On the total synthesis of terpenes containing quaternary stereocenters
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ABSTRACT: Mycobacterium tuberculosis is a disease causing bacterium which infects several million people and leads to over 1.5 million deaths on an annual basis. The success of Mycobacterium tuberculosis as world’s most important bacterial pathogen can be attributed to two key factors. First, an intricate intracellular survival mechanism protects it from immune responses during a decades long infection process. Secondly, its unusually hydrophobic, multi-layered, cell wall protects it even further from penetration by drugs. Despite of over a century of active research into Mycobacterium tuberculosis, the function of the lipid components which make up for its cell wall, remain largely unknown. Additionally, no definite, tuberculosis specific chemical marker based diagnostic test for this disease exists. Discovery of new lipids can help to understand Mycobacterium tuberculosis’ survival and virulence mechanism, and when the lipids are specific to pathogenic Mycobacterium tuberculosis, these can be used as chemical markers for the disease. This chapter gives an overview of our previous efforts regarding the asymmetric total synthesis of Mycobacterium tuberculosis lipids. In the final paragraph an outline of this dissertation is presented.

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CHAPTER 1

1.1 Introduction

Tuberculosis is an infectious disease caused by the bacterium *Mycobacterium tuberculosis* (*Mtb*).\(^{[1]}\) It is a disease which primarily attacks the pulmonary tract (e.g. lungs and throat), hence the “typical” tuberculosis cough, but can also attack the kidney, spine, and/or brain.\(^{[2a]}\) Tuberculosis is a fatal human disease and is contagious as the bacterium can easily be transmitted from individual to individual simply by coughing and sneezing. It is therefore not surprising that tuberculosis manifests itself as one of world’s leading pathogens causing over five million infections and over one and a half million deaths annually.\(^{[1]}\) The majority of casualties from this disease are inhabitants of the developing countries, and more specifically from regions with high HIV (human immunodeficiency virus) infection rates. It is the decrease in immune response in HIV patients which make these individuals more vulnerable for tuberculosis infection.\(^{[2b]}\) Luckily not everybody infected with tuberculosis becomes sick and as a result there are basically two tuberculosis-related conditions. One can either have latent tuberculosis infection or have the active tuberculosis disease (Figure 1).\(^{[2c]}\) Treatment\(^{[2d,e,3]}\) of both forms is possible but, as the high number of fatalities indicates, is far from trivial. Patients diagnosed with latent tuberculosis infection do have tuberculosis bacilli in their body but these are not active. Such individuals are therefore not contagious and do not exhibit the tuberculosis symptoms. However, the danger of developing active tuberculosis is associated with having latent tuberculosis infection, and it is for this reason that treatment of the latter is initiated. Typical treatment involves the use of either isoniazid, rifampicin/rifampin or rifapentine for several months (first-line drugs).\(^{[2d,3]}\)

![Figure 1. Tuberculosis risk factors, causing active Tb disease from latent Tb infection.](image-url)
Figure 1 (continued). 

Tuberculosis symptoms.

When the immune system in a latent TB infected patient is unable to stop the tuberculosis bacteria from growing, it multiplies and causes the active Tb disease. The patient becomes sick, showing typical tuberculosis symptoms and more importantly becomes contagious. Due to multiplication of the bacterium inside the human host there are more bacteria compared to those having latent tuberculosis infection, making treatment harder. Drug-susceptible tuberculosis involves a treatment with the previously mentioned first-line drugs, pyrazinamide and ethambutol (also first-line drugs), generally in a combinatorial fashion, for several months.\cite{2e,3}

Since tuberculosis is caused by a living organism it is subjected to evolutionary principles causing, inevitably, drug resistance.\cite{4} It goes without saying that the regimen for treatment of (multi- or extensively-) drug resistant tuberculosis\cite{5} complicates the situation, necessitating, dependent on the pattern of drug resistance, a regimen of up to two years.\cite{2e,3} This involves the use of a combination of first-line drugs, fluoroquinolone based drugs, injectable agents (e.g. streptomycin, amikacin, kanamycin, or capreomycin) and alternative agents (e.g. cycloserine, p-aminosalicylic acid, clarithromycin, amoxicillin-clavulanate). As medicines are not exonerated from adverse effects the extensive use of tuberculosis drugs will take its toll on the patient, in the worst case forcing discontinuation of treatment.\cite{6}

