusion

The synthesis of a new 5,7-O-silylated Kdo thioglycoside donor was accomplished. Introduction of a double protecting silyl clip was sucessful and reactivity conditions could be optimized in order to increase yield. Additionally, new access to 4,8-disubstituted Kdo acceptors *via* a dibutyl tin acetal intermediate product and dimethoxy protection could be developed. It was proved that stereochemical hindrance of chosen protective groups provided stability of donor. Synthesis and characterization of five novel substances was accomplished.

Nevertheless the thioglycoside Kdo donor was unstable for glycosylation reactions. Reaction only caused additional formation of the unfavoured elimination product. Consequently, the results of the working group of Oscarson [8] were confirmed.

However, there are several new aspects to exploit the thio donor. Because of the reactivity of thio-leaving group conversion to other Kdo donors can be made. Trihaloacetimidate as described by Shimoyama [5] serves as a new attractive leaving group even under harsh glycosylation conditions. On the other hand a simple step conversion into Kdo halide donors like Kdo bromide could also be an alternative.

# 4 Experimental

## 4.1 General methods

For thin layer chromatography silica gel 60 F254 precoated glass plates (Merck) were used and for HPTLC silica gel 60 F254 HPTLC precoated glass plates with 2.5 cm concentration zone (Merck). For detection of substances TLC/HPTLC was dipped in anisaldehyde-$H_2SO_4$-acetic acid reagent and heated to 200°C on a hot plate.

Column chromatography was performed on silica gel 60 (230-400 mesh, Merck).

Melting points are uncorrected and measured by using Kofler-type Reichert Thermovar micro hot stage microscope.

Optical rotation was determined using Perkin Elmer 243 B polarimeter. $[\alpha]^{D}_{20}$-Values are given in units of $10^{-1}$ deg $cm^3$ $g^{-1}$.

Combustion analysis was performed at the micro analytical laboratory of the Faculty of Chemistry, University of Vienna, under the direction of Mag. Johannes Theiner.

Nuclear magnetic resonance spectra were recorded on a Bruker DPX 300 spectrometer or a Bruker Avance III 600 instrument. Reference material was TMS or the solvent signal in $CDCl_3$ ($^1H$: δ 7.26 ppm, $^{13}C$: δ 77.0 ppm).

**Preparation of solutions/solvents**

Preparation of chemicals was perfomed as described elsewhere. All other chemicals were used as purchased from commercial supplier.

Acetonitrile was dried by using $P_2O_5$ and distilled. Residual amount of water was continually checked by Karl-Fischer titration on a Mitsubishi Karl Fischer moisture meter model CA-21 before using solvents.

**Stock solutions/solvents**

5% $I_2$/KI-solution

15.6 mg solid potassium iodide was dissolved in 100 ml $H_2O$

1,5 M HCl solution

40.5 ml of 37% HCl was filled up to 1000 ml with RO-$H_2O$

1,5 M NaOH solution

60 g solid NaOH was filled up to 1000 ml with RO-$H_2O$

Equilibration of cation-exchanger DOWEX 50

100 g Cation-exchanger DOWEX 50 was filled into a suction flask. Equilibration occurred under vacuum filtration. DOWEX 50 was purged with 500 ml 1.5 M HCl and 500 ml 1.5 M NaOH. Then cation-exchanger was pre-conditioned with wet MeOH. Equilibration was finished by purging and storage in dry MeOH.

0,1 M Sodiummethoxide NaOMe (50 ml)

0.115 g solid sodium was dissolved in 50.0 ml MeOH.

## 4.2 *Methyl* (phenyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-2-thio-*α/β-D-manno*-oct-2-ulopyranosid)*onate* (3)

**Reaction at room temperature**

A 50 ml flask was dried by storing for 10 minutes at 50°C. Compound **(1)** (49.8 mg, 0.108 mmol) was dissolved in dry DCM (10 ml). Thiophenol (8.5 µl, 0.083 mmol) was added and after 1 hour $BF_3.Et_2O$ (36.4 µl, 1.664 mmol) was added and stirred at RT. After 75 min the reaction mixture was cooled to 0°C. 10 min later the solution was warmed to RT and washed with saturated aqueous $NaHCO_3$ (50 ml). Organic phase was washed with RO-$H_2O$ (50 ml). Aqueous solution of 5% $I_2$/KI was added as indicator until the phase was coloured. Organic phase was dried with $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography (30 g, 2:1 toluene-ethylacetate) to give **(3)** (18.6 mg, 0.036 mmol, 33.7% of α/β-anomer). Yield of product **(4)** was 7.9 mg (0.02 mmol, 18%).

After pooling two main fractions could be isolated. First main fraction contained product. Second main fraction contained the product glycal ester **(4)**.

Table 3: Rf-values of synthesis 4.2

| TLC | |
|---|---|
| Toluene:Ethylacetate 1:1 (α/β) | 0.71 |
| Toluene:Ethylacetate 1:1 (glycal ester) | 0.63 |

$^1$H-NMR (600 MHz, $CDCl_3$ ref. 7.36 ppm): δ 7.57-7.28 (m, 14, Ar-H thiophenyl group) 5.45-5.42 (m, 2H, H4α, H5α), 5.28 (d, J = 2.8 Hz, 1H, H5β), 5.25-5.20 (m, $J_{8βa,7α,β}$ = 4.3, $J_{7α,8αa}$ = 9.6, $J_{7β,\ 8αb}$ = 5.0, $J_{7β,6β}$ = 9.5, 2H, H7α, H7β), 4.87 (ddd, $J_{5β,\ 4β}$ = 2.9 $J_{3(equ)β,4β}$ = 4.7, $J_{3(ax)β,4b}$ = 12.2 Hz, $J_{3,4}$ = 2.91 Hz, 1H, H4β), 4.71 (dd, $J_{5β,6α}$ = 1.4 Hz, $J_{7α,6α}$ = 9.6 Hz, 1H, H6α), 4.47 (dt, 2H, H8aα, H8aβ), 4.14 (dd, $J_{7β,8aα}$ = 5.04 Hz, $J_{8b,β,8bα}$= 12.2 Hz, 1H, H8bα), 4.00 (dd, $J_{7α,\ 8β}$ = 4.4, $J_{8bα,8bβ}$ = 12.3 Hz, 1H, H8bβ), 3.92 (dd, $J_{6β,7β}$ = 9.5 Hz, 1H, H6β), 3.58 (s, 3H, OMe (α)), 3.54 (s, 3H, OMe (β)), 2.56 (dd, $J_{3(eq)β,4β}$ = 4.7 Hz, $J_{3(eq)β,3(ax)β}$ = 12.6 Hz, 1H, H3β(equ.)), 2.43 (dd, $J_{3(eq)α,4β}$ = 4.9, $J_{3(eq)α,\ 3(ax)α}$= 13.7 Hz, 1H, H3α(equ.)), 2.36-2.31 (m, $J_{3(eq)α,3(ax)α}$= 13.5 Hz, 2H, H3α), 2.22 (t, $J_{3(eq)β,3(ax)β}$ = 12.6 Hz, 1H, H3β), 2.09-1.98 (m, 29H, OAc)

$^{13}$C-NMR: δ 20.65-20.76 (C-Ac) 28.9 (C-3(ax)β) 30.4 (C-3(eq)β), 32.1, 32.4 (C-3(eq.,ax.)α) 52.5, 52.6 ($OCH_3$,α,β), 62.2 (C-8α), 62.8 (C-8β), 63.5 (C-5β), 64.4 (C-4α), 66.8 (C-5α), 67.4 (C-4β), 67.7 (C-7α), 67.9 (C-7β), 69.5 (C-6α), 72.3 (C-6β) ), 160-120 (Ar-C).

