Synthesis of Structures Related to the Capsular Polysaccharide of *Neisseria meningitidis* Serogroup A and to Mycothiol

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Abstract

This thesis describes the synthesis of structures related to the capsular polysaccharide of Neisseria meningitidis serogroup A and the synthesis of analogues of mycothiol, a compound produced by Mycobacterium tuberculosis. The first part of the thesis describes the synthesis of structural elements present in the native capsular polysaccharide of Neisseria meningitidis serogroup A. In this part, an improved synthesis of 2-azido-2-deoxy-D-mannopyranose is included. The second part of the thesis describes the formation of stable C-phosphonate analogues related to the capsular polysaccharide. The last part outlines the formation of analogues of mycothiol, where the syntheses of a bicyclic analogue and a thioglycosidic analogue are described.
List of publications

This thesis is based on the following papers, which will be referred to by their Roman numerals I-V.

I **Synthesis of structures corresponding to the capsular polysaccharide of *Neisseria meningitidis* group A**

II **Improved synthesis of 1,3,4,6-tetra-α-O-acetyl-2-azido-2-deoxy-α-D-mannopyranose**

III **Synthesis of stable C-phosphonate analogues of *Neisseria meningitidis* group A capsular polysaccharide structures using modified Mitsunobu reaction conditions**

IV **Synthesis of fused bicyclic thioglycosides of N-acylated glucosamine as analogues of mycothiol**

V **Synthesis of 1-S-(2-amino-2-deoxy-α-D-glucopyranosyl)-1-deoxy-1-thio-D-myo-inositol, a precursor to the thioglycoside analogue of mycothiol**
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*Manuscript*

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Abbreviations

AgOTf  silver triflate (trifluoromethanesulfonate)
AIBN  azobisobutyronitrile
All   allyl
CAN   cerium ammonium nitrate
mCPBA m-chloroperoxybenzoic acid
CPS   capsular polysaccharide
DBU   1,8-diazabicyclo[5.4.0]undec-7-ene
DCC   N,N-dicyclohexylcarbodiimide
DDQ   2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIAD  diisopropyl azodicarboxylate
DMTST dimethyl(methylthio)sulfonium triflate
DPPA  diphenylphosphonic azide
EDCI  1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
Glc   glucose
HOBt  1-hydroxybenzotriazole hydrate
MenA  Neisseria meningitidis serogroup A
NIS   N-iodosuccinimide
NPhth phthalimido
Piv-Cl pivaloyl chloride
PMB   p-methoxybenzyl
TBAF  tetra-n-butylammonium fluoride
TFA   trifluoroacetic acid
TMSBr trimethylsilyl bromide
TMSOTf trimethylsilyl triflate
TREAT-HF triethylamine tris(hydrogen fluoride)
Ts    p-toluenesulfonyl
Z     benzyloxy carbonyl
1 Introduction

Meningitis is a viral or bacterial infection of the meninges, the thin lining that surrounds the brain and spinal cord. This is one of the most feared infections due to its rapid progression and the serious brain damage it causes. Mainly three bacterial species are associated with meningitis: *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis*, which is the most important because of its potential to cause epidemics of meningitis. 5-15% of the human population carries this bacteria without having any symptoms. A major step in disease progression is when the bacteria colonise the nasopharynx and invade the circulatory system. In its most severe form, heightened levels of bacteria in the blood make their way to the meninges in the brain and induce inflammation. 10-15% of these infections are fatal even with antibiotic treatment, and approximately 10% of patients who recover are left with permanent neurological defects.

*Neisseria meningitidis* is a Gram-negative bacterium with both an outer and an inner cell membrane that are separated by a peptidoglycan cell wall. A capsular polysaccharide (CPS) surrounds the outer cell membrane and this surface polysaccharide, apart from being an important antigen, also protects the bacteria from phagocytosis by the host’s immune defence. From the chemical composition of the capsular polysaccharide, 13 serogroups of *N. meningitidis* have been defined and five of these serogroups: A, B, C, W135 and Y account for approximately 90% of the disease burden.

The prevalence of these different serogroups varies geographically, with B and C responsible for meningitis in industrialised countries in Europe and North America, whereas serogroup A is responsible for the massive epidemics of meningitis that sporadically affect sub-Saharan Africa. This region from Senegal to Ethiopia is known as the meningitis belt where up to 2% of the population can be affected during an epidemic. The size of these epidemics can be enormous, as in 1996, when 200 000 cases and 20 000 deaths were reported. A well-working vaccine to control the disease is therefore urgently required in this region.
1.1 Glycoconjugate vaccines

The first vaccines against meningococcal disease were developed and tested in humans in the early 1900s. These initial vaccines that were based on heat-killed whole-cell meningococci showed adverse side effects and variable efficiency. Gotschlich and co-workers achieved the first major success in vaccination against meningococcus in the 1960s. They developed a method for the production of highly purified meningococcal polysaccharide and demonstrated that the polysaccharide was immunogenic and protective in adult volunteers. Unfortunately, the duration of the induced immunity was limited. Furthermore, purified polysaccharide proved to be poorly immunogenic in infants and young children, the age group at greatest risk of infection. The reason for this is that polysaccharide antigens induce an immune response without the involvement of T-cells: the antigen is called T-cell independent. Such a response lacks several important features that characterise the T-cell dependent immune response, such as immunological memory, a class switch from IgM to IgG, and affinity maturation.

Linkage of the polysaccharide to an immunogenic carrier protein creates glycoconjugates, which are T-cell-dependent antigens capable of giving protection to young children and inducing immunological memory. Various protein carriers have been used and the best results have been achieved using detoxified versions of strongly immunogenic proteins like diphtheria and tetanus toxins. During the 1990s, four *Haemophilus influenzae* type b (Hib) glycoconjugate vaccines were introduced with great results. The meningitis caused by Hib has virtually been eradicated in countries with high immunisation coverage. Development of such glycoconjugate vaccines against *Neisseria meningitidis* are still in progress, but for serogroup C, three well-working vaccines have recently been produced and used in several countries with excellent results. A tetravalent glycoconjugate vaccine against serogroups A, C, Y and W135 has recently been developed by Sanofi-Pasteur and licensed in the USA. This vaccine has shown a 100% efficiency at rising bacterial antibody titres in people between the ages of 11 to 18, but the cost of this vaccine is too high for the developing countries in Africa. The urgent need for a MenA glycoconjugate vaccine for Africa has recently resulted in a partnership between the WHO and the Meningitis Vaccine Project (MVP). The plan is to develop an affordable vaccine and hopefully start mass vaccination by the year 2009 for all people up to 29 years of age living in the African meningitis belt. The problem with serotype B is that the capsular polysaccharide is not immunogenic because it consists of specific sialic acid residues, structures that are found in humans.
The carbohydrate part of a glycoconjugate vaccine is normally a functionalised bacterial CPS. These naturally derived carbohydrates are heterogeneous mixtures that may include impurities and contaminants. The use of synthetic carbohydrate structures, which can be produced as single compounds in a controlled manner without batch-to-batch variability, can eliminate these problems. A successful example is the synthetic *Haemophilus influenzae* type b (Hib) glycoconjugate vaccine, which elicited a higher anticapsular immune response in humans than the commercial polysaccharide vaccine.\(^{11}\) A similar finding was observed with a synthetic oligosaccharide protein conjugate for *Shigella dysenteriae* type 1, which were more immunogenic than a similar product prepared from the native polysaccharide.\(^ {12}\) Synthesis of the carbohydrate part also allows for modifications of the structure that may be impossible to perform on the native material. An important issue when making a glycoconjugate vaccine based on the capsular polysaccharide of serogroup A is the labile nature of the capsular polysaccharide itself.\(^ {9}\) The main goal with this work was to synthesise stable analogues of the capsular polysaccharide of MenA and investigate whether these structures are suitable vaccine candidates.
This chapter describes the synthesis of well defined analogues related to the capsular polysaccharide of Neisseria meningitidis serogroup A (MenA). The capsular polysaccharide of MenA is composed of a monosaccharide repeating unit, 2-acetamido-2-deoxy-\(\alpha\)-d-mannopyranose linked together via \(1\rightarrow6\) phosphodiester bridges. 13-70-90% of the ManNAc residues in the native CPS are O-acetylated, with acetyl groups predominantly at 3-OH, but also at 4-OH probably due to acetyl migration (Figure 2.1).

