

Synthesis of mycobacterial phenolic glycolipids Dijk, J.H.M. van

Citation

Dijk, J. H. M. van. (2022, October 13). *Synthesis of mycobacterial phenolic glycolipids*. Retrieved from https://hdl.handle.net/1887/3480227

Version:	Publisher's Version
License:	<u>Licence agreement concerning inclusion of doctoral</u> <u>thesis in the Institutional Repository of the University</u> <u>of Leiden</u>
Downloaded from:	https://hdl.handle.net/1887/3480227

Note: To cite this publication please use the final published version (if applicable).

Chapter 8

Summary and Future Prospects

Mycobacteria, such as *Mycobacterium tuberculosis* and *M. leprae*, have a thick, highly lipophilic outer membrane called the mycomembrane. This protective barrier contains many (glyco)lipids which are thought to aid the bacterium in the evasion and manipulation of the host immune response. One group of glycolipids present in many pathogenic mycobacteria are the phenolic glycolipids (PGLs). These are mostly rhamnose containing, partially *O*-methylated oligosaccharides which are connected to phenolphthiocerol on the 'reducing end', which also carries two mycocerosic acid moieties. Chemical synthesis of complete PGLs may help to gain understanding of the exact interaction of these molecules with the host immune system and thereby also their role in the pathogenicity of the corresponding mycobacteria. Therefore this thesis has described the synthesis of PGLs originating from the *M. tuberculosis* complex (MTBC), *M. leprae*, *M. haemophilum*, *M. kansasii* and *M. gastri*, so that these can be used in immunological studies to unravel their exact mode of action, with the ultimate goal of finding a therapeutic target or vaccine candidate.

Chapter 1 has provided a concise overview of the current knowledge of the interactions of PGLs at the molecular level. It has described the previously reported syntheses of truncated PGLs, and the application of these molecules. Only one synthesis

of a complete PGL has been reported to date, and the global synthetic strategy used throughout this dissertation has been based on this synthesis. The reported total synthesis of PGL-tb1, shown in Figure 1, was based on the use of an iodophenol bearing trisaccharide that can be attached to a phthiocerol alkyne derivative by means of a Sonogashira cross coupling. The resulting diol was then esterified with two equivalents of mycocerosic acid. Thereafter hydrogenation led to the global deprotection and concurrent reduction of the conjugated internal alkyne which was formed in the Sonogashira reaction.



Figure 1. General synthetic strategy of this thesis which is based on the synthesis of PGL-tb1 as reported by Barroso et al.¹

Chapter 2 has described the synthesis of protein-glycoconjugates carrying *M. leprae* glycans, that can be used for the detection of anti-PGL-I antibodies in sera to diagnose leprosy infections (See Figure 2). In addition to the standard disaccharide conjugate (so-called ND-O-BSA) which is normally used for diagnosis, this chapter has describes the synthesis of BSA-conjugates of three different *M. leprae* PGL trisaccharides. While these conjugates did not appear to be an improvement over the currently used diagnostic standard in terms of diagnostic potential, a trisaccharide was identified that can be used for this purpose and the devised synthetic route could be scaled up to provide the product on gram-scale. Furthermore, the trisaccharide conjugates reaffirmed that the C-3 methyl of the terminal glucose of PGL-I is a highly important determinant for antibody binding.



Figure 2. Synthesis of glycoconjugates of *M. leprae* PGL glycans as described in Chapter 2.

Chapter 3 has described the synthesis of a phthiocerol alkyne derivative which is required for the synthesis of the complete PGLs. Although a synthesis of the alkyne had been described before, the starting compound, 7-hexadecyn-1-ol, was no longer available. Therefore, a new route had to be devised for iodide **11**, which made use of a Corey-Fuchs homologation of an aldehyde derived from pentadecanolide (See Figure 3). This way, the synthesis could be easily scaled up which was required for the large amounts of phenolic glycolipids to be synthesized in the ensuing chapters.



Figure 3. Retrosynthetic analysis of the iodide building block which was synthesized in Chapter 3.

Chapter 4 has described the synthesis of all known complete PGLs of the MTBC. It was found that making use of a carboxybenzyl (Cbz) carbonate as a participating protecting group which can be removed by hydrogenation increased the overall efficiency of the synthetic route compared to a strategy based on the use of benzoyl ester protection (See Figure 4). The use of an additive based glycosylation method increased the selectivity and overall efficiency of the 1,2-*cis* fucosylations and it further reduced the number of required steps.