It is evident from the preceding paragraphs that treatment of tuberculosis is apparently very demanding. The origin of hampered treatment of tuberculosis lies for a large part within its cell envelope (cell wall). Consisting of a complex array of (glyco)lipids, polysaccharides and peptidoglycans, the cell wall exhibits low permeability of drugs into the mycobacterial cell.\cite{7} In figure 2, based on our current understanding, one sees a molecular representation of the cell envelope of Mycobacterium tuberculosis, showing an exquisite architecture build-up of many different layers.
Figure 2. A schematic molecular representation of the cell envelope of *Mycobacterium tuberculosis* (© and reproduced with permission from Elsevier, reference 7c).
On the inside of the cell envelope lays the so-called plasmic membrane (PM). This bilayer consists primarily of glycerol based lipids and phosphatidyethanolamines (cephalins), a class of glycerophospholipids. Here one also finds the well-known phosphatidylinositol mannosides (PIMs). Connected in a non-covalent manner to the plasma membrane is the lipoarabinomannan (LAM), a complex oligosaccharide which is a known virulence factor of tuberculosis. LAM consists of a phosphatidylinositol moiety linked to mannopyranan core to which is attached an arabinan, spanning the peptidoglycan (PG) into the arabinogalactan (AG) region of the membrane. The peptidoglycan determines the cellular shape and plays an important role in surviving osmotic pressure. The amino saccharide backbone of the peptidogalactan is cross-linked with peptide bonds which enhances rigidity of the cell wall. Covalently connected to PG sugar moieties is the arabinogalactan, with as the backbone a 30-mer galactofuran linker. This functionality is linked at the 8, 10 and 12 position, with arabinofurans. These polymeric saccharides span a large part of the overall cell wall and eventually branch out connecting with mycolic acids. The mycolate based glycolipid structures form the outer membrane (OM), an interwoven network of the long aliphatic (C60-C90) mycolic acid chains. The cell-wall is topped by an outermost compartment, a loosely bound structure called a capsule (not shown in Figure 2), primarily consisting mainly of polysaccharides and peptides.

Due to the high lipid content the cell envelope is very hydrophobic. This therefore forms an almost unsurpassable barrier for drugs, making the treatment of the tuberculosis disease difficult. To complicate the situation even further, intracellular survival of the bacterium is granted by successful infection of endosomal phagocytes. Residing in phagosomes, and actively inhibiting pH dependent killing mechanisms, it is protected from both drugs and immune responses.\[8\]

Because of over a century of active research on *Mycobacterium tuberculosis,* many of the lipid components which make up for its cell membrane are largely known.\[7\] It is however surprising how little is known about the function and antigenic properties of these lipids.\[7e\] As the mycobacterial cell envelope is at the interface with the human host, it plays a key role in *Mycobacterium tuberculosis’* virulence but also in the immune response. The identification of new lipids is therefore instrumental to understand *Mycobacterium tuberculosis’* survival and virulence, and provides an opportunity to develop anti-mycobacterial drugs. Moreover, when specific to *Mycobacterium tuberculosis,* these lipids can serve a potential source of chemical markers for the tuberculosis disease, making them useful for developing diagnostic tests.\[9\]

The discovery of biologically active “small molecules” (secondary metabolites) and in particular the studies thereof are counteracted by small isolated quantities. In order to facilitate investigation, more specifically the structure elucidation and assessment of their biological activity, the need for significant quantities of material is undeniable and for *Mycobacterium tuberculosis* total synthesis is the technology to serve these needs.
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In this chapter an overview of our in-house executed total syntheses, of *Mycobacterium tuberculosis* produced lipids, is presented. The emphasis lies on the synthetic strategies employed, with a brief description of the molecule’s biological activity.