The main goal was to develop a synthetic pathway that would allow the formation of stable analogues of the native CPS of MenA either by per-acetylation or by introducing different electron-withdrawing acetamido substituents. To investigate various issues such as the dependence of antigenicity and immune response on oligosaccharide size, acetylation and the mode of phosphorylation of a glycoconjugate vaccine, four different substituted trimers of the CPS repeating unit were synthesised. Two of the target trimers were acetylated at positions 3-OH and 4-OH to test whether acetylation is important or not for the immune response. When the native phosphodiester linkage undergoes hydrolysis, a terminal phosphate is formed, therefore two trisaccharides with a terminal phosphate were
synthesised (Figure 2.2). The target structures were all equipped with a spacer to facilitate conjugation to a carrier protein for immunogenic studies.

![Chemical structures](image)

**Figure 2.2** The target structures related to the native CPS of MenA

The synthesis is inspired by earlier work by Oscarson and co-workers,\(^{14}\) where 2-azido-2-deoxy mannose derivatives were used during the synthesis to stabilise the anomeric phosphodiester linkages. The electron withdrawing properties and the non-participating effect of the azido groups stabilised these linkages as expected. Pozgay and co-workers also used this strategy for the synthesis of the non-acetylated trimer target structure using 3,4-di-\(O\)-benzyl protection during the synthesis.\(^{15}\) Our strategy was to use acetate esters instead of benzyl ethers for protection at 3-OH and 4-OH. Acetate esters were also used for protection at 3-OH and 4-OH in the first synthesis of a dimeric analogue of the repeating unit of MenA.\(^{16}\) This approach allowed us to conveniently obtain the target structures both with and without acetates. Another reason was to avoid the usually acidic conditions for removal of the benzyl ethers during the deprotection. We also believed that the acetyl groups would have a stabilising effect on the phosphodiester linkages that would become important later in the synthesis when the azido groups had been converted to the less stabilising acetamido groups.
Figure 2.3 Retrosynthetic analysis
2.1 Synthesis of 1,3,4,6-tetra-O-acetyl-2-azido-2-deoxy-α-D-mannopyranose precursor (Paper II)

A common mannosamine precursor was needed for the synthesis of the structures related to the native CPS of MenA. 2-Azido-2-deoxy-mannose is an important precursor for the synthesis of various mannosamine derivatives, but there were no methods to synthesise it in a simple and high-yielding way. A common method for the synthesis of this compound is the azido-nitration of glucal using cerium ammonium nitrate and sodium azide, which gives both 2-azido-2-deoxy-glucose and 2-azido-2-deoxy-mannose.\textsuperscript{17}

Triflatation and azide displacement of various selectively protected 2-OH glucose derivatives is another method, which has been successfully applied on more complex structures.

An obvious synthetic route would be to use 1,3,4,6-tetra-O-acetyl-α-D-glucopyranose \textsuperscript{1} as the precursor for the inversion of configuration, as this crystalline compound is easily synthesised from glucose in a one-pot reaction.\textsuperscript{18} Compound \textsuperscript{1} was triflated with triflic anhydride to give compound \textsuperscript{2} in 97\% yield, and subsequent triflate displacement with sodium azide gave a clean conversion into 2-azido-2-deoxy-mannose according to TLC and MALDI-TOF (Scheme 2.4). However, during work-up, the product started to decompose and several unidentified by-products started to form, decreasing the isolated yield to about 20\%. Similar results and even lower yields have been reported by other groups.\textsuperscript{19}

\begin{center}
\begin{tikzpicture}
  \node[draw] (1) at (0,0) {\includegraphics[width=0.9\textwidth]{Scheme24.png}};
  \node[draw] (2) at (2,0) {\includegraphics[width=0.9\textwidth]{Scheme24.png}};
  \node[draw] (3) at (4,0) {\includegraphics[width=0.9\textwidth]{Scheme24.png}};

  \draw[->] (1) -- (2) node[midway,above] {\textit{i}) \text{TF}_2\text{O, pyridine}};
  \draw[->] (2) -- (3) node[midway,above] {\textit{ii}) \text{NaN}_3, \text{DMF}};

\end{tikzpicture}
\end{center}

\textbf{Scheme 2.4} Synthesis of 1,3,4,6-tetra-O-acetyl-2-azido-2-deoxy-α-D-mannopyranose 3 \textit{i}) \text{TF}_2\text{O, pyridine} \textit{ii}) \text{NaN}_3, \text{DMF}

Several different work-up procedures were tried, but without improving the yield. Interestingly, the decomposition increased when water was used, and therefore a water-free work-up procedure was developed. When the displacement reaction was complete, most of the DMF was removed under reduced pressure and the residue diluted with toluene was transferred directly to a column containing a slurry of dry silica in dry toluene. The water was removed from the silica by heating it over an open flame until the efflux of water ceased, and it was then stored in an oven at 150 °C overnight and cooled under argon before use. The product was then eluted using a
2.2 Synthesis of the building blocks

The peracetylated 2-azido-2-deoxy-mannose 3, synthesised as described in the previous chapter, was first converted to the ethyl thioglycoside 4 in 68% yield, by treatment with ethane thiol and BF$_3$-etherate (Scheme 2.5). For the temporary protection of the 6-OH group, a TBDMS-ether was chosen. Initially, the idea was to use a dimethoxytrityl ether as the protective group, but the silyl ether was found to give a higher yield in the regioselective protection and also proved to be more stable in subsequent transformations. Subsequent acetylation (one-pot reaction) afforded compound 5 in 99% yield.

Scheme 2.5 Synthesis of the precursor 5 i) EtSH, BF$_3$-etherate, CH$_2$Cl$_2$ ii) 1. NaOMe, MeOH 2. TBDMSCl, pyridine 3. Ac$_2$O

To obtain the first acceptor, the precursor 5 was coupled to a benzyloxy-carbonyl-protected ethanolamine spacer using NIS as the promotor. A high $\alpha$-selectivity was observed, giving compound 6 in 86% yield (Scheme 2.6). Interestingly, if the two acetates were replaced with benzyl ethers, a much lower $\alpha$-selectivity was observed (see page 19). In order to investigate whether the benzyl ethers or the silyl ether was responsible for the low $\alpha$-selectivity, the coupling was performed with compound 4 as the donor. The result was similar as compared to when donor 5 was used, with a high ratio of the $\alpha$-anomer formed. The TBDMS-ether in derivative 6 was then removed to give the acceptor 7 in 97% yield. The risk of acetyl migration to the 6-OH group during cleavage of the TBDMS-ether was avoided by using TREAT-HF as the desilylating reagent. TBAF could not be used due to acetyl migration, facilitated by the more basic conditions.

The same precursor 5 was used to prepare the H-phosphonate elongating monomer by first hydrolysing the thioglycoside in wet methylene chloride with NIS as the promotor to give compound 8. When the reaction was
performed at –20 °C, the α-anomer was formed exclusively in 82% yield, but at room temperature a high percentage of the β-anomer was also formed. Phosphonylation of the hemiacetal using PCl₃/imidazole,²⁰,²² proceeded without any concomitant anomerisation, giving exclusively the α-H-phosphonate donor 9 in 97% yield.