**Reaction at higher temperature (I)**

Compound **(1)** (50.0 mg, 0.108 mmol) was dissolved in dry DCM (10 ml). Thiophenol (8.5 µl, 0.083 mmol) was added under argon. After 45 min $BF_3.Et_2O$ (36.5 µl, 1.671 mmol) was added and stirred at RT. After 2.5 h, the reaction mixture was gradually heated for 1 hour at 60°C. Reaction mixture turned brown. Reaction was stopped by cooling to 0°C. The solution was washed with saturated aqueous $NaHCO_3$ (50 ml). Organic phase was washed with RO-$H_2O$ (50 ml). An aqueous solution of 5% $I_2$/KI was added as indicator until the phase was coloured. Organic phase was dried over $MgSO_4$, filtered and concentrated in vacuo. The concentrated residue was purified by silica gel chromatography (2:1 toluene-ethylacetate) to give **(3)** (8.4 mg, 0.0164 mmol, 15%).

**In-Situ-NMR**

Compound **(1)** (11.0 mg, 0.024 mmol) was dissolved in dry deutered DCM (0.33 ml) in a NMR-tube. Thiophenol (1.9 µl, 0.019 mmol) was added and mixed thoroughly. $BF_3.Et_2O$ (10.0 µl, 0.048 mmol) was added to the solution which was measured after 10, 13 and 16 min, respectively.

## 4.3 *Methyl* (phenyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-2-thio-*β*-*D*-*manno*-oct-*2*-ulopyranosid)*onate* (3) *via Methyl* (4,5,7,8-tetra-*O*-acetyl-3-deoxy-2-thio-*α*-*D*-*manno*-oct-*2*-ulopyranosyl bromide)*onate* (2)

**1.Reaction to Kdo bromide**

Compound **(1)** (5.0 g, 10.81 mmol) was dissolved in dry DCM (50 ml). $TiBr_4$ (6.13 g, 16.69 mmol) was added to the stirred solution at 4°C and kept for 20 h. The solution was extracted with $CHCl_3$ (100 ml), cooled saturated aqueous $NaHCO_3$ (100 ml) and concentrated to give 5.53 g (11.44 mmol) of crude bromide **(2)**.

**Reaction with Kdo bromide**

Bromide **(2)** (51.5 mg, 0.107 mmol) was dissolved in dry acetonitrile (2.0 ml). Thiophenol (6.0 µl, 0.059 mmol) and $Et_3N$ (3.3 µl, 0.05 mmol) were added. The solution was stirred over the weekend at RT. The solution was diluted with DCM (50 ml), washed with saturated aqueous $NaHCO_3$ (50 ml) and $H_2O$ (50 ml). An aqueous solution of 5% KI/$I_2$ was added in order to oxidize thiophenol. The organic phase was dried, concentrated and the residue was purified by silica gel chromatography (2:1 toluene-ethylacetate) to give **(3)** (23.1 mg, 0.0451 mmol, 42%) as syrup.

Rf-value (TLC): 0.72 (toluene:ethylacetate 1:1 (β-anomer))

$^1$H-NMR (300 MHz, $CDCl_3$ ref. 7.32 ppm): δ 7.56-7.27 (m, 6H, H-Ar thiophenyl group), 5.28 (t, $J_{4,5}$ = 2.9 Hz, 1H, H5), 5.22 (ddd, $J_{7,8}$ = 2.3 Hz, $J_{7,8}$ = 5.1 Hz, $J_{6,7}$ = 9.5 Hz, 1H, H7), 4.88 (ddd, $J_{4,5}$= 2.9 Hz, $J_{3(eq),4}$ = 4.8 Hz, $J_{3(ax.),4}$ = 12.5 Hz, 1H, H4), 4.48 (dd, $J_{7,8}$ = 2.3 Hz, $J_{8,8}$ = 12.3 Hz, 1H, H8), 4.14 (dd, $J_{7,8}$ = 5.1 Hz, $J_{8,8}$ =12.2 Hz, 1H, H8), 3.93 (d, $J_{6,7}$ = 9.5 Hz, 1H, H6), 3.54 (s, 3H, OMe), 2.60 (dd, $J_{3(eq),4}$ = 4.8 Hz, $J_{3(eq.),3(ax)}$ = 12.6 Hz, 1H, H3(equ)), 2.22 (t, $J_{3(equ),3(ax)}$ = 12.6 Hz, 1H, H3(ax)), 2.09-1.92 (m, 12H, OAc)

NMR-data are in agreement with results by Oscarson [8] and Waglund [16].

**Reaction with Kdo bromide(II)**

Kdo bromide **(2)** (5.28 g, 10.9 mmol) was dissolved in dry acetonitrile (15.0 ml). Thiophenol (0.53 ml, 5.19 mmol) and $Et_3N$ (0.34 ml, 5.19 mmol) were added. The solution was stirred for 1 hour at RT then at 0°C for 1 hour. Work-up and purification as described above gave 3.23 g (6.29 mmol, 58%) of **(3)**.

## 4.4 *Methyl* (phenyl 3-deoxy-2-thio-*β*-*D*-*manno*-oct-2-ulopyranosid)*onate* (5)

**1st approach**

Compound **(4)** (422.7 mg, 0.825 mmol) was dissolved in dry methanol (25.0 ml). To the stirred solution NaOMe 0.1 M (0.0343 ml, 0.825 mmol) was added. After 1 h the pH was tested. Because the solution was still acidic, 7.0 ml NaOMe were added. The reaction was stirred over night. Cation-exchange resin was added until the pH was neutral. TLC showed no further conversion and some weak intermediate product spots. The filtrate was concentrated in vacuo to give **(5)** (286.0 mg, 0.83 mmol, ~100%).

**2nd approach**

In a 100 ml flask provided with drying tube, compound **(3)** (2.85 g, 5.57 mmol) was dissolved in MeOH (73.0 ml). To the stirred solution NaOMe was added (11.0 ml, 264.4 ml). After 0.5 h pH was checked. Because the solution was too acidic (pH-1) 11.5 ml NaOMe was added until the pH reached 8. After 4.5 h 0.5 ml NaOMe were added again. After further 1.5 h the reaction was finished and DOWEX 50 $H^+$ was added until pH was 7. Mixture was filtered and the filtrate was concentrated. TLC purity check showed only one spot in pure ethylacetate. The product was dried by HV-pump over the weekend to give **(5)** as a solid (1.89 g, 5.50 mmol, 98.9%).

Rf-value (TLC): 0.85 (methanol: ethylacetate 1:2)

## 4.5 *Methyl* (phenyl 8-*O*-*tert*butyldimethylsilyl-3-deoxy-2-thio-*β*-*D*-*manno*-oct-*2*-ulopyranosid)*onate* (6)

Compound **(5)** (213.6 mg, 0.618 mmol) was dissolved in dry acetonitrile (50 ml) and DABCO (91.0 mg, 0.812 mmol) was added. tBDMSCl (103.6 mg, 0.688 mmol) was added to the stirred solution at RT and a white granulous precipitate was formed immediately. Additional amounts of DABCO (2 x 13.9 mg, 2 x 27.8 mg) and tBDMSCl (2 x 18.6 mg, 2 x 37.2 mg) were added in the course of 3 days. After 67.5 h mixture the filtrate was concentrated in vacuo.