One interesting result of this chapter has been the apparent influence of a distal methyl or benzyl ether on the stereoselectivity of glycosylation reactions of the used disaccharide acceptors. Although it is generally assumed that methyl and benzyl ethers behave similarly in a glycosylation reaction, this result indicates that significant differences can be encountered. It will be of interest to investigate the effect of (distal) protecting groups on the reactivity and selectivity of the (disaccharide) acceptors. Generating a set of disaccharide acceptors with different combinations of methyl and benzyl ethers and coupling these to the same donor (Figure 5A) can provide insight into the influence of these groups on the conformation and reactivity of the disaccharide in the glycosylation reaction. Along this line, it was also found that replacing the C-3 methyl ether of a fucose donor for a benzyl ether had a large influence on selectivity of the donor. It will therefore be of interest to generate a set of donors with different combinations of methyl and benzyl ethers and to couple these to the same acceptor (See Figure 5B). Both sets of experimental results can be complemented with calculated conformational landscapes of reactive intermediates, such as oxocarbenium ions, to computationally probe the effect of the methyl vs benzyl ethers.²



Figure 5. Proposed model reactions for investigating the effect of (distal) methyls and benzyls on the selectivity of donors and disaccharide acceptors.

Chapter 5 has described the synthesis of all known PGLs originating from *M. leprae* and a PGL of *M. haemophilum* as well as a hypothesized biosynthetic intermediate thereof (Figure 6). The Cbz was used again as a hydrogenation-labile, participating protecting group and this increased the efficiency of the synthesis of *M. leprae* trisaccharides compared to the syntheses described in Chapter 2 by circumventing the debenzoylation and benzylation steps required in the trisaccharide stage. However, it was also found that the use of a Cbz carbonate led to a lower yield and selectivity of glycosylation reactions when sterically hindered acceptors were used. For example, when the *M. haemophilum* disaccharide acceptor **36** was used in combination with donor **35** carrying a C-2 Cbz, the 1,2-*cis* linked trisaccharide was produced as the major anomer. While this problem was circumvented by coupling the disaccharide acceptor to a peralkylated donor under the agency of IDCP, the results do warrant further investigation (*vide infra*).



Figure 6. Synthesis of the PGLs originating from *M. leprae* and *M. haemophilum* as described in Chapter 5.

Chapter 6 has described the synthesis of all known PGLs originating from *M. kansasii* and *M. gastri* (Figure 7). Again, the Cbz-carbonate was applied as a participating protecting group, this time from a remote position on the glycosyl donor's ring, as a common occurrence in the desired glycans is the presence of a methyl ether on the C-2 position of 1,2-trans linked saccharides. The C-3 Cbz carbonate proved to be effective to steer the selective formation of the desired α -linkages. In the case of C-2 deoxy donor **44** it was found that the use of a C-3 Cbz-protected donor led to excellent stereoselectivity when coupled to an electron rich acceptor but to a lower stereoselectivity when an acceptor with a proximal electron-withdrawing substituent was used.



Figure 7. Synthesis of PGLs originating from *M. kansasii* and *M. gastri* as described in Chapter 6.

A proposed mechanistic rationale for the results described in Chapters 5 and 6 is described in Scheme 1. When a donor is activated, an array of reactive intermediates can form, with covalently bound anomeric triflates, participating in S_N2 -type substitution

Chapter 8

reactions, and more reactive oxocarbenium ions, which likely adopt a half-chair conformation, taking part in S_N1 -type substitution reactions, ³ While these oxocarbenium ions can be approached from two sides, it has been proposed that the half-chair intermediates are approached preferably from the side that leads to a chair like transition state, as attack on the other diastereotopic face would lead to a twist-boat conformation, which is energetically less favorable.⁴ When a participating protecting group is present it is able to intramolecularly "trap" the oxocarbenium ion and form a more stable dioxolenium ion.⁵ A donor with a C-2 participating group can lead to the formation of a 1,2-dioxolenium ion (64, Scheme 1A). Opening of this dioxolenium ion produces the 1,2trans α product 65. If the acceptor is not reactive enough to substitute the 1,2dioxolenium ion, either due to steric factors or due to decreased electron density, it will prefer to react with either of the half-chair oxocarbenium ion like intermediates (62 & 63), as these are both more reactive and sterically more accessible. The rhamnosyl oxocarbenium ion shown in Scheme 1A, will preferentially adopt a ⁴H₃ half-chair conformation 63^2 and attack on the ${}^{4}H_{3}$ half-chair leads to the β -product 66. The glycosylation of the C-2-O-Cbz rhamnosyl donor and the sterically hindered axial acceptor **36** predominantly gave the β product, which may be accounted for by attack on the ⁴H₃ half-chair oxocarbenium ion. The C-3-O-Cbz group in donor **41** can trap the ⁴H₃-half chair **70** to form a 1,3-dioxolenium ion (**71**, Scheme **1B**).^{3,6} This intermediate will be less stable and more reactive than a *cis*-fused 1,2-dioxolenium ion. Substitution of this species will lead to the desired α product.