A 6-O-phosphorylated donor 13 was also prepared to be used in the synthesis of target structures with a terminal phosphate group, because attempts to phosphorylate the desilylated trisaccharide 16 failed (see page 11). The TBDMS-ether was removed from compound 5 (75%) and a dibenzyl-protected phosphate was introduced at 6-OH using phosphoramidite chemistry.²¹ The reaction of 10 with dibenzyl N,N-diisopropylphosphoramidite and oxidation with mCPBA gave compound 11 in 67% yield. The thioglycoside was then hydrolysed (79%) as described above for the formation of compound 8 and subsequent phosphonylation using PCl₃/imidazole provided the second H-phosphonate donor 13 in 92% yield.

Scheme 2.6 Synthesis of the building blocks i) NIS, AgOTf, HO(CH₂)₂NHZ, CH₂Cl₂ ii) TREAT-HF, THF iii) NIS, AgOTf, wet CH₂Cl₂ iv) PCl₃, imidazole, Et₃N, MeCN v) 1. dibenzyl N,N-diisopropylphosphoramidite, tetrazole, CH₂Cl₂ 2. mCPBA
2.3 Formation of di- and trisaccharides

Formation of the phosphodiester linkages were performed using standard H-phosphonate chemistry with pivaloyl chloride as the promotor. The H-phosphonate diester formed was then oxidised under mild conditions by adding iodine and water to the reaction mixture. Thus, H-phosphonate donor was condensed with acceptor 7 and oxidised to give the disaccharide 14 in 96% yield (Scheme 2.7).

![Scheme 2.7 Formation of the dimer 14](image)

Scheme 2.7 Formation of the dimer 14 i) 1. Piv-Cl, pyridine 2. I₂, H₂O

The TBDMS ether could then be removed using TREAT-HF to form the disaccharide acceptor 15 in 91% yield. The coupling of this acceptor to donor 9 using the same conditions afforded the trisaccharide 16 in 62% yield (Scheme 2.8). The use of donor 13 in a corresponding coupling gave the second trisaccharide 17 (59%). The decrease in yield for the formation of the trimers could be explained by the decomposition of the anomeric phosphodiester linkages already present in the acceptor, a finding frequently observed with anomeric phosphodiesters.
2.4 Deprotection

The azido groups in trisaccharides 16 and 17 were reduced using sodium borohydride with nickel chloride hexahydrate as a catalyst. The free amino groups were then acetylated in situ using acetic anhydride, which afforded the two trisaccharides 18 and 22 in 89% and 64% yields respectively (Scheme 2.9 and 2.10). The Z-group was then removed from trisaccharide 18 prior to the TBDMS ether. Basic ion-exchange resins were added to prevent hydrolysis of the phosphodiester linkages during the catalytic hydrogenolysis, which gave compound 19 in 83% yield. The silyl ether was then cleaved again using TREAT-HF to give target compound 20 (85%). The presence of acetamido groups instead of azido groups shortened the reaction time significantly for the desilylation. Compound 20 was then deacetylated using sodium methoxide in methanol to obtain target structure 21 in 94% yield.

As mentioned in the introduction, different electron-withdrawing substituents at C-2 were considered to stabilise the phosphodiester linkages. The idea was to introduce trifluoroacetamides after reduction of the azido groups to free amines, either by in situ acetylation (using trifluoroacetic anhydride or ethyl trifluoroacetate) or by first isolating the derivative
containing the free amino groups. Unfortunately these attempts only resulted in decomposition of the starting material.

Scheme 2.9 Formation of target compounds 20 and 21 i) 1. NaBH₄, NiCl₂(H₂O)₆, MeOH 2. Ac₂O ii) Pd/C, H₂, MeOH iii) TREAT-HF, THF iv) NaOMe, MeOH

To obtain target structure 23, the Z-group and the benzyl groups on the phosphate triester was removed from compound 22 in 85% yield using catalytic hydrogenolysis as described for compound 18. However, final deacetylation to obtain the target structure 24 failed. Reversal of the order of deprotection, i.e., initial removal of the acetate esters followed by hydrogenolysis, solved this problem and afforded the target structure 24 in 51% yield over two steps.
Scheme 2.10 Formation of target compounds 23 and 24 i) 1. NaBH₄, NiCl₂(H₂O)₆, MeOH 2. Ac₂O ii) Pd/C, H₂, MeOH iii) NaOMe, MeOH

2.5 Conclusion

An improved synthesis of 1,3,4,6-tetra-O-acetyl-2-azido-2-deoxy-α-D-mannopyranose has been developed. This compound was used as a precursor for the synthesis of the different building blocks needed for the synthesis of structures corresponding to the capsular polysaccharide of Neisseria meningitidis serogroup A. Four different trisaccharides with the native phosphodiester linkages were synthesised starting from a common precursor. The synthetic pathway allowed the formation of target structures with a terminal phosphate and acetyl esters at 3-OH and 4-OH. Conjugation of the structures to a carrier protein and immunogenic studies of the formed conjugates are in progress.
The anomeric phosphodiester linkages in the CPS of MenA are known to be labile. The electron pairs on the ring oxygen or the participating acetamido group at C-2 can displace the anomeric phosphate or phosphodiester under slightly acidic conditions (Figure 3.1). In nature, glycosyl phosphates are key intermediates in the biosynthesis of oligosaccharides and the use of anomeric phosphates as leaving groups has even been exploited in glycosylation reactions. Replacing the anomeric phosphodiester linkage with a stable C-phosphonate linkage, thus creating C-analogues of the CPS of MenA would solve this instability problem.

![Figure 3.1](image-url)  
*Figure 3.1* The instability of the native CPS of MenA and a stable C-analogue.

The phosphonate moiety is assumed to be a stable isosteric analogue of the natural phosphate, incapable of being hydrolysed by the ordinary enzymes involved in phosphate cleavage, and replacement of the anomeric O-P bond with a C-P bond has been employed to make inhibitors of various enzymes. An even better oxygen mimic is the CF₂-moiety because of the electronic properties of the fluorine atom. However, only a few CF₂-isosteres of anomeric phosphates have been synthesised using radical chemistry on anomeric alkenes, which strongly favoured the β-product and gave much lower yields on pyranoses than on furanoses. Synthesis of such analogues...
related to the CPS of MenA was considered to be too complicated, and so C-phosphonate analogues were chosen as target structures. This chapter describes the synthesis of two such analogues: a trimer and a tetramer (Figure 3.2). To investigate whether these analogues could be possible vaccine candidates, they were equipped with a spacer to facilitate conjugation to a carrier protein to form glycoconjugates. Immunogenic studies would then tell whether these analogues were suitable vaccine candidates.

Figure 3.2 Target trimer 47 and target tetramer 50
Figure 3.3 Retrosynthetic analysis of the target trimer 47.