The residue was purified by silica gel chromatography (1:2 toluene-ethylacetate) to give **(6)** (127.9 mg, 0.28 mmol, 45%).

**Table 4: Rf-values of synthesis 4.5**

| **TLC** | |
|---|---|
| Pure Ethylacetate | 0.79 |
| Ethylacetate:Ethanol 2:1 | 0.98 |
| Hexane:Ethylacetate 1:1 | 0.23 |
| Toluol: Ethylacetate 1:2 | 0.39 |
| **HPTLC** | |
| Toluene:Ethylacetate 1:1 | 0.30 |

$[\alpha]_D^{20}$ + 28.2° (**c** 1.1, $CHCl_3$)

$^1$H-NMR (600 MHz, $CDCl_3$ ref. 7.26 ppm): δ 7.51 (d; 2H; H-Ar phenyl group), 7.37 (tt, 1H, H-Ar phenyl group), 7.30 (t, 1H, H-Ar phenyl group), 4.01 (d, $J_{4,5}$= 2.8 Hz, 1H, H5), 3.94 (ddd, $J_{7,8a}$ = 4.4 Hz, $J_{6,7}$ = 6.2 Hz, $J_{7,8b}$ = 10.6 Hz, 1H, H7), 3.87 (dd, $J_{7,8a}$= 4.3 Hz, $J_{8a,8b}$= 10.1 Hz, 1H, H8a), 3.62 (ddd, $J_{4,5}$ = 3.2 Hz, $J_{3(eq),4}$ = 4.8 Hz, $J_{3(ax.),4}$ = 11.8 Hz, 1H, H4), 3.59 (dd, $J_{7,8}$ = 5.9 Hz, $J_{8a,8b}$ = 10.0 Hz, 1H, H8b), 3.49 (s, 3H, OMe), 3.37 (d, $J_{6,7}$ = 7.4 Hz, 1H, H6), 2.64 (dd, $J_{3(eq),4}$ = 4.7 Hz, $J_{3(eq),3(ax)}$ = 12.7 Hz, 1H, H3(eq)), 2.06 (t, $J_{3(ax),3(eq)}$ = 12.2 Hz, 2H, H3(ax), $OCH_2$-EtOAc), 0.91-0.89 (m, 10H, O-silyl group), Anal. Calculation for $C_{21}H_{34}O_7SSi$: C, 54.99; H, 7.47; S, 6.99. Found: C, 53.83; H, 7.38; S, 6.29, MS (solvent: acetonitrile): HRMS (ESI) m/z calculated for [M + Na+] $C_{21}H_{34}O_7SSiNa^+$: 418.1687. Found: +ESI m/z = 418.1688 (Δ 0.22 ppm)

## 4.6 *Methyl* (phenyl 4,5,7-tri-*O*-acetyl-8-*O*-*tert*butyldimethylsilyl-3-deoxy-2-thio-*β*-*D*-*manno*-oct-*2*-ulopyranosid)*onate* (10)

Compound **(6)** (23.0 mg, 0.0457 mmol) was dissolved in 2 ml dry pyridine and stirred at RT. DMAP (55.8 mg; 0.457 mmol) and $Ac_2O$ (43.2 mg; 0.457 mmol) were added. The reaction was stirred over night at RT.

The TLC showed that conversion to product was not fully completed. In order to stop reaction 4 droplets methanol were added and stirred again for 15 min. The solution was coevaporated with toluene and concentrated. Product was purified by silica chromatography (toluene:ethylacetate 5:1) to give 12.1 mg, (0.0212 mmol, 46%) of **(10)** as syrup.

Table 5: Rf-values of synthesis 4.6

| **TLC** | |
|---|---|
| Toluene: Ethylacetate 1:1 | 0.56 |
| **HPTLC** | |
| Toluene:Ethylacetate 5:1 | 0.88 |

$^1$H-NMR (600 MHz, $CDCl_3$ ref. 7.26 ppm): δ 7.55 (d, 2H, H-Ar phenyl group), 7.40 (tt, 1H, H-Ar phenyl group), 7.33 (t, 1H, H-Ar phenyl group), 5.26 (d, $J_{4,5}$ = 2.9 Hz, 1H, H5), 5.10 (ddd, $J_{7,8a}$ = 2.5 Hz; $J_{7.8b}$ = 5.8 Hz, $J_{6,7}$ = 9.2 Hz, 1H, H7), 4.88 (ddd, $J_{4,5}$ = 2.9 Hz, $J_{3(eq),4}$ = 4.8 Hz, $J_{3(ax.),4}$ = 12.5 Hz, 1H, H4), 3.90 (dd, $J_{7,8a}$ = 2.6 Hz; $J_{8a,8b}$ = 11.3 Hz, 1H, H8a), 3.84 (dd, $J_{5,6}$ = 1.3 Hz; $J_{6,7}$ = 9.4 Hz; 1H, H6), 3.68 (d, $J_{7,8b}$ = 5.9 Hz, $J_{8a,8b}$ = 11.3 Hz, 1H, H8b), 3.54 (s, 3H, OMe), 2.57 (dd, $J_{3(eq),4}$ = 4.8 Hz, $J_{3(eq),3(ax)}$ = 12.5 Hz, 1H, H3(eq)), 2.14 (t, $J_{3(ax),3(eq)}$ = 12.5 Hz, 1H, H3(ax)), 2.01 (s, 3H, OAc), 1.97-1.6 (m, 6H, OAc), 0.86 (m, 10H, $CH_3$-silyl group)

## 4.7 Synthesis of *Methyl* (phenyl 8-*O*-*tert*butyldiphenylsilyl-3-deoxy-2-thio-*β*-*D*-*manno*-oct-*2*-ulopyranosid)*onate* (7)

### 1st approach

Compound **(5)** (199.8 mg, 0.579 mmol) was dissolved in 3.0 ml dry acetonitrile. DABCO (91.0 mg, 0.811 mmol) and TBDPSCl (165.8 ml, 0.637 mmol) were added at RT. After 30 min TLC showed formation of product. Additional amounts of DABCO (4 x 0.0139 g) and TBDPSCl (4 x 0.0186 ml) were added in the course of 3 days. After 67 h the suspension was filtered and the filtrate was concentrated in vacuo. Reaction mixture was purified by silica chromatography column (toluene:ethylacetate 1:2) to give **(7)** (12.1 mg, 0.0212mmol, 46.%).

**2nd approach**

Compound **(5)** (452.4 mg, 1.314 mmol) was dissolved in 10.0 ml dry acetonitrile. DABCO (206.3 ml, 1.839 mmol) and TBDPSCl (0.375 ml, 1.445 mmol) were added at RT. Additional amounts of DABCO (1 x 0.0737 g, 1 x 0.0369 g, 3 x 0.0147 g) and TBDPSCl (1 x 0.1708 ml, 1 x 0.0854 ml, 3 x 0.034 ml) were added in the course of 4 days. Reaction was stopped after 94.5 h. Mixture was filtered over Celite and the filtrate was concentrated. The product was purified by silica gel chromatography (toluene ethylacetate 1:2) to give **(7)** (535.9 mg, 0.919 mmol, 70%).