Scheme 1. Proposed mechanistic rationale of the results of Chapters 5 and 6.

It would be of interest to couple the *M. haemophilum* disaccharide acceptor **36** with the C-3-*O*-Cbz donor **41** used for the synthesis of *M. kansasii* glycans, to further explore this hypothesis. The decreased α -selectivity in glycosylations of C-2 deoxy donor **44**, used

Chapter 8

in Chapter 6, with electron-poor acceptors may be explained with the ³H₄ half-chair oxocarbenium ion as product forming intermediate. Deoxy donors more readily form oxocarbenium ions, as they carry less electron withdrawing substituents. It would be of interest to do perform a series of glycosylations to determine how effective the C-3-*O*-Cbz is in providing long range anchimeric assistance with a systematic series of acceptors, of which the nucleophilicity is well understood. The knowledge obtained could be of value for the synthesis of complex oligosaccharides.

Chapter 7 has described the synthesis of structurally simplified aglycone analogues of different PGLs to gain an understanding how structural details of the lipid part of the phenolic glycolipids impact the detection and/or presentation of the compounds by the host immune system. Three different lipid analogues have been assembled, changing the complexity on the phthiocerol and/or mycocerosic acids and these have been attached to the PGL-tb1 and PGL-I glycans (Figure **8A**). The glycans were also attached to a C₁₈ aglycone and directly hydrogenated to deliver a phenolic aglycone. In a similar fashion, multiple aglycon analogues have been synthesized of PGL-III, which has been revealed to be a Mincle ligand using the synthetic PGL,⁷ to generate an analogue that is better soluble in aqueous solutions. These analogous may be useful for crystallization studies to unravel the atomic details of PGL-Mincle binding. It would be of interest to take the same set of simplified aglycones displayed in Figure **8A** and combine these with the PGL-III glycan to investigate the role of hydrophobic interactions in Mincle binding.



Figure 8. Aglycone analogues synthesized in chapter 7. **A**: Aglycone analogues of PGL-tb1 and PGL-I. **B**: Aglycone analogues of PGL-III.

Future prospects

The synthetic PGLs and variations thereof described in this thesis are currently being investigated for their immunomodulatory capabilities, with the ultimate goal of finding a therapeutic target or vaccine candidate.

In the future it could be interesting to make photoaffinity labeling probes of PGLs as it is unknown with which receptors the PGLs interact. The hydrazide intermediate that was synthesized in Chapter 2 to generate the protein conjugates, can be used to attach a linker containing a photoreactive diazirine group as well as an alkyne that can be used to attach a reporter tag or a biotin moiety to capture the PGL binding partners.^{8,9}



Scheme 2. Proposed synthesis of the PGL-III glycan with a bifunctional linker which contains a photolabile diazirine group for labelling and an alkyne to attach a reporter.

Alternatively the route of the phthiocerol can be altered in such a way that it gives access to phthiodiolone containing PGLs (Scheme 3). By oxidizing the secondary alcohol of **85** and then masking the resulting ketone as a ketal with ethylene glycol under acidic conditions, the same route could be followed as was described for phthiocerol to obtain protected phthiodiolone alkyne **88**, as no acidic conditions are used in the rest of the route. After the Sonogashira, esterification and hydrogenation steps the ketone can be deprotected in the presence of esters using molecular iodine in acetone.¹⁰ Not only can these phthiodiolone containing PGLs then be investigated for their immunomodulatory capabilities, the ketone could also function as a conjugation handle to attach the bifunctional linker **84** by means of reductive amination.



Scheme 3. Proposed synthesis of phthiodiolone containing PGLs.