The retrosynthetic analysis (Figure 3.3) shows a linear synthetic route, using a C-phosphonate monoester as elongating monomer and a spacer-equipped acceptor in the coupling reactions.
3.1 Synthesis of the C-phosphonate building block

A straightforward procedure for making C-phosphonate building blocks using a one step Horner-Emmons/Michael reaction has been reported by Nikolaev and co-workers.\textsuperscript{26} They synthesised \(\alpha\)-D-mannosyl methane-phosphonates using various isopropylidene-protected mannose derivatives as precursors. The stereoselectivity depended on the protective group pattern and choice of reaction conditions. When this reaction was tried on 2,3,4,6-tetra-O-benzyl-\(\alpha\)-D-glycopyranose (with the idea of later inversion at C-2 to obtain the mannosamine derivative), the result was only anomerisation of the starting material. Instead a longer synthetic route towards the C-phosphonate building block was chosen. The alkene \textsuperscript{26,27} previously synthesised from 2,3,4-tri-O-benzyl-D-arabinose \textit{via} vinylation at C-1 using divinylzinc, was obtained from the readily accessible derivative \textsuperscript{25} via a Wittig reaction\textsuperscript{29} in 91\% yield (Scheme 3.4). Subsequent stereoselective cyclisation of the obtained alkene with mercury acetate\textsuperscript{27} afforded exclusively the \(\alpha\)-linked C-glucoside \textsuperscript{27} (80\%) and reaction of the mercury chloride with iodine gave the corresponding stable iodide \textsuperscript{28} in 99\% yield.

Conversion of \textsuperscript{28} into the phosphonate required temporary protection of the free hydroxyl group at C-3 (former C-2) in order to prevent the formation of a cyclic phosphonate.\textsuperscript{30} A TBDMS ether was introduced and a subsequent Arbuzov reaction with triethyl phosphite gave the desired C-phosphonate \textsuperscript{29} in 77\% yield over two steps. The silyl ether was then removed using TBAF to give \textsuperscript{30} in 99\% yield.

Scheme 3.4 Synthesis of the C-phosphonate building block

\textit{i) BuLi, methyl triphenylphosphonium bromide \textit{ii) Hg(AcO)}\textsubscript{2}, KCl, THF \textit{iii) I\textsubscript{2}, CH\textsubscript{2}Cl\textsubscript{2} \textit{iv) 1. TBDMSCl, pyridine, imidazole 2. P(OEt)}\textsubscript{3} \textit{v) TBAF, THF}
Several attempts to introduce the azido functionality as a precursor for the acetamido group at position C-3 were made. The obvious procedure using the triflate of compound 30 for the displacement reaction with azide ion proceeded in low yield (30%). However, the use of Mitsunobu conditions\textsuperscript{31} instead and DPPA\textsuperscript{32} as the azide source gave the azido derivative 31 in high yield (Scheme 3.5). The primary benzyl ether was then replaced with an orthogonal acetyl ester using acetylation conditions. A high regioselectivity was observed when a mixture of acetic anhydride and acetic acid (1:1) was used together with sulfuric acid. The azido group was then reduced with sodium borohydride and nickel chloride hexahydrate, and \textit{in situ} acetylation of the resulting amine gave 33 in 76% yield.

The ethyl phosphonate esters had to be replaced with methyl esters since selective cleavage of one of the ethyl groups was difficult to perform. Removal of both ethyl groups with bromotrimethyl silane\textsuperscript{29} afforded the phosphonic acid 34 in quantitative yield. The dimethyl phosphonate 35 was then formed using acetic acid and trimethyl orthoacetate (84%).\textsuperscript{33} One methyl group was then selectively removed using triethylamine and phenyl mercaptan,\textsuperscript{34} which afforded the monomethyl phosphonate 36 in 92% yield.

\begin{center}
\textbf{Scheme 3.5} Synthesis of the C-phosphonate building block 36

\begin{itemize}
  \item i) Ph\textsubscript{3}P, DIAD,
  \item ii) HOAc, Ac\textsubscript{2}O, H\textsubscript{2}SO\textsubscript{4}
  \item iii) 1. NaBH\textsubscript{4}, NiCl\textsubscript{2}(H\textsubscript{2}O)\textsubscript{6}, MeOH 2. Ac\textsubscript{2}O
  \item iv) Me\textsubscript{3}SiBr, CH\textsubscript{2}Cl\textsubscript{2}
  \item v) CH\textsubscript{3}C(OMe)\textsubscript{3}, HOAc
  \item vi) PhSH, Et\textsubscript{3}N
\end{itemize}
\end{center}
3.2 Synthesis of the monosaccharide acceptor

To obtain the monosaccharide acceptor, benzylation of deacetylated compound 6 was first tried (see page 9), but this approach was abandoned because of concomitant benzylation of the carbamate nitrogen in the spacer. Instead, compound 37 (obtained from 2-azido-2-deoxy-mannose in four steps) was used in an NIS-promoted coupling to a Z-protected ethanolamine spacer (Scheme 3.6). However, this reaction gave a low stereoselectivity compared to the coupling using donor 5 (see page 9), forming the α-glycoside in only 50% yield. As discussed earlier, the benzyl ethers at 3-O and 4-O were the reason for the rather low α/β-ratio observed. The 50% yield of compound 38 was eventually accepted and we continued with reduction of the azido group and subsequent acetylation of the free amine to give compound 39 in 85% yield. Finally, cleavage of the TBDMS ether using TBAF formed the acceptor 40 in 99% yield.

Scheme 3.6 Synthesis of the acceptor 40

- i) NIS, AgOTf, HO(CH₂)₂NHZ, CH₂Cl₂
- ii) 1. NaBH₄, NiCl₂(H₂O)₆, MeOH 2. Ac₂O
- iii) TBAF, THF

3.3 Synthesis of phosphonic acid esters

Esterification of a phosphonic acid can be accomplished with condensing agents, such as DCC³⁵ or trichloroacetonitrile³⁶ together with the appropriate alcohol. A large excess of both the condensing agent and the alcohol is required, and the yields vary depending on the components being coupled. First, we tried to couple the phosphonic acid 34 directly to the acceptor 40 using DCC with poor results. The coupling of the monomethyl phosphonate 36 and the acceptor was then tried using a number of coupling reagents: DCC, 2,4,6-trisopropylbenzenesulfonyl chloride (TIPS-Cl) and 1-(2-mesitylenesulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT), but all procedures
resulted in low yields caused either by the formation of the pyrophosphonate as the major product or by the low reactivity of the starting materials. The best isolated yield of 41 was 20%, which was achieved with 5 equivalents of DCC in pyridine.

Another method to form phosphonic acid esters is to use the Mitsunobu reaction with triphenylphosphine and a dialkyl azodicarboxylate.\textsuperscript{37} Using standard Mitsunobu conditions in the coupling of 36 to 40, the dimer 41 was formed in about 50% yield. This yield was further improved by using the modified Mitsunobu procedure developed by Campbell and co-workers using trichlorotriphenylphosphine instead of triphenylphosphine together with a large access of triethylamine.\textsuperscript{39} Utilising this procedure, the yield was increased to a reproducible 89% (Scheme 3.7). An advantage of using the monoester 36 in these reactions, was that the reaction products were not charged, which simplified the purification. The only drawback was the formation of diastereoisomers that made characterisation by NMR spectroscopy more problematic. The work by Russo and co-workers,\textsuperscript{38} describing the formation of phosphonic acid esters and their synthesis of the phosphono analogue of the dimeric subunit of MenA was not available at the time of this research. Interestingly they report the formation of a similar dimer to 41 in 97% yield using standard Mitsunobu conditions.

\begin{center}
\begin{tikzpicture}
\node (i) at (0,0) {36 \text{AcO}};
\node (ii) at (2,0) {40 \text{NHAc}};
\node (iii) at (1,-1) {41 (89\%)};
\node (iv) at (0,-2) {AcO \text{NHAc}};
\node (v) at (2,-2) {HO \text{NHAc}};
\node (vi) at (1,-3) {\text{O} \text{O}};
\node (vii) at (0,-4) {\text{BnO} \text{BnO} \text{BnO}};
\node (viii) at (2,-4) {\text{BnO} \text{BnO} \text{BnO}};
\node (ix) at (0,-5) {\text{OMe}};
\node (x) at (2,-5) {\text{NHZ}};
\node (xi) at (0,-6) {\text{O} \text{O}};
\node (xii) at (2,-6) {\text{BnO} \text{BnO} \text{BnO}};

\draw[->, thick] (i) -- (ii);
\draw[->, thick] (ii) -- (iii);
\draw[->, thick] (iii) -- (i);
\draw[->, thick] (i) -- (iv);
\draw[->, thick] (iv) -- (v);
\draw[->, thick] (v) -- (vi);
\draw[->, thick] (vi) -- (vii);
\draw[->, thick] (vii) -- (viii);
\draw[->, thick] (viii) -- (ix);
\draw[->, thick] (ix) -- (x);
\draw[->, thick] (x) -- (xi);
\draw[->, thick] (xi) -- (xii);
\end{tikzpicture}
\end{center}

\textbf{Scheme 3.7} Formation of the dimer 41 i) (pClPh)\textsubscript{3}P, DIAD, Et\textsubscript{3}N, THF
The disaccharide acceptor 42 was then formed by removal of the acetate ester using KOH in methanol. Subsequent coupling of this acceptor 42 to the monomethyl phosphonate 36 using the same conditions as for acceptor 40 afforded the trimer 43 in 92% yield (Scheme 3.8).