Table 6: Rf-values of synthesis 4.7

| **TLC** | |
|---|---|
| Pure Ethylacetate | 0.85 |
| Ethylacetate:Ethanol 2:1 | 0.99 |
| Hexan:Ethylacetate 1:1 | 0.28 |
| Toluene:Ethylacetate 1:2 | 0.51 |
| **HPTLC** | |
| Toluene :Ethylacetate 1:1 | 0.30 |

$[\alpha]_D^{20}$ + 20.6° (**c** 1.3, $CHCl_3$)

$^1$H-NMR (600 MHz, $CDCl_3$ ref. 7.26 ppm): δ 7.68 (ddd; 6H, H-Ar tBDPS/phenyl group), 7.46-7.35 (m, 12H, H-Ar tBDPS/phenyl group), 7.26 (t, 1H, H-Ar phenyl group), 7.03 (t, 2H, H-Ar phenyl group), 4.10 (ddd, $J_{7,8a}$ = 4.1 Hz; $J_{7.8b}$= 7.2 Hz; $J_{6,7}$= 14.5 Hz, 1H, H7), 4.03 (d, $J_{4,5}$ = 3.0 Hz, 1H, H5), 3.97 (dd, $J_{7,8a}$ = 4.1 Hz; $J_{8a,8b}$ = 10.4 Hz, 1H, H8a), 3.64 (dd, $J_{7,8b}$ = 7.1 Hz, $J_{8a,8b}$ = 10.4 Hz, 1H, H8b), 3.60 (ddd, $J_{4,5}$ = 3.1 Hz, $J_{3(eq),4}$ = 4.9 Hz, $J_{3(ax.),4}$ = 11.8 Hz, 1H, H4), 3.36 (dd, $J_{5,6}$ = 1.0 Hz, $J_{6,7}$ = 7.4 Hz, 1H, H6), 3.34 (s, 3H, OMe), 2.65 (dd, $J_{3(eq),4}$ = 4.6 Hz, $J_{3(eq),3(ax)}$ = 12.7 Hz, 1H, H3(eq)), 2.62 - 2.48 (-OH group), 2.03 (t, $J_{3(ax),3(eq)}$ = 12.2 Hz, 4H, H3(ax), OAc), 1.07 (m, 14H, tBDPS/$CH_3$-silylgroup), Anal. Calculation for $C_{31}H_{38}O_7SSi$: C, 63.89; H, 6.57; S, 5.50. Found: C, 62.24; H, 6.74; S, 4.71, MS (solvent: acetonitrile): HRMS (ESI-TOF) *m/z* calculated for $[M + NH_4]^+$ $C_{31}H_{42}NO_7SSi^+$ : 600.2446. Found: +ESI m/z = 600.2444 = Δ 0.61 ppm

## 4.8 *Methyl* (phenyl 8-*O*-*tert*butyldimethylsilyl-3-deoxy-4-*O*-*p*-methoxybenzyl-*2*-thio-*β*-*D*-*manno*-oct-*2*-ulopyranosid)*onate* (12)

Compound **(6)** (50.0 mg, 0.109 mmol) and dibutyl tin oxide (DBTO) (56.5 mg, 0.227 mmol) were dissolved in 5.0 ml toluene. Boiling stones were added. The reaction mixture was heated for 6 hours using Dean-Stark apparatus in order to separate water. The solution reaction was cooled to RT, concentrated in vacuo and dried by HV-pump over night.

On the next day the reaction mixture was dissolved in 3.0 ml dry DMF. Molecular sieves (200 mg) were added. Tetrabutylammonium bromide (TBAB) (71.1 mg, 0.22 mmol) and p-methoxybenzyl chloride (0.0296 ml, 0.218 mmol) were added and stirred at RT. After 2 h 20 min the reaction was heated to 50°C and after 3 h 20 min reaction was heated to 90°C. Reaction was cooled to RT and stirred over night for 7 h 40 min.

On the next day reaction was heated to 90°C. After 2 h 45 min reaction was cooled to RT. 0.5 ml MeOH were added and stirred for 30 min. Altogether reaction was stopped after 25 h 40 min. The solution was concentrated and the product was purified by silica gel chromatography (hexane: ethylacetate 1:2) to give 9.1 mg (0.0157 mmol, 14%) of **(12)**.

Rf (hexane:ethylacetate 1:2, TLC) 0.52

$^1$H-NMR (600 MHz, $CDCl_3$ ref. 7.25 ppm) 7.50 (d, 2H-Ar thiophenyl group), 7.36 (t, 1H, H-Ar thiophenyl), 7.30-7.22 (m, 10H, H-Ar tBDMS/phenyl group), 6.88 (d, 5H, H-Ar,phenyl group), 4.52 (s, $J_{4,OCH2}$ = 1.3 Hz, 2H, $OCH_2$ methoxybenzyl group), 4.43 (dq, AB-system, J = 11.4 Hz, $\Delta\vartheta_{AB}$ = 28.3 Hz, $OCH_2$, methoxybenzyl group) 4.16 (ddd, $J_{7,8a}$ = 3.4 Hz; $J_{7.8b}$ = 6.0 Hz, $J_{6,7}$ = 8.5 Hz, 1H, H7), 4.12 (s, $J_{4,5}$ = 2.5 Hz, 1H, H5), 3.80-3.78 (m, 7H, $OCH_3$, tBDMS group), 3.73 (dd, $J_{7,8a}$ = 3.5 Hz; $J_{8a,8b}$ = 6.5 Hz, 1H, H8a), 3.49 (dd, $J_{7,8b}$ = 9.9 Hz, $J_{8a,8b}$ = 6.1 Hz 1H, H8b), 3.41 (s, 3H, OMe), 3.40 (ddd, $J_{4,5}$ = 2.8 Hz, $J_{3(eq),4}$= 4.7 Hz, $J_{3(ax.),4}$ = 11.8 Hz, 1H, H4), 3.34 (d, $J_{6,7}$ = 8.3 Hz; 1H, H6), 2.65 (dd, $J_{3(eq),4}$ = 4.5 Hz, $J_{3(eq),3(ax)}$ = 12.7 Hz, 1H, H3(eq)), 2.13 (t, $J_{3(ax),3(eq)}$ = 12.3 Hz, 1H, H3(ax))

## 4.9 *Methyl* (phenyl 8-*O*-*tert*butyldiphenylsilyl-3-deoxy-4-O-*p*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-*2*-ulopyranosid)*onate* (13) and *Methyl* (phenyl 3-deoxy-4,8–di-*O*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-*2*-ulopyranosid)*onate* (8)

Compound **(7)** (124.2 mg, 0.312 mmol) and dibutyltin oxide (DBTO) (143.4 mg, 0.576 mmol) were dissolved in 50 ml toluene. The mixture was refluxed for 6 hours with Dean-Stark apparatus and reflux condenser in order to separate water. Reaction was cooled to RT and filtered over Celite. The filtrate was concentrated in vacuo and dried by HV-pump over night.

The reaction mixture was dissolved in 5 ml dry DMF. Molecular sieves (200 mg) were added. Tetrabutylammonium bromide (TBAB) (137.5 mg, 0.427 mmol) and p-methoxybenzyl chloride (0.058 ml, 0.427 mmol) were added and stirred at RT. After 48 h, the reaction was heated to 65°C for 2 h and to 90°C for 4 hours. Reaction mixture was cooled to RT and stirred over night.