1.2 β-Mannosyl phosphomycoketide

In 2000 Moody and co-workers, isolated a new compound from *Mycobacterium tuberculosis* and *Mycobacterium avium*, named mannosyl phosphomycoketide (MPM). This polyketide (not terpenoid!) was found to be a potent mycobacterial antigen for a CD1c-restricted mycobacterial T-cell line. Antigen presentation of MPM by CD1c was postulated to arise from binding of the chain within a hydrophobic groove of the CD1c protein. The hydrophilic mannosyl phosphate functionality is recognized by the T-cell receptor. The importance of the hydrophobic tail in T-cell response was assessed, showing T-cell response to be dependent on the length of the chain, with an optimum around C35. Also the hydrophilic head group was shown to be crucial for T-cell response as a glucose, instead of mannose, was not recognized.

The structure elucidation did not include the assignment of the stereochemical elements present in the MPM chain, due to its limited availability. In 2002, Crich and Dudkin confirmed the overall chemical structure of MPM by means of a stereorandom total synthesis. It was confirmed that MPM has a β-glycosidic bond but the stereochemistry in the chain remained unresolved. In 2005, the Minnaard/Feringa laboratory set out to perform this task (Scheme 1 and 2). However, before the synthesis was started, a hypothesis had to be made about the stereochemistry (out of the 32 possibilities!). For this the biosynthesis of the mycoketides was considered leading to the conclusion that the methyl groups are introduced through the iterative action of polyketide synthase pks12, providing the all-\(\text{syn}\) compound. Subsequently, all-(S)-MPM was arbitrarily chosen as the target.

Starting from cyclooctanone (Scheme 1), a double oxidation employing IBX gave diene 2 in 69% yield. This compound was then subjected to the first of two conjugate additions, in which 5 mol% of Cu(OTf)\(_2\) and 10 mol% Feringa ligand L1 were utilized. The conjugate addition proceeded smoothly giving enone 3 in an excellent ee of 98%. The second conjugate addition was performed using half the catalyst loading, and using ent-L1. Trapping of the in situ formed enolate as its TMS enol ether gave, after ozonolysis and esterification, alcohol 5 in 60% over the four steps with an excellent de and ee of >99%. 5 was then converted into alcohol 6 and aldehyde 7 employing standard chemistry. These building blocks were united with a Julia-Kocienski olefination. To achieve this, alcohol 6 was subjected to a Mitsunobu reaction in the presence of thiol 8. Oxidation of 9 with mCPBA delivered sulfone 10 which was used in the Julia-Kocienski reaction with aldehyde 7 to obtain alkene 11 as an, inconsequential, mixture of isomers (\(E:Z = 2:1\)). Complete reduction of the ester with DIBAL-H followed by tosylation and subsequent alkylation, using CuBr•SMe\(_2\) and C\(_5\)H\(_{11}\)MgBr, gave tetramethyl alkane 12 in 82% yield over the three steps.
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Completion of the MPM side chain only required the installation of an additional methyl functionality (Scheme 2). This was achieved by constructing sulfone building block 18 from 1,4-butanediol derived aldehyde 13. A Wittig olefination and subsequent DMAP-catalyzed isomerization afforded thioester 14. At this stage the final stereocenter of the MPM side chain was introduced via the asymmetric copper-catalyzed conjugate addition of methylmagnesium bromide. Employing 5 mol% of CuBr•SMe₂ in combination with josiphos ligand L₂ provided the methyl-substituted stereocenter in 15 in 94% yield with 92% ee₁². Reduction of the thioester, Mitsunobu functionalization and sulfone formation afforded enantiopure building block 18. Unification of this molecule with tetramethyl substrate 12 was again achieved via a Julia-Kocienski olefination, after TBDPS deprotection and Ley-Griffith oxidation of 11. The alkene functionalities and benzyl group were then removed by a Pd/C-catalyzed hydrogenolysis/hydrogenation to give the MPM side chain 19.

Completion of β-mannosyl phosphomycoketide was effected by connecting the MPM-side chain with mannose (Scheme 2). For this, hemiacetal 20 was reacted with diphenyl chlorophosphate to give predominantly the β-anomer of mannosyl phosphate 21. Removal of the phenyl groups using Adams’ catalyst and quenching with pyridine afforded pyridinium mannosyl phosphate 22. Linking this fragment with MPM side chain 19, with the assistance of 2,4,6-trisopropylbenzenesulfonyl chloride (TPSCI), provided, after removal of the acetyl protecting groups, all-(S)-β-mannosyl phosphomycoketide, its first asymmetric total synthesis.
Scheme 2. Completion of β-mannosyl phosphomycoketide.