The methods that were used in this thesis can also be applied in the synthesis of other complex mycobacterial molecules such as glycopeptidolipids (GPLs). These tetrapeptide glycolipids are thought to play a significant role in the biofilm formation, environmental spread and immunomodulation of mycobacteria belonging to the *Mycobacterium avium-intracellulare-scrofulaceum* (MAIS) complex.^{11–15} GPLs have a relatively well conserved core to which haptenic oligosaccharides are attached. The β-hydroxy lipid tails attached to the N-terminus of the tetrapeptide can be accessed from the corresponding β-keto ester by means of an asymmetric hydrogenation with Noyori's ruthenium catalyst, as was applied in Chapters 3 and 7. This keto-ester can in turn be synthesized either by means of coupling ethyl diazoacetate to an aldehyde or by condensation of the corresponding ketone with diethyl carbonate, as was performed in Chapter 7. The GPL oligosaccharides mostly feature 1,2-*trans* linkages and the formation of these can be achieved with a C-2-*O*-Cbz as a participating protecting group. This way, hydrogenation could be used a single global deprotection step to complete the synthesis.



Figure 9. Retrosynthetic analysis of a glycopeptidolipid (GPL) originating from Mycobacterium avium.

References

- 1. Barroso, S. *et al.* Total Synthesis of the Triglycosyl Phenolic Glycolipid PGL-tb1 from Mycobacterium tuberculosis. *Angew. Chemie* **124**, 11944–11947 (2012).
- Hansen, T. *et al.* Defining the SN1 Side of Glycosylation Reactions: Stereoselectivity of Glycopyranosyl Cations. *ACS Cent. Sci.* 5, 781–788 (2019).
- 3. Hansen, T. *et al.* Characterization of glycosyl dioxolenium ions and their role in glycosylation reactions. *Nat. Commun.* **11**, 1–9 (2020).
- Stevens, R. V. Nucleophilic additions to tetrahydropyridinium salts. Applications to alkaloid syntheses. Acc. Chem. Res. 17, 289–296 (1984).
- Pittman, C. U., McManus, S. P. & Larsen, J. W. 1,3-Dioxolan-2-ylium and related heterocyclic cations. *Chem. Rev.* 72, 357–438 (1972).
- Kleijne, F. F. J., Elferink, H., Moons, S. J., White, P. B. & Boltje, T. J. Characterization of Mannosyl Dioxanium Ions in Solution Using Chemical Exchange Saturation Transfer NMR Spectroscopy. *Angew. Chemie Int. Ed.* 61, (2022).
- 7. Unpublished results of Sho Yamasaki and Shige Ishizuka of Osaka University.
- Li, Z. et al. Design and Synthesis of Minimalist Terminal Alkyne-Containing Diazirine Photo-Crosslinkers and Their Incorporation into Kinase Inhibitors for Cell- and Tissue-Based Proteome Profiling. Angew. Chemie 125, 8713–8718 (2013).
- Hill, J. R. & Robertson, A. A. B. Fishing for Drug Targets: A Focus on Diazirine Photoaffinity Probe Synthesis. J. Med. Chem. 61, 6945–6963 (2018).
- 10. Sun, J. *et al.* Highly Efficient Chemoselective Deprotection of 0,0 -Acetals and 0,0 -Ketals Catalyzed by Molecular Iodine in Acetone. *J. Org. Chem.* **69**, 8932–8934 (2004).
- Schorey, J. S. & Sweet, L. The mycobacterial glycopeptidolipids: Structure, function, and their role in pathogenesis. *Glycobiology* 18, 832–841 (2008).
- Fujiwara, N. *et al.* Structural analysis and biosynthesis gene cluster of an antigenic glycopeptidolipid from mycobacterium intracellulare. *J. Bacteriol.* 190, 3613–3621 (2008).
- 13. Whang, J. *et al.* Mycobacterium abscessus glycopeptidolipids inhibit macrophage apoptosis and bacterial spreading by targeting mitochondrial cyclophilin D. *Cell Death Dis.* **8**, e3012 (2017).
- 14. Fujiwara, N. *et al.* Glycopeptidolipid of Mycobacterium smegmatis J15cs affects morphology and survival in host cells. *PLoS One* **10**, 1–11 (2015).
- Chatterjee, D. & Khoo, K. Cellular and Molecular Life Sciences The surface glycopeptidolipids of mycobacteria : *Cell. Mol. Life Sci.* 58, 2018–2042 (2001).