Scheme 3.8 Formation of the trimer 43 and tetramer 45 i) KOH, MeOH ii) (pClPh)_3P, DIAD, Et_3N, THF
Finally, deacetylation of 43 (84%) to form acceptor 44 and Mitsunobu reaction with the C-phosphonate monomer 36 afforded the target protected tetramer 45 in 85% yield. As hoped, no drop in yields were observed, when larger structures were used in the Mitsunobu reactions in contrast to the decreased yield when forming the native trisaccharide (see page 11).

3.4 Deprotection

The methyl esters in compound 44 were removed using the stronger base DBU (instead of triethylamine) to give 46 in 75% yield. Subsequent hydrogenolysis, using palladium on charcoal together with HCl to prevent poisoning of the catalyst, removed the benzyl ethers and the benzyloxy-carbonyl group to form the target trimer 47 in 83% yield (Scheme 3.9).

Scheme 3.9 Deprotection of the trimer i) PhSH, DBU, MeCN ii) Pd/C, H₂, HCl, MeOH
The tetramer 45 was deprotected similarly by first removing the acetate ester to give 48 in 73% yield, followed by demethylation using the same conditions as before to obtain compound 49 (Scheme 3.10). Finally, catalytic hydrogenolysis formed the target tetramer 50 in 85% yield.

Scheme 3.10 Deprotection of the tetramer i) KOH, MeOH ii) PhSH, DBU, MeCN iii) Pd/C, $\text{H}_2$, HCl, MeOH

3.5 Polycondensation

An attractive route to larger structures would be a polycondensation between the deacetylated phosphonic monomer 36 together with the spacer equipped acceptor 40. According to MALDI-TOF, these attempts resulted in clean formations of oligomeric mixtures from dimers up to heptamers all containing the spacer equipped acceptor with no or little decomposition in between the oligomers. However, the dimers and trimers were always the dominant components of the mixtures, with smaller amount of tetramers to heptamers.
Attempts to increase the formation of the larger structures were made using more of the elongating monomer 36 and reagents, but this only resulted in similar oligomeric mixtures but without including the spacer-equipped acceptor. This area of research will be continued and hopefully the right conditions for the polycondensation will be found.

3.6 Conclusion

An efficient synthesis of stable C-phosphonate analogues, a trimer and a tetramer, related to the capsular polysaccharide of Neisseria meningitidis serogroup A has been developed. The key step was the formation of the inter-saccharide phosphonate ester linkage between an elongating C-phosphonate monomer and a spacer equipped acceptor, using a modified Mitsunobu reaction. The methodology allowed iterative synthesis of larger structures and conjugation and immunogenic studies to investigate whether these structures are possible candidates for a glycoconjugate vaccine are in progress.
4 Synthesis of Structures Related to Mycothiol

Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis*. Tuberculosis most commonly attacks the lungs but can also affect the central nervous system, the lymphatic system, the circulatory system, the bones and the skin. Over one-third of the world's population carries the TB bacterium and about 10% of these will develop the active disease. Each year approximately 2 million people die of TB and about 8 million new cases are diagnosed. In Africa, the estimates for 2002 were 2.3 million cases with 550,000 deaths. The high mortality rates for Africa have been attributed to the association of TB with HIV and AIDS, and TB is now recognised as a leading cause of death among people who are HIV-positive. Another concern is the emergence of multidrug resistant strains of the bacteria, therefore a major priority is to develop alternative drug therapies.

Low molecular-weight thiols play a key role in maintaining a reducing environment in the cell. In eukaryotes and Gram-negative bacteria, the tripeptide glutathione protects the cell from oxidative stress and alkylating agents. *Mycobacterium tuberculosis* in common with other actinomycetes, instead produces the pseudodisaccharide mycothiol, 1D-1-\(O\)-(2-\([N\text{-acetyl-}L\text{-cysteinyl}]\)amino-2-deoxy-\(\alpha\text{-}D\text{-glucopyranosyl}\)-myo-inositol (Figure 4.1). Mycothiol is believed to play an analogous role to glutathione in maintaining a reducing intracellular environment in these Gram-positive bacteria.

![Figure 4.1 The structure of mycothiol](image-url)
The increased sensitivity of mycothiol-deficient mutants towards electrophiles, free radicals and antibiotics suggest that the enzymes involved in the biosynthesis are potential targets for drugs. The complete biosynthetic pathway of mycothiol has recently been elucidated and the two enzymes MshA and MshC, have been identified as potentially important drug targets (Figure 4.2).

![Biosynthetic pathway of mycothiol](image)

**Figure 4.2** Biosynthetic pathway of mycothiol

### 4.2 Synthesis of a fused bicyclic donor related to mycothiol (Paper IV)

Although mycothiol is a rather small molecule, its synthesis involves several challenges: regioselective protection and resolution of myo-inositol, formation of the α-D-glucosamine glycosidic linkage, and introduction of the cysteine moiety. Up until now, three total syntheses of mycothiol have been developed. Analogues of mycothiol, with variations either in the inositol or in the cysteine component have also been synthesised, and these analogues have shown interesting inhibition properties for the enzymes involved in the mycothiol biosynthesis. To prepare analogues with variations in the inositol component, a bicyclic thioglucoside donor was
considered (see page 28). The idea was inspired by the work of Boons and co-workers who discovered neighbouring group participation of a (1S)-phenyl-2-(phenylsulfanyl)ethyl moiety at C-2 of a glucosyl donor, creating 1,2-cis glycosides via an intermediate β-sulfonium ion (Figure 4.3). Activation of the thioglucoside 54 with a thiophilic reagent in the presence of an acceptor would hopefully yield the 1,2-cis glycoside through an SN2-type displacement reaction.

![Figure 4.3](image)

**Figure 4.3** The formation of 1,2-cis glycosides via an intermediate β-sulfonium ion

Two possible approaches were considered for the synthesis of the bicyclic donor 54, either by first forming the amide bond between a glucosamine derivative and L-cysteine followed by the construction of the thioglycosidic linkage or vice versa. Several problems were encountered with the first approach. The peptide coupling of various 2-amino-2-deoxy-glucose derivatives with cysteine moieties proceeded in poor yields. The best yield (34%) was achieved when S-trityl-N-acetyl-L-cysteine and free 2-amino-2-deoxy-D-glucose were used in the coupling, but then after detritylation, no conditions would be found to form the thioglycosidic linkage. This approach was therefore abandoned and attention was instead concentrated on the second route, where the thioglycosidic linkage is formed first.