The completion of the total synthesis allowed the elucidation of the relative and absolute stereochemistry by proving sufficient material for biological evaluation. Gratifyingly, the hunch to make the all-(S) MPM paid off since immunological essays, performed by the Moody laboratory, showed that the synthetic material exhibited a similar antigenic T-cell response compared to the natural isolate. Interestingly, the stereorandom mixture of MPMs was significantly less potent, indicating a strong, somewhat unexpected, influence of lipid moiety on T-cell response. Additionally it was reported that T-cell response was even dependent on the stereochemistry of the C4-methyl (the first methyl-branched stereocenter from the left), where (S)-stereochemistry proved to be significantly more active than the (R)-isomer. The study therefore indicates a strong influence of the lipid stereochemistry, more specifically the chiral methyl ramification, on T-cell response.

The total synthesis of all-(S)-β-mannosyl phosphomycoketide was recently also reported by the Piccirilli group. Whereas in our synthesis asymmetric catalysis stood central, the Piccirilli synthesis mined from the chiral pool, synthesizing the natural product with >96% stereopurity.

1.3 Asymmetric catalytic deoxypropionate synthesis

After our synthesis of β-mannosyl phosphomycoketide, bearing the chiral 1,5-methyl array, the focus shifted towards other Mycobacterium tuberculosis lipids comprising asymmetric methyl branches. Our attention was drawn in particular by the complex glycolipids Ac2SGL and its “big brother” sulfolipid-1. Of special interest was the so-called deoxypropionate unit (1,3-methyl ramification) on the lipid side chains. Both
glycolipids contain lipid chains with up to eight repeating 1,3-methyl groups making it a challenging moiety for the asymmetric synthesis community. Also the somewhat smaller molecules PDIM-A, PGL-tb1 and mycoside B isolated from \textit{Mycobacterium tuberculosis} bear this structural feature, though shorter in length, and therefore also became part of our synthesis program.

Deoxypropionates are polyketides and are an abundant moiety in a wide variety of natural products.\cite{18} It is therefore not surprising that many synthetic strategies towards the 1,3-methyl array have been reported.\cite{19} In order to construct deoxypropionates, an iterative synthetic sequence is an especially appealing strategy. In such way the 1,3-methyl ramification can be introduced relatively fast, reliable and efficient, albeit at the cost of the route’s convergence. Since the discussion of the different iterative methodologies to construct deoxypropionates falls outside the scope of this chapter we like to refer to our review on this specific topic.\cite{19}

One of the methodologies which does need to be mentioned in light of this overview is the copper-catalyzed asymmetric Michael addition of methylmagnesium bromide to $\alpha,\beta$-unsaturated thioesters, by Feringa and Minnaard in 2005.\cite{15} For the iterative construction of the deoxypropionate units in the \textit{Mycobacterium tuberculosis} lipids, $\alpha,\beta$-unsaturated thioester 23 was used as the starting material (Scheme 3). The asymmetric conjugate addition of MeMgBr to this substrate proceeded in 94% yield and an excellent 98% ee. The thioester was converted into the corresponding aldehyde by the aid of DIBAL-H. A Horner-Wadsworth-Emmons olefination then provided $\alpha,\beta$-unsaturated thioester 26 in 86% over the two steps. Repetition of this sequence also proved to be highly stereoselective as each consecutive methyl introduction led to increased diastereoselectivity. This feature, the high yield and ease of execution of the steps made this methodology ideal to synthesize long deoxypropionates (\textit{vide infra}).

\begin{center}
\textbf{Scheme 3. General iterative deoxypropionate synthesis strategy used in the construction of Mtb lipids.}
\end{center}

1.4 Phthiocerol dimycocerosate A

The first Mtb-borne lipid successfully crafted in our laboratory applying the described iterative asymmetric conjugate addition sequence was phthiocerol dimycocerosate A (PDIM A).\cite{20} In 1999 two independent studies found that \textit{Mycobacterium tuberculosis}...
mutant strains, deficient in PDIM A, showed attenuated virulence.\textsuperscript{[21]} This finding strongly suggested an important role for the PDIM A lipid as a virulent determinant. The structure and absolute configuration of the phthiocerol and mycocerosic acid chains were proposed as early as in 1963 and 1973 respectively.\textsuperscript{[22]} Confirmation of the structure, by means of rigorous \textsuperscript{1}H-NMR analysis, was provided by Daffé and co-workers.\textsuperscript{[23]} However, absolute proof of the structure by means of total synthesis was not provided until our group embarked upon an asymmetric total synthesis of PDIM A (Scheme 4).