After 71 h 45 min dry MeOH was added dropwise. The mixture was filtered over Celite and washed with 50 ml ethylacetate and 100 ml water. The aqueous phase was washed with 2x100 ml ethylacetate. All organic phases were pooled and washed with 100 ml water. The organic phase was dried with $Na_2SO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane: ethylacetate 1:2) to give 38.9 mg (0.055 mmol, 26%) of **(13)** and 37.3 mg (0.064 mmol, 30%) of **(8).**

**2nd approach**

The 2nd approach was made with **(7)** (495.6 mg, 0.850 mmol), dibutyltin oxide (DBTO) (577.9 mg, 2.322 mmol), tetrabutylammonium bromide (TBAB) (548.3 mg, 1.701 mmol) and p-methoxybenzylchloride (0.231 mg, 1.701 mmol). After 29 h 30 min work-up and purification gave 174.4 mg (0.248 mmol, 29%) for **(13)** and 25.2 mg (0.0431 mmol, 5 %) for **(8)**. Recovery of educt **(7)** gave ~70 mg (0.1201 mmol, 14%).

**3rd approach**

The 3rd approach was made with **(7)** (392.2 mg, 0.673 mmol), DBTO (452.3 mg, 1.817 mmol), TBAB (433.9 mg, 1.346 mmol) and p-methoxybenzylchloride (0.183 ml, 1.346 mmol) The solution was filtered over Celite and washed with 50 ml ethylacetate and 50 ml water. The aqueous phase was washed with 2x100 ml ethylacetate. All organic phases were pooled and washed with 150 ml water (small amount of saturated aqueous $NaHCO_3$ and brine were added). The organic phase was dried with solid $Na_2SO_4$. The organic phase was concentrated and purified by silica gel chromatography (hexane: ethylacetate 1:2) to give 87.5 mg (0.125 mmol, 18.5%) for **(13)** and 150.4 mg (0.257mmol, 38%) for **(8)**.

Table 7: Rf-values of synthesis 4.9

| **TLC** | |
|---|---|
| Rf-value | **(13)** / **(8)** |
| hexane: ethylacetate 1:2 | 0,44/0,92 |
| hexane: ethylacetat 2:1 | n.s../0,46 |
| hexane:ethylacetate 3:2 | n.s../0,48 |
| **HPTLC** | |
| hexane:ethylacetate 3:1 | 0.16/ n.s. |
| hexane: ethylactetate 2:3 | n.s./ 0.24 |
| Hexane:ethylacetate 1:3 | n.s./0.32 |

**(13)** $[\alpha]_D^{20}$ + 26.7° (**c** 1.02, $CHCl_3$)

**(8)** $[\alpha]_D^{20}$ + 26.4° (**c** 1.3, $CHCl_3$)

**(13)**

$^1$H-NMR (600 MHz, $CDCl_3$ ref. 7.26 ppm): δ 7.67 (dd, 4H, H-Ar thiophenyl group), 7.47-7.41 (m, 4H, H-Ar tBDPS/ phenyl group), 7.37-7.34 (m, 4H, H-Ar phenyl group), 7.26-7.21 (m, 4H, H-Ar phenyl group), 6.97 (t, 2H, H-Ar phenyl/methoxybenzyl group), 6.90-6.87 (m, 2H, H-Ar phenyl/methoxybenzyl group)), 4.53 (s, 2H, methoxybenzyl group), 4.20 (ddd, $J_{7,8a}$ = 3.6 Hz; $J_{7.,8b,6}$ = 7.8 Hz, 1H, H7), 4.16 (d, $J_{4,5}$ = 2.4 Hz, 1H, H5), 3.99 (dd, $J_{7,8a}$ = 4.2 Hz; $J_{8a,8b}$ = 10.8 Hz, 1H, H8a), 3.81 (s, 3H, $OCH_3$/methoxybenzyl group), 3.58 (dd, $J_{7,8b}$ = 7.8 Hz, $J_{8a,8b}$ = 10.2 Hz 1H, H8b), 3.41, (ddd, $J_{4,5}$ = 3.0 Hz, $J_{3(eq),4}$ = 4.8 Hz, $J_{3(ax.),4}$ = 12.0 Hz, 1H, H4), 3.26 (s, 3H, OMe) 3.24 (dd, $J_{6,7}$ = 7.8 Hz; 1H, H6), 2.68 (dd, $J_{3(eq),4}$ = 4.8 Hz, $J_{3(eq),3(ax)}$ = 12.6Hz, 1H, H3(eq)), 2.12 (t, $J_{3(ax),3(eq)}$ = 12.0 Hz, 1H, H3(ax)), 1.06 (s, 7H, tBDPS/$CH_3$-silylgroup), Anal. Calculation for $C_{39}H_{46}O_8SSi$: C, 66.64; H, 6.60; S, 4.56. Found: C, 65.35; H, 6.38; S, 3.95. MS (solvent: acetonitrile): HRMS (ESI-TOF) m/z calculated for [M + NH4]+ C39H50NO8SSi + : 720.3021 Found: +ESI m/z = 720.3021 = Δ -0.76 ppm

Data for **(8)**

$^1$H-NMR (600 MHz, $CDCl_3$ ref. 7.25 ppm): δ 7.66 (dd, 1H, H-Ar phenyl group), 7.50 (d, 2H, H-Ar thiophenyl group), 7.38 (t, 2H, H-Ar thiophenyl group), 7.30-7.22 (m, 8H, H-Ar methoxybenzyl group, $CDCl_3$), 6.88 (t, 4H, H-Ar methoxybenzyl group), 4.52 (s, 2H, -$OCH_2$/ methozybenzyl group), 4.43 (AB-system, J = 11.4 Hz, $\Delta\vartheta_{AB}$ = 27.5 Hz, methoxybenzyl group) 4.16 (ddd, $J_{7,8a}$ = 3.2 Hz; $J_{7.8b}$ = 5.9 Hz, $J_{7,6}$ = 8.6Hz, 1H, H7), 4.12 (s, 1H, H5), 3.80 (s, 6H, -$OCH_3$/methoxybenzyl group) 3.73 (dd, $J_{7,8a}$ = 3.3 Hz, $J_{8a,8b}$ = 9.8 Hz, 1H, H8a), 3.49 (dd, $J_{7,8b}$ = 6.0 Hz, $J_{8a,8b}$ = 9.9 Hz, 1H, H8b), 3.43 (s, 3H, OMe) 3.40 (ddd, $J_{4,5}$ = 3.0 Hz, $J_{3(eq),4}$ = 4.8 Hz, $J_{3(ax.),4}$ = 11.9 Hz, 1H, H4), 3.36, (s, 1H, OH group), 3.33 (d, J $_{6,7}$ = 8.3 Hz, 1H, H6), 2.65 (dd, $J_{3(eq),4}$ = 4.4 Hz, $J_{3(eq),3(ax)}$ = 12.8 Hz, 1H, H3(eq)), 2.13 (t, $J_{3(ax),3(eq)}$ = 12.2 Hz, 1H, H3(ax)), 2.00 – 1.45 (OH group), 1.25 (s, 2H, tBDPS/silyl group residues) 1.06 (s, 3H, tBDPS/silylgroup residues)

## 4.10 *Methyl* (phenyl 3-deoxy-4,8–di-*O*-*p*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-2-ulopyranosid)*onate* (8)

### 1st approach

Compound **(5)** (48.4 mg, 0.141 mmol) was treated with DBTO (77.0 mg, 0.309 mmol), TBAB (90.6 mg, 0.281 mmol) and p-methoxybenzyl chloride (0.0381 ml, 0.281 mmol) as described. HPTLC showed conversion of product and one new higher spot. Reaction was stopped by adding 0.5 ml MeOH. Solution was filtered over Celite and washed with 200 ml ethylacetate and 150 ml $H_2O$. The organic layer was dried with solid $Na_2SO4$ and concentrated in vacuo.