To ensure creation of a β-linkage, peracetylated 2-deoxy-2-phthalimido glucose 51, was used as the donor. The coupling with N-acetyl-L-cysteine as the acceptor was promoted by borontrifluoride etherate and gave the desired β-thioglycoside 52 in 68% yield (Scheme 4.4). This compound was treated with ethylene diamine, to remove the phthalimid and acetyl groups, and form the zwitterion 53. The intramolecular peptide coupling was achieved using HOBT and EDCI as the condensation agents, which formed the bicyclic compound 54 isolated as its tri-O-acetate in 50% yield. Attempts were made to improve this yield, but the polar nature of compound 53 and its low solubility in organic solvents made this complicated, and eventually, the 50% yield in the condensation reaction was accepted. In the literature, similar cyclisation reactions have been reported for the corresponding O-serine glycosides. These compounds were formed as by-products either during N-acetylation or during removal of the 2-amino protecting group. 51-53
Scheme 4.4 Synthesis of the bicyclic donor 54 i) N-acetyl-L-cysteine, BF$_3$-etherate ii) ethylene diamine, MeOH iii) 1. EDCI, HOBr, DMF, H$_2$O 2. Ac$_2$O, pyridine iv) NaOMe, MeOH

The idea was to use NIS or DMTST as promoters for the glycosylation and to create a free thiol or a methyl disulfide in the product. First, an inositol acceptor was used, which did not result in any product formation. The same observation was made with less sterically hindered acceptors, such as a benzyloxycarbonyl-protected ethanolamine spacer, and even under forcing conditions (high temperatures and large access of promoter) with methanol as the acceptor, only traces of product were detected by MALDI-TOF. Although the donor seemed to be very stable it eventually started to decompose under the forcing glycosylation conditions. Compound 54 was then deprotected to give 55, which will be evaluated as an inhibitor of enzymes in mycothiol biosynthesis.

Figure 4.5 Crystal structure of compound 55
4.3 Synthesis of a thioglycoside analogue of mycothiol (Paper V)

The goal was to develop a methodology for preparing stable analogues of the biosynthetic intermediates of mycothiol because of evidence that potential inhibitors were being hydrolysed by glycosidases during biological studies. Inhibitors with stable glycosidic linkages would solve this problem, and a thioglycoside analogue to mycothiol was considered as an interesting target compound (Figure 4.6). Thioglycosides, in which the glycosidic oxygen has been replaced with a sulfur atom, are known to be stable glycoside analogues and act as competitive inhibitors of many glycosidases.

![Thioglycoside analogue of mycothiol](image)

**Figure 4.6** Thioglycoside analogue of mycothiol

To synthesise the target compound both general procedures for introduction of thioglycosidic linkages were investigated (Scheme 4.9 and 4.10). A GlcNAc mercaptan was reacted with an inositol derivative in a $S_N^2$ displacement reaction and a thioinositol acceptor was prepared and reacted with a suitable glucosamine donor.

The first step was a chiral resolution of myo-inositol as described by Pietrusiewicz and co-workers, where crystalline myo-inositol camphor acetals were prepared directly from myo-inositol. The preparation of D- or L-camphor dimethyl acetals and subsequent reaction with myo-inositol, produced the D-myositol-2,3- or 1,2-camphor acetal, respectively. An optimised procedure was used, where the camphor dimethyl acetal was produced in situ. Using this approach, the chirally pure myo-inositol-D-1,2-L-camphor acetal 56 was obtained (Scheme 4.7). The remaining free hydroxyl groups were protected as benzyl ethers and acidic hydrolysis of the acetal formed the known diol 57 in 73% yield over two steps.
Scheme 4.7 Resolution of myo-inositol using L-camphor dimethyl acetal, forming myo-inositol-D-1,2-camphor acetal 56 i) 1. L-camphor dimethyl acetal, H$_2$SO$_4$, DMSO 2. TsOH, DMSO, CHCl$_3$, MeOH ii) 1. BnBr, NaH, DMF 2. TFA, CHCl$_3$

To prepare the 1-O-triflyl-D-chiro-inositol (the triflate of compound 60), needed for the displacement reaction with the α-GlcNAc mercaptan, the diol 57 synthesized as described, was converted to the alcohol 58 by a three-step procedure: regioselective allylation of the equatorial alcohol \textit{(via the dibutylstannylene acetal)},$^{58}$ benzylaion of the axial alcohol and then removal of the allylic ether formed compound 58, in 87% yield over three steps (Scheme 4.8). Then an epimerization reaction at position C-1 of compound 58 was necessary to create an axial alcohol. Mitsunobu conditions$^{31}$ using p-nitrobenzoic acid was tested first, but without any success. Then 58 was converted to the corresponding triflate, and subsequent treatment with sodium nitrite afforded the D-chiro-inositol derivative in about 50% yield, but a large amount of elimination products was also formed. An oxidation/reduction procedure was then tested by first forming the myo-inosose derivative using Swern conditions$^{59}$ to give 59 in 90% Yield. To form the axial hydroxyl group, the hydride had to be introduced equatorially to the ketone. A large reducing agent such as L-selectride$^{60}$ afforded the equatorial alcohol exclusively, whereas the smaller reducing agent NaBH$_4$ in THF/MeOH 7:2 gave a 1:1 mixture of isomers. However, when the solvent was changed to a less polar solvent (CH$_2$Cl$_2$/MeOH 20:1) an increase in stereoselectivity was observed with NaBH$_4$ giving the desired isomer 60 in 70% yield.
Scheme 4.8 Synthesis of the S-acceptor 62

\[ \text{Scheme 4.8: Synthesis of the S-acceptor 62} \]

\[ 1. \text{Bu}_3\text{SnO, toluene} \]
\[ 2. \text{AllBr, CsF, DMF} \]
\[ 3. \text{BnBr, NaH, DMF} \]
\[ 4. (\text{Ph}_3\text{P})_3\text{RhCl, DBU, EtOH} \]
\[ 5. \text{HCl, acetone} \]

\[ \text{Scheme 4.9 Displacement reaction } \]

\[ \text{i) NaH, DMF} \]

The $\alpha$-GlcNAc mercaptan 63 was synthesised as described by Knapp and co-workers. By treating 2-acetamido-2-deoxy-$\beta$-D-glucopyranose tetraacetate with the Lawesson’s reagent the thiazoline was formed and subsequent hydrolysis gave the $\alpha$-GlcNAc mercaptan 63. Attempted couplings of 63 with the 1-$O$-triflate of compound 60 were carried out by first deprotonating the thiol with a base and then reacting it with the inositol derivate (Scheme 4.9). However, these reactions resulted only in the elimination of the triflate or the formation of disulfides. This approach was therefore abandoned and our attention turned instead to the preparation of a thioinositol acceptor to be used in a glycosylation reaction with an appropriate glycosyl donor.
The triflate of compound 60 was used as the starting material for the reactions with various sulfur nucleophiles. First, potassium thioacetate or tetrabutylammonium thioacetate were tried, which resulted mainly in formation of elimination products and low yields of the desired product 61 (Scheme 4.8). The best yield (22%) was obtained when the displacement reaction was carried out in DMF at room temperature with KSAc as the nucleophile. Other sulfur nucleophiles such as thiourea and thiocyanate were also tried but resulted in even lower yields. Another approach was to use the myo-inosose derivative 59 and to convert the carbonyl to a thio carbonyl using Lawesson’s reagent. However, the reduction of the thiocarbonyl was not stereoselective and also caused degradation of the starting material.

Similar low-yielding displacement reactions of myo-inositol with various sulfur nucleophiles have been reported before. Le Gall and co-workers developed an alternative route to introduce the sulfur by using an intramolecular sulfur–delivery reaction to produce 1-deoxy-1-thio-myoinositol in relatively high yield.