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Scheme 4. Asymmetric total synthesis of PDIM A.

PDIM A initially attracted our attention mainly due to the mycocerosic acid side chains, but the molecule contains an additional four stereocenters in the phthiocerol backbone. The stereogenic methyl-branch with a vicinal asymmetric methoxy stereocenter prompted us to employ another asymmetric conjugate addition strategy. Treatment of cycloheptenone with Me\textsubscript{2}Zn, in the presence of Cu(OTf)\textsubscript{2} (0.5 mol%) and Feringa ligand \textit{L}\textsubscript{1} (1 mol%),\textsuperscript{[14]} followed by trapping of the \textit{in situ} formed enolate with ethyl iodide provided 29 with 95\% \textit{ee} and >20:1 \textit{de}. Opening of the ring via a Baeyer-Villiger oxidation/hydrolysis sequence gave after further transformations aldehyde 30. An asymmetric 1,2-addition of 2-methyl-3-butyn-2-ol following Carreira’s procedure gave propargylic alcohol 32 with an excellent selectivity of 95\% \textit{de}.\textsuperscript{[24]} Multiple steps led to alkyne 33 which was hydroxilylated to furnish vinyl silane 34 in 86\% yield. A Tamao-Fleming oxidation and subsequent directed reduction of the ketone...
with tetramethylammonium triacetoxyborohydride selectively produced phthiocerol. Esterification with mycocerosic acid, constructed using the iterative asymmetric conjugate addition sequence,\textsuperscript{[25,15]} produced PDIM A thereby confirming its proposed chemical structure. The produced material also aided in the development of an analytical procedure to detect PDIM A in \textit{Mtb} samples.\textsuperscript{[26]} Moreover, the synthetic material helped to establish the role of PDIM A as virulence factors of \textit{Mycobacterium tuberculosis}.\textsuperscript{[27]} It was shown that \textit{Mtb} strains lacking PDIM A are susceptible to killing in early stages of infection indicating that PDIM A protects \textit{M. tuberculosis} from an early innate host response.

1.5 Phenolic glycolipid (PGL-tb1)

A compound closely related to PDIM A, is the phenolic glycolipid PGL-tb1. This compound was first isolated and characterized by Daffé \textit{et al.}, and was found in the outer layer of the cell envelope in several strains of \textit{Mtb}.\textsuperscript{[28]} Where the closely related DIM/PDIMs are required for the multiplication and persistence of \textit{Mtb} \textit{in vivo},\textsuperscript{[29]} PGL-tb1 has also been suspected to be involved in hypervirulence of specific \textit{Mtb} strains.\textsuperscript{[30]} Given its presence in specific \textit{Mtb} strains, PGL-tb1 is one of the most unusual virulence factors modulating its defense systems and causing disease. This feature can be exploited since there is a great need for \textit{Mtb}-specific compounds that permit to distinguish between prior BCG vaccination and infection. Steps towards such a distinction have been made with the recent development of an enzyme-linked immunosorbent assay (ELISA) based on PGL-tb1, a potential method for the diagnosis of TB in HIV-infected patients.\textsuperscript{[31]} To facilitate further studies, there is a need for pure synthetic material since the natural isolate comes only in minute quantities.

Our synthetic endeavor on the synthesis of PGL-tb1 (Scheme 5) involved the preparation of the previously mentioned aldehyde 30 (Scheme 4).\textsuperscript{[20]} Conversion into keto-ester 36 was followed by a Noyori asymmetric hydrogenation, installing the third stereocenter in $>99\%$ de. Ester 37 was transformed into Weinreb amide 3 which was alkylated with alkyl iodide 40. The intermediate ketone was reduced and the alkyne was liberated from its protecting group to give phthiocerol analogue 41.