Product was purified by silica gel chromatography (hexane: ethylacetate 1:2) to give 9.1 mg (0.0156 mmol, 11%) of **(8)**. NMR was not analysed because of the presence of tin contaminants.

**2nd approach**

Compound **(5)** (451.2 mg, 1.310 mmol) was treated with DBTO, (717.5 mg, 2.882 mmol), TBAB (844.8 mg, 2.62 mmol) and p-methoxybenzyl chloride (0.3553 ml, 2.62 mmol)) as described. After adding MeOH the reaction mixture was filtered over Celite and washed with 200 ml ethylacetate and 150 ml $H_2O$. The organic layer was dried with solid $Na_2SO_4$ and concentrated in vacuo. The material was immediately used for the next reaction without a purification step.

Rf (hexane:ethylacetate 1:2, TLC) 0.42

## 4.11 *Methyl* (phenyl 5,7-di-*O*-acetyl-4,8–di-*O*-*p*-methoxybenzyl-3-deoxy-2-thio-*β*-*D*-*manno*-oct-*2*-ulopyranosid)*onate* (9)

**1st approach**

Compound **(8)** (9.8 mg, 0.028 mmol) was dissolved in 2.0 ml dry pyridine and stirred at RT. DMAP (34.8 mg, 0.285 mmol) and $Ac_2O$ (0.027 ml, 0.285 mmol) was added. The reaction was stirred for 18 h and stopped by adding 4 droplets methanol. The mixture was stirred again for 15 min. Pyridine was evaporated with toluene. The residue was dissolved again in toluene and filtered over Celite and concentrated in vacuo. Product was purified by silica gel chromatography (toluene: ethylacetate 5:1) to give 8.7 mg (0.0136 mmol, 48 %) of **(9)** as solid.

**2nd approach**

Compound **(8)** (726.7 mg, 1.31 mmol) was treated with DMAP (1.58 g, 13.1 mmol) and $Ac_2O$ (1.24 ml, 13.1 mmol). As a consequence of many different intermediate products which seemed to have similar Rf-values on TLC/HPTLC the product was additionally purified by using a gradient 9:1 to 4:1 (toluene:ethylacetate).
HPTLC resulted in good purification of product which gave 106.0 mg (0.166 mmol, 13%) of **(9)**.

Table 8: Rf-values from 4.11

| **TLC** | |
|---|---|
| Rf-value | |
| Toluol: Ethylacetate 1:2 | 0,87 |
| Toluol: Ethylacetate 5:1 | 0,37 |

$^1$H-NMR (600 MHz, $CDCl_3$ ref. 7.26 ppm): δ 7.52 (d; 2H; H-Ar thiophenyl group), 7.39 (t, 1H, H-Ar thiophenyl group), 7.30-7.28 (m, 2H, H-Ar thiophenyl group), 7.20 (dd, 4H, H-Ar phenyl/methoxybenzyl group), 6.85 (t, 4H, H-Ar phenyl/methoxybenzyl group ), 5.42 (d, $J_{5.6}$ = 1.3 Hz 1H, H5), 5.18 (ddd, $J_{7,8a}$ = 2.3 Hz; $J_{7.8b}$ = 4.8 Hz, $J_{7,6}$ = 9.5Hz, 1H, H7), 4.61 (d, $J_{CH2}$= 10.9 Hz, 1H, $OCH_2$, methoxybenzyl group), 4.37 (AB-system, J = 11.9 Hz, $\Delta\vartheta_{AB}$ = 40.0 Hz, 2H, -$OCH_2$/methoxybenzyl group), 3.86 (dd, $J_{6,5}$ = 1.2 Hz; $J_{5,6}$ = 9.5 Hz, 1H, H6), 3.80 (s, 6H, $CH_3$/methoxybenzyl group) 3.71 (dd, $J_{7,8a}$ = 2.3 Hz; $J_{8a,8b}$ = 11.1 Hz, 1H, H8a), 3.58 (dd, $J_{7,8b}$ = 4.8 Hz, $J_{8a,8b}$ = 11.1 Hz, 1H, H8b), 3.47 (ddd, $J_{3(eq),4}$ = 3.6 Hz, 1H, H4), 3.45 (s, 3H, OMe) 2.61 (dd, $J_{3(eq),4}$ = 3.8 Hz, $J_{3(eq),3(ax)}$ = 12.8 Hz, 1H, H3(eq)), 2.57 (s, 1H), 2.11 (t, $J_{3(ax),3(eq)}$ = 12.5 Hz, 1H, H3(ax)), 2.08-2.02 (m, 7H, OAc), 1.56 (s, 2H, OAc)

## 4.12 *Methyl* (phenyl 8-*O*-*tert*butyldiphenylsilyl-5,7-O-*tert*-butyl-silylene-1,1-diyl-3-deoxy-4-*O*-*p*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-2-ulopyranosid)*onate* (15)

**1st approach**

Dried compound **(13)** (35.5 mg) was dissolved in 3.0 ml dry pyridine and cooled to 0°C on ice water. *Di-tert*-butylsilyl bis(trifluoromethanesulphonate) (19.6 µl, 0.061 mmol) was added. First TLC showed a small high running spot on HPTLC. Addition of 2 x 9.8 µl reagent was made during a period of 26.5 h. DMAP (61.7 mg, 0.505 mmol) was added as catalyst. After 146.5 h the reaction was heated to 60° for 2.5 h. Then the mixture was cooled to RT.

On the next day the reaction was heated to 60°C again for 2.5 h. After 168 h 0.6 equ. (9.8 µl, 0.030 mmol) reagent were added. Because of no further conversion reaction was finished after 171.5 h by adding MeOH dropwise and toluene. The solution was filtered over Celite and concentrated in vacuo. Product was purified by silica gel chromatography (hexane: ethylacetate 3:1) to give 16.0 mg (0.0201 mmol, 40%) of **(15)** as bright yellow syrup.

**2nd approach**

Dried compound **(13)** (86.8 mg, 0.123 mmol) was dissolved in 10.0 ml dry pyridine under argon at RT. *Di-tert*-butylsilyl bis(trifluoromethanesulphonate) (48.0 µl, 0.148 mmol) was added. Reaction was completely finished after 1 h and was stopped by adding 1 ml of dry MeOH. Pyridine was diluted with dry toluol and concentrated in vacuo. The residue was resuspended in DCM (50 ml) washed with saturated aqueous Brine (50ml) and dried with solid $Na_2SO_4$. The filtrate was concentrated in vacuo. Product was purified by silica gel chromatography (hexane: ethylacetate 3:1) to give 78.3 mg (0.0983 mmol, 79.5%) of **(15)**.