After some time, the poor yield (22%) was accepted and the deacetylation was carried out in 96% yield to form the target acceptor 62. The glycosylation with 3,4,6-tri-O-acetyl-2-azido-2-deoxy-α,β-D-glucopyranosyl trichloroacetimidate as donor gave a good α-selectivity and formed the thioglycoside 64 in 73% yield (Scheme 4.10). Debenzylation was carried out using Birch reduction as required for sulphur-containing compounds. This was performed prior to the introduction of various cysteine derivatives, since amide linkages are not compatible with the deprotection conditions. Thus, removal of the acetyl groups (74%) and then Birch reduction, gave the target compound 66 in 83% yield, ready to be coupled to cysteine or labelled acetic acid for biological studies.
Scheme 4.10 Glycosylation and deprotection i) TMSOTf, diethyl ether ii) NaOMe, MeOH iii) NH$_3$ (l), Na, THF
4.4 Towards the synthesis of a C-glycoside analogue of mycothiol (Appendix A)

C-glycosides are compounds where the interglycosidic oxygen is replaced with a methylene group. These analogues are interesting because of their possible activity as enzyme inhibitors in combination with their stability towards enzymatic degradation. As mentioned in the previous chapter, stable analogues of the biosynthetic intermediates of mycothiol were needed, and this chapter describes an attempt to synthesise the C-glycoside of mycothiol (Figure 4.11).

![Figure 4.11 Stable C-analogue of mycothiol](image-url)

Since the report on the first synthesis of a C-disaccharide, several synthetic procedures have been developed. The intermolecular reaction of a nucleophilic anomeric radical with an alkene is a widely used procedure to form C-glycosides. Giese and Witzel developed a method where a glycosyl halide and a Michael acceptor containing an exo-cyclic double bond reacts under radical conditions to form a C-C bond (Figure 4.12). The idea was to synthesise the Michael acceptor derived from myo-inositol and to react it with a suitable glycosyl halide under these radical conditions to form a C-glycoside analogue of mycothiol.

![Figure 4.12 Formation of a C-glycoside](image-url)

Figure 4.12 Formation of a C-glycoside i) 1. AIBN, Bu₃SnH 2. Ac₂O
The diol 57 was subjected to regioselective allylation (via the dibutyl-stannylene acetal) of the equatorial hydroxyl group to form compound 67 in 89% yield (Scheme 4.13). A 4-methoxybenzyl ether was installed as an orthogonal protective group for the axial hydroxyl group and subsequent removal of the allyl group using Wilkinson’s catalyst and DBU formed the alcohol 68 in 91% yield over two steps. Oxidation of the alcohol was achieved using Swern conditions forming the myo-inosose derivative 69 in 94% yield. The following Wittig reaction initially resulted in low yields because elimination of benzyl ethers occurred during the reaction. Interestingly this problem was solved by using forcing conditions (high temperatures, large access of the base and the phosphonium salt), which resulted in complete avoidance of elimination products and formation of compound 70 in 86% yield.

Scheme 4.13 Formation of the Michael acceptor 72 i) 1. Bu₂SnO, toluene 2. AllBr, CsF, DMF ii) 1. 4-methoxybenzyl bromide, NaH, DMF 2. (Ph₃P)₃RhCl, DBU, EtOH 3. HCl, acetone iii) DMSO, oxalyl chloride, CH₂Cl₂, Et₃N iv) methyl-triphenylphosphonium bromide, tBuOK, toluene v) TFA, CHCl₃

Removal of the 4-methoxybenzyl ether also caused problems. Standard procedures using either DDQ or CAN resulted in no reaction or degradation of the starting material. However, pure acidic conditions cleaved the 4-methoxybenzyl ether cleanly and efficiently (92%) and subsequent Swern oxidation gave the desired Michael acceptor 72 in 92% yield.
The prepared Michael acceptor and acetobromoglucose were used in the radical reactions using similar conditions as described by Vogel and co-workers. The alkene, tributyltin hydride and AIBN were dissolved in benzene and slowly added to a heated solution of acetobromoglucose in benzene. However the desired product could not be detected. Instead, a by-product was observed on MALDI-TOF that corresponded to the weight of two molecules of compound \( \text{72} \) (Scheme 4.14). This product was exclusively formed during all attempts, and NMR analysis showed the formation of a hetero Diels-Alder product as a single isomer \( \text{73} \). The Diels-Alder product was also formed when the Michael acceptor was stored at room temperature. A similar reaction has been observed when the simpler molecule 2-methylene-cyclohexanone was left in storage.

![Scheme 4.14 Formation of the hetero Diels-Alder product \( \text{73} \)](image)

To avoid the formation of the hetero Diels-Alder product \( \text{73} \) a different approach may be required. The use of compound \( \text{71} \) instead of the Michael acceptor \( \text{72} \) in an intramolecular radical cyclisation, would hopefully lead to a C-glycoside analogue of mycothiol.
4.5 Conclusion

In conclusion, a synthesis of a bicyclic thioglycoside 55 that mimics mycothiol was developed. This mimic will be evaluated as an inhibitor for the various enzymes involved in the biosynthesis of mycothiol. However, glycosylation reactions of donor 54 with various acceptors to create more mimics failed, probably because of the great stability of the bicyclic system. A thioglycosidic analogue of a biosynthetic intermediate of mycothiol was prepared using a thioanalogue to myo-inositol in glycosylation reactions with 3,4,6-tri-O-acetyl-2-azido-2-deoxy-α,β-D-glucopyranosyl trichloroacetimidate. The target compound is ready to be coupled to cysteine or labelled acetic acid for following biological studies.

The attempted formation of a C-glycoside analogue of mycothiol using radical chemistry failed. However, a high yielding synthesis of the target Michael acceptor 72 was developed and further exploration of the radical reaction will hopefully allow synthesis of a C-glycoside analogue of mycothiol.
Appendix A

1-O-Allyl-3,4,5,6-penta-O-benzyl-D-myoinositol (67).
The diol 57 (1.0 g, 1.85 mmol) and Bu₂SnO (0.51 g, 2.03 mmol) were dissolved in toluene (20 mL) and refluxed for 2 h. The reaction mixture was then cooled to room temperature and concentrated. The residue was dissolved in DMF (10 mL) and allyl bromide (0.20 mL, 2.22 mmol) was added. The mixture was cooled to 0 °C before caesium fluoride (0.843 g, 5.55 mmol) was added and the mixture was allowed to attain room temperature and stirred over night. The mixture was diluted with (EtOAc) and washed with water. The organic layer was filtered through a silica plug and concentrated. Purification by silica gel chromatography (toluene/EtOAc 1:0 → 10:1) gave 67 (0.953 g, 1.64 mmol, 89%); ¹³C NMR δ 138.8, 138.8, 138.1, 134.8, 128.6-127.6 (aromatic C), 117.5, 83.2, 81.3, 81.3, 80.0, 79.7, 76.0, 76.0, 76.0, 72.9, 72.0, 67.8; ¹H NMR δ 7.43-7.26 (m, 20H), 6.02-5.95 (m, 1H), 5.34 (dq, J = 1.65, 17.2 Hz, 1H), 5.24 (dq, J = 1.47, 10.4 Hz, 1H), 4.97-4.85 (m, 8H), 4.35 (t, J = 2.56 Hz, 1H), 4.24-4.22 (m, 2H), 4.05 (t, J = 9.52 Hz, 1H), 4.01 (t, J = 9.52 Hz, 1H), 3.53-3.45 (m, 2H), 3.35 (dd, J = 2.75, 9.7 Hz, 1H), 2.56 (s, 1H).