**Scheme 5. Asymmetric synthesis of the phthiocerol backbone in PGL-tb1.**
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The trisaccharide building block was synthesized starting from L-rhamnose, which in multiple steps was converted into glycoside acceptor 42 (Scheme 6). Glycosylation of this compound with rhamnoside 43, by the aid of N-thiophenyl-ε-caprolactam, Tf₂O in a tri-t-butylylimidazoline (TTBP) buffered solution, provided the α-linked di-rhamnoside 44 with full stereocontrol in 71% yield. The third sugar functionality was introduced in 52% yield, following a similar procedure, this time with 46, to give after two more steps trisaccharide 48.

Scheme 6. Construction of trisaccharide 48 for the PGL-tb1 synthesis.

The unification of the two aforementioned building blocks, 41 and 48 came about in the form of a Sonogashira coupling (Scheme 7). Careful optimization led to the isolation of 51% of the desired cross-coupled product 49, with the recovery of 36% of 41. After introduction of the mycocerosic side chains, deprotection concluded the first asymmetric total synthesis of PGL-tb1. The synthetic material is currently under investigation for the development of diagnostic tools for the detection of hypervirulent strains of Mtb. Also its role in virulence is being studied.
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Scheme 7. Completion of the PGL-tb1 total synthesis.

1.6 Diacylated sulfoglycolipid (Ac₃SGL)

In 2004, Gilleron et al. communicated the isolation and characterization of the Mycobacterium tuberculosis specific lipid Ac₃SGL, which is present in the outer cell membrane. They found that Ac₂SGL is a promising candidate for the development of a new tuberculosis vaccine due to its antigenic properties. It was shown that T-cells activated by Ac₂SGL release interferon-γ, recognize M. tuberculosis infected antigen-presenting cells, and kill intracellular mycobacteria in vitro.

In 2008, Prandi and co-workers constructed and biologically evaluated a set of Ac₃SGL analogues containing the trehalose-sulfate functionality, but varying in the acyl side chain. T-cell receptor recognition and T-lymphocyte activation was shown to be dependent on the number and stereochemistry of the methyl substituents, and the respective location of the acyl chains on the trehalose core. Despite the finding that Ac₂SGL-specific T-cell activation occurred at low concentration (<100 nM, ~0.1 μg mL⁻¹) for the analogues, their antigenicity was significantly attenuated compared to the naturally occurring Ac₂SGL.

Due to the limited access of Ac₂SGL from the pathogenic Mycobacterium tuberculosis (~1 mg/L) culture, and the painstaking isolation procedure, the development of a
vaccine program is depending on total synthesis efforts. Our group embarked on the first total synthesis of naturally occurring Ac$_2$SGL, which started with the construction of the long deoxypropionate lipids phthioceranic acid and hydroxyphthioceranic acid. The former was constructed in a straightforward manner using the iterative conjugate addition sequence to install the seven methyl groups in a linear fashion in 8% yield over 18 steps (Scheme 3). Thioester 50 was subsequently reduced to the alcohol, tosylated, substituted by the long alkyl chain followed by desilylation and oxidation to give phthioceranic acid (Scheme 8).

**Scheme 8. Asymmetric synthesis of phthioceranic acid.**

For the synthesis of hydroxyphthioceranic acid we adopted a slightly different strategy since a chiral hydroxy group is present in this molecule. In our first generation synthesis (Scheme 9) we decided to install this hydroxyl group by means of a copper/taniaphos-catalyzed allylic alkylation of ester 51 with C$_{15}$H$_{31}$MgBr. The desired terminal olefin 52 was obtained in 76% yield and an excellent ee of $>98\%$. Ring-closing metathesis afforded lactone 53 which was diastereoselectively methylated using a cuprate addition, preparing 54. A straightforward four step protocol afforded $\alpha,\beta$-unsaturated thioester 55, which set the stage for the iterative introduction of the remaining seven methyl groups. The first methyl group to be installed using the iterative sequence gave thioester 56 in 91% yield and a 98% de. Six iterations (18 steps!) on this molecule provided deoxypropionate 