Rf (hexane:ethylacetate 3:1, TLC) 0.76

$[\alpha]_D^{20}$ + 40.8° (**c** 0.83, $CHCl_3$)

$^1$H-NMR (600 MHz, $CDCl_3$ ref. 7.26 ppm): δ 7.61 (dd, 4H, H-Ar tBDPS/phenyl group), 7.54-7.52 (m, 4H, H-Ar thiophenyl group), 7.41-7.26 (m, 12H, H-Ar tBDPS/thio-phenyl group/methoxybenzyl group, $CDCl_3$)), 6.87 (d, 2H, H-Ar methoxybenzyl group), 6.90-6.87 (m, 2H, H-Ar phenyl/methoxybenzyl group), 4.49 (AB-system 2H, $J_{CH2}$=11.2 Hz, $\Delta\vartheta_{AB}$ = 109.1 Hz, 2H, $OCH_2$ methoxybenzyl group), 4.34 (s, J = 1.9 Hz, 1H, H5), 4.31 (dd, $J_{7,8a}$ = 4.6 Hz; $J_{7.8b}$ = 6.8 Hz, 1H, H7), 3.76 (s, OMe), 3.74 (dd, $J_{7,8a}$ = 4.6 Hz; $J_{8a,8b}$ = 10.6 Hz, 1H, H8a), 3.69 (dd, $J_{7,8b}$ = 6.9 Hz, $J_{8a,8b}$ = 10.5 Hz 1H, H8b), 3.64 (s, 1H, H6), 3.33 (s, 1H, -$CH_3$, methoxybenzyl group), 3.29, (ddd, $J_{3(eq),4}$ = 4.2 Hz, $J_{3(ax.),4}$ = 11.8 Hz, 1H, H4), 2.60 (dd, $J_{3(eq),4}$ = 4.1 Hz, $J_{3(eq),3(ax)}$ = 12.3Hz, 1H, H3(eq)), 2.42 (t, $J_{3(ax),3(eq)}$ = 12.1 Hz, 1H, H3(ax)), 1.19 (s, 9H, tBDPS/ silyl group), 1.07-1.00 (m, 27H, $CH_3$-silylgroup)

## 4.13 *Methyl* (phenyl 5,7-*O*-*tert*-butyl-silylene-1,1-diyl-3-deoxy-4,8–di-*O*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-2-ulopyranosid)*onate* (14)

Reaction was done under the same conditions as described. Compound **(8)** (17.1 mg, 0.031 mmol) was treated with *Di-tert*-butylsilyl bis(trifluoromethanesulphonate) (12.0 µl, 0.037 mmol), and DMAP (37.7 mg, 0.308 mmol). No conversion to product was detectable on TLC. Educt **(8)** didn't react under these conditions.

## 4.14 *Methyl* (phenyl 3-deoxy-4,8–di-*O*-*p*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-2-ulopyranosid)*onate* (8) from *Methyl* (phenyl 5,7-di-*O*-acetyl-3-deoxy-4,8–di-*O*-*p*-methoxybenzyl-2-thio-*β*-*D*-*manno*-oct-2-ulopyranosid)*onate* (9)

Compound **(9)** (72.9 mg, 0.114 mmol) was dissolved in 5.0 ml dry MeOH. To the stirred solution 0.1 M NaOMe (0.225 ml, 5.4 mmol) was added. pH was checked to be around 8. After 1 h 20 min conversion to product was detectable. After 46 h the reaction was finished and DOWEX 50 $H^+$ was added until the pH was 7. The suspension was filtered and the filtrate was concentrated. The residue was purified by silica gel chromatography (touluene: ethylacetate 2:1) to give 52.9 mg (0.095 mmol, 83%) of **(8)**. Product formed white spicular crystals. Melting point: 129°C- 132°C

Table 9: Rf-values of synthesis 4.14

| **TLC** | |
|---|---|
| Rf-value | |
| Toluol: Ethylacetate 1:2 | 0.87 |
| Toluol: Ethylacetate 5:1 | 0.37 |

$[\alpha]_D^{20}$ + 25.8° (**c** 0.13, $CHCl_3$)

$^1$H-NMR (600 MHz, $CDCl_3$ ref. 7.24 ppm) 7.50 (d, 1H, H-Ar thiophenyl/group), 7.36 (t, 1H, H-Ar thiophenyl group), 7.26-7.23 (m, 7H, H-Ar methoxybenzyl group, $CDCl_3$), 7.18-7.14 (m, 4H, H-Ar thiophenyl/methoxybenzyl group), 6.88 (t, 2H, H-Ar methoxybenzyl group), 4.52 (d, J = 1.6 Hz 1H, $OCH_2$/methoxybenzyl group), 4.44 (AB-system, J = 11.5 Hz, $\Delta\vartheta_{AB}$ = 28.6 Hz, 1H, methoxybenzyl group), 4.17 (ddd, $J_{7,8a}$ = 3.3 Hz; $J_{7.8b}$ = 5.9 Hz, $J_{7,6}$ = 8.5 Hz, 1H, H7), 4.13 (s, 1H, H5), 4.12 (AB-system, J = 7.2 Hz, $\Delta\vartheta_{AB}$ = 12.4 Hz, 1H, methoxybenzyl group), 3.80 (s, 2H, methyoxybenzyl group), 3.71 (dd, $J_{7,8a}$ = 3.4 Hz; $J_{8a,8b}$ = 9.8 Hz, 1H, H8a), 3.49 (dd, $J_{7,8b}$ = 5.9 Hz, $J_{8a,8b}$ = 9.8 Hz 1H, H8b), 3.42 (s, 3H, OMe) 3.40 (ddd, $J_{3(eq),4}$= 4.6 Hz, 1H, H4), 3.33 (dd, $J_{6,7}$ = 8.3 Hz, 1H, H6), 2.66 (dd, $J_{3(eq),4}$ = 4.2 Hz, $J_{3(eq),3(ax)}$ = 12.1 Hz, 1H, H3(eq)), 2.15 (t, $J_{3(ax),3(eq)}$ =11.6 Hz, 1H, H3(ax))

NMR-data are in agreement with results of synthesis 4.9.

## 4.15 Allyl 2-acetamido-2-deoxy-*β*-*D*-glucopyranoside (17)

In a 100 ml flask compound **(16)** (49.9 mg, 0.129 mmol) was dissolved in 4.0 ml dry MeOH and 0.1 M NaOMe (5.4 µl, 0.129 mmol) was added. During 3.5 h 0.4, 0.2 and 1 equivalents of 0.1 M NaOMe (2.2 µl, 1.1 µl, and 5.4 µl) were added until pH was 8. The reaction was finished after 4.5 h and cation exchange resin (DOWEX 50) was added until the pH was neutral. The suspension was filtered, the filtrate was concentrated in vacuo and dried over night at HV-pump. Reaction gave 30.8 mg (0.118 mmol, 91.5%) of **(17)** as solid product.

Under same conditions a scale-up reaction was made with **(16)** (1.56 g, 4.06 mmol) and 0.1 M NaOMe (0.439 ml, 10.56 mmol)). Reaction gave 1.1 g (4.05 mmol, 99.6%) of product **(17)**.