3,4,5,6-Penta-O-benzyl-2-O-(4-methoxybenzyl)-D-myoinositol (68).
A solution of compound 67 (3.73 g, 6.43 mmol) in DMF (40 mL) was added dropwise to 60% NaH (386 mg, 9.65 mmol) at 0 °C. After the addition, the mixture was stirred for 30 min at 0 °C, then 4-methoxybenzyl bromide (1.39 mL, 9.64 mmol) was added. The reaction mixture was allowed to reach room temperature and stirred for 1 h. The reaction was quenched with MeOH and toluene was added to dilute the mixture, which was washed with water and filtrated through a plug of silica. Further purification using silica gel chromatography (toluene/EtOAc 1:0 → 10:1) gave 1-O-allyl-3,4,5,6-penta-O-benzyl-2-O-(4-methoxybenzyl)-D-myoinositol (4.48 g, 6.39 mmol, 99%). To a solution of this compound (1.03 g, 1.47 mmol) in EtOH (15 mL) were added DBU (22 μL, 0.147 mmol) and tris(triphenylphosphine)rhodium chloride (14 mg, 0.015 mmol). The reaction mixture was refluxed for 1 h. The solvent was removed and the residue was dissolved in acetone (20 mL) and 1M HCl (1 mL) and refluxed for 10 min. The mixture was diluted with EtOAc and washed with water and filtered through silica. Purification by silica gel chromatography (toluene/EtOAc 1:0 → 10:1) gave 68 (0.895 g, 1.36 mmol, 92%); ¹³C NMR δ 159.4, 138.9, 138.8, 138.8, 138.4, 131.0-
127.7 (aromatic C), 113.9, 83.7, 82.4, 82.0, 81.3, 76.0, 75.9, 75.7, 74.5, 73.1, 72.6, 55.4; $^1$H NMR δ 7.36-7.28 (m, 22H), 6.90-6.87 (m, 2H), 4.97-4.64 (m, 10H), 4.09-4.03 (m, 2H), 3.82 (s, 3H), 3.80-3.77 (m, 1H), 3.80-3.77 (m, 1H), 3.51-3.45 (m, 3H), 2.21-2.18 (m, 1H).

3,4,5,6-Penta-O-benzyl-2-O-(4-methoxybenzyl)-D-myoinosose (69).
A solution of oxalyl chloride (223 µL, 2.55 mmol) in CH$_2$Cl$_2$ (10 mL) was cooled to -78 °C and DMSO (363 µL, 5.11 mmol) was added. The reaction mixture was stirred under argon for 15 min and then compound 68 (0.846 g, 1.28 mmol) dissolved in CH$_2$Cl$_2$ (12 mL) was added dropwise. The reaction mixture was stirred for 1 h at -78 °C and then Et$_3$N (1.78 mL, 12.77 mmol) was added. After 30 min of additional stirring at -78 °C the mixture was diluted with CH$_2$Cl$_2$ and washed with water. Filtration through a plug of silica and further purification by silica gel chromatography (toluene/EtOAc 1:0 → 10:1) gave 69 (0.795 g, 1.21 mmol, 94%); $^{13}$C NMR δ 206.3, 159.8, 138.7, 138.4, 137.8, 137.7, 130.4, 128.7-127.8 (aromatic C), 114.1, 83.5, 82.2, 81.1, 79.4, 76.2, 76.1, 73.6, 72.8, 72.8, 55.4; $^1$H NMR δ 7.38-7.28 (m, 20H), 7.19-7.17 (m, 2H), 6.86-6.84 (m, 2H), 4.94-4.76 (m, 4H), 4.69-4.56 (m, 4H), 4.45-4.39 (m, 3H), 4.28 (t, J=9.15 Hz, 1H), 3.96 (d, J=2.93 Hz, 1H), 3.79 (s, 3H), 3.51 (t, J=9.15 Hz, 1H), 3.44 (dd, J=6.59, 9.52Hz, 1H).

3,4,5,6-Penta-O-benzyl-1-deoxy-1-methylene-D-myoinositol (71).
Potassium tert-butoxide (343 mg, 3.06 mmol) and methyltriphenyl phosphonium bromide (1.09 g, 3.05 mmol) were refluxed in toluene (20 mL) for 1 h. The mixture was cooled to 80 °C and then compound 69 (503 mg, 0.764 mmol), dissolved in toluene (20 ml) was added. After stirring for 30 min at 80 °C the reaction mixture was diluted with toluene and washed with water. The organic phase was filtered through a plug of silica and concentrated. Purification by silica gel chromatography (toluene/EtOAc 1:0 → 10:1) gave 70 (430 mg, 0.655 mmol, 86%). The compound 70 (422 mg, 0.643 mmol) was then dissolved in CHCl$_3$/TFA (10:1, 15 mL). After stirring for 1 h at room temperature the reaction was completed (TLC toluene/EtOAc 5:1). The mixture was diluted with CHCl$_3$ and washed with NaHCO$_3$, filtered (silica) and purified by silica gel chromatography (toluene/EtOAc 1:0 → 5:1) to obtain 71 (318 mg, 0.593 mmol, 92%).

Michael acceptor (72)
A solution of oxalyl chloride (76 µL, 0.87 mmol) in CH$_2$Cl$_2$ (3mL) was cooled to -78 °C and DMSO (123 µL, 1.73 mmol) was added. The reaction mixture was stirred under argon for 15 min and then compound 71 (232 mg, 0.433 mmol) dissolved in CH$_2$Cl$_2$ (4.5 mL) was added dropwise. The reaction mixture was stirred for 1 hour at -78 °C and then Et$_3$N (603 µL, 4.33 mmol) was added. After 30 min of additional stirring at -78 °C the mixture
was diluted with CH₂Cl₂ and washed with water. Filtration through a plug of silica and further purification by silica gel chromatography (toluene/EtOAc 1:0 → 10:1) gave 72 (214 mg, 0.40 mmol, 92%); $^{13}$C NMR $\delta$ 196.9, 140.5, 138.3, 138.0, 137.9, 137.6, 128.6-127.8 (aromatic C), 125.7, 83.5, 82.5, 81.9, 78.4, 74.2, 74.1, 72.9, 71.5; $^1$H NMR $\delta$ 7.43-7.21 (m, 20H), 6.29 (m, 1H), 5.56 (m, 1H), 4.97 (d, $J$=11.35 Hz, 1H), 4.80 (d, $J$=11.35 Hz; 1H), 4.69-4.48 (m, 7H), 4.24-4.23 (m, 1H), 3.96 (t, $J$=4.76 Hz, 1H), 3.81 (dd, $J$=4.76, 9.15 Hz).

**Diels-Alder product (73)**

A solution of compound 72 (29 mg, 0.054 mmol), Bu₃SnH (19 µL, 0.072 mmol) and a catalytic amount of AIBN in 200 µL benzene was prepared. This solution was added via a syringe during 1.5 h to a 75 °C solution of acetobromo-α-D-glucose (29 mg, 0.071 mmol) in benzene (300 µL). After the addition the mixture was stirred for 1 h at 75 °C followed by purification by silica gel chromatography (toluene/EtOAc 1:0 → 10:1) to give 73 (23 mg, 0.021 mmol, 78%); $^{13}$C NMR $\delta$200.7, 145.9, 139.0, 138.9, 138.8, 138.4, 138.3, 138.2, 138.1, 137.3, 131.1, 128.9-127.5 (aromatic C), 105.6, 85.7, 84.0, 82.8, 82.4, 81.8, 81.7, 81.4, 80.2, 79.6, 76.3, 76.2, 75.5, 75.4, 74.7, 74.0, 73.7, 71.9, 24.4, 16.3; MALDI-TOF calcd for C₇₀H₆₈NaO₁₀ [M+Na]$^+$ 1091.5, found 1092.3.
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