**Table 10: Rf-values of synthesis 4.16**

| **TLC** | |
|---|---|
| Rf-value | |
| methanol : ethylacetate 1:1 | 0.28 |
| **HPTLC** | |
| pure methanol | 0.27 |

## 4.16 Synthesis of Allyl 2-acetamido-2-deoxy-4,6-*O*-(1,1,3,3-*tetra*isopropyldisiloxane-1,3-diyl)-*β*-*D*-glucopyranoside (18)

**1st approach**

In a 50 ml flask purged with argon compound **(17)** (29.8 mg, 0.114 mmol) was dissolved in 1.5 ml dry pyridine. TIPS-Cl (35.6 µl, 0.114 mmol) was added dropwise and the reaction was cooled with ice water. After 4 h 25 min 0,1 equ. (0,0046 ml) TIPS-Cl were added. The reaction was stirred over night and stored in cooled room (4°C). After 21 h the reaction was

stopped by adding 0.5 ml 2-propanol. Mixture was stirred for 15 min at RT. The solution was washed with ice-cooled 0,5 M HCl (44 ml), saturated aqueous $NaHCO_3$ (50 ml) and $H_2O$ (50 ml), dried with $Na_2SO_4$ and concentrated in vacuo. The residue was purified by column chromatography with toluene:ethylacetate 1:1 to give 31.3 mg (0.062 mmol, 54.5%) of solid product **(18)**.

**2nd approach**

In a 100 ml flask, fluted with argon, **(17)** (500 mg, 1.914 mmol) was dissolved in 4,5 ml dry pyridine and TIPS-Cl (0.598 ml, 1.914 mmol) was added dropwise after the reaction was cooled with ice water. After 3 h 0,1 equ. (0,0568 ml) TIPS-Cl were added. After 4 h reaction was stopped by stirring at RT and 2.0 ml 2-propanol was added. Mixture was washed with ice-cooled 0.5 M HCl (124 ml), saturated aqueous $NaHCO_3$ (150 ml) and $H_2O$ (150 ml), dried by $Na_2SO_4$ and concentrated in vacuo. The reaction product was purified with silica gel chromatography (toluene: ethylacetate 1:1) to give 1.058 g (2.10 mmol, 109.7%) of solid product **(18)**.

**3rd approach**

Compound **(17)** (549.2 mg, 2.10 mmol) was treated with 5.0 ml pyridine and TIPS-Cl (0.722 ml, 2.31 mmol) as described.

After 4 h the reaction was stopped by adding 2 ml 2-propanol at RT. Extraction was done as described before. There was no purification step before next reaction started. Reaction gave 1.35 g (2.68 mmol, 127.5%) of **(18)** as syrup.

Table 11: Rf-value of synthesis 4.17

| **TLC** | |
|---|---|
| Rf-value | |
| toluene : ethylacetate 1:1 | 0.26 |
| methanol : chloroform 1:4 | 0.84 |

## 4.17 Allyl 2-acetamido-2-deoxy-3,4-*O*-(1,1,3,3-*tetra*isopropyldisiloxane-1,3-diyl)-*β*-*D*-glucopyranoside (19)

**Reagent *p*-Toluenesulfonic acid**

Compound **(18)** (31.3 mg, 0.062 mmol) was dissolved in dry DMF (5 ml). Molecular sieves were added and at last *p*-toluenesulfonic acid (PTSA 9.6 mg, 0.056 mmol) was added. During a time of 52.5 h 0.2, 1.0 and 1.1 (2.1 mg, 10.5 mg and 13.2 mg) of PTSA were added and the solution was filtered over Celite. The filtrate was washed with ethylacetate and saturated aqueous $NaHCO_3$ (50 ml). Aqueous phase was washed with ethylacetate (200 ml)

for 4 times. The organic phase was washed with brine and $NaHCO_3$ for 3 times and dried with $Na_2SO_4$. It was filtered and concentrated in vacuo. No product was formed.

**Reagent camphorsulfonic acid (I)**

Dried compound **(18)** (30.7 mg, 0.061 mmol) was dissolved in dry DMF and camphorsulfonic acid (CSA) (14.9 mg, 0.064 mmol) was added and stirred at RT. After 3 h 15 min the reaction was finished and the solution was washed with $NaHCO_3$ and ethylacetate. The aqueous phase was washed 2 times with ethylacetate. The organic phase was washed with $NaHCO_3$ and brine 3 times. The organic phase was dried over $Na_2SO_4$, filtered and concentrated in vacuo which gave 25.6 mg, 0.051 mmol, 83.4% of **(19)** as solid product.

**Reagent camphorsulfonic acid (II)**

Reaction was done under same conditions as 1$^{st}$ approach using (**(18)** (1.06 g, 2.1 mmol), CSA (0.51 g, 2.21 mmol). After 19 h 0.1 equ. (0.051 g) CSA was added. Reaction was stopped after 46 h. The solution was washed with 50 ml $NaHCO_3$ and 50 ml ethylacetate. The aqueous phase was washed 2 times with 75 ml ethylacetate. The organic phase was washed with 75 ml aqu. $NaHCO_3$ and 75 ml brine. The organic phase was dried over $Na_2SO_4$, filtered and concentrated in vacuo. The product was purified by silica gel chromatography (toluene: ethylacetate 1:2) to give 348 mg (0.69 mmol, 33%) of **(19)**.

**Reagent camphorsulfonic acid (III)**

Reaction was done under same conditions as before using **(18)** (1.350 g, 2.68 mmol) and CSA (684.9 mg, 2.948 mmol). After 4 h 35 min 0.137 g CSA was added and after 23h 0.137 g CSA was added. After 25 h 45 min 0.137 g CSA was added. There was no further conversion of the educt. Reaction was stopped after 27h. The solution was washed and purified as described above to give 402.3 mg (0.80 mmol, 30%) of **(19)**.

Rf (toluene:ethylacetate 1:1, TLC) 0.33.

## 4.18 Glycosylation experiments

**Promotorsystem NIS/TfOH**

Flask was dried at 50°C for 1 hour. Additionally it was flame-dried. Molecular sieves were added and cooled to RT via HV-pump. Donor **(15)** (22.8 mg, 0.029 mmol) was added and acceptor **(19)** (14.4 mg, 0.029 mmol) were dissolved in 1.0 ml dry DCM and molecular sieves were added. Mixture was stirred for 2 minutes. *N*-Iodosuccinimide (NIS) (6.4 mg, 0.029 mmol) was added and flask was cooled to 0°C. Stock solution out of 1.0 ml dry DCM and

10.0 µl Trifluoromethanesulfonic acid (TfOH) was made and an aliquote on the stock solution (13.0 µl, 0.029 mmol) was added. First TLC's showed no conversion to product.

On the next day concentrated assay showed different spots on TLC. After 95 h the reaction was stopped with 2 droplets triethylamine, diluted with DCM and filtered over Celite. The filtrate was washed with 5% aqueous $Na_2S_2O_3$ (20 ml). Inorganic phase was washed with 10 ml DCM. Organic phases were pooled and washed with 15 ml saturated aqueous $NaHCO_3$, which was washed with 10 ml DCM. HPTLC showed no product in anorganic phases. Organic Phase was dried with solid $Na_2SO_4$, filtered and concentrated. The reaction was purified by gradient silica gel chromatography (started with hexane: ethylacetate 5:1, finished with pure ethylacetate). NMR spectra were made of all fractions. No product was detectable.

**Promotorsystem NIS/Ytterbium(III)trifluoromethanesulfonate**

The 2nd approach was done under the same conditions. Compound **(15)** (13.1 mg, 0.016 mmol) and **(19)** (8.4 mg, 0.017 mmol)) were treated with NIS (4.2 mg, 0.019 mmol) and Ytterbium(III)trifluoromethanesulfonate (1.5 mg, 0.002 mmol) as promotor-system. After 68 h 55 min 0.15 equ. NIS were added. After 98 h the reaction was stopped by 2 droplets triethylamine. Filtrate was washed and purified as described before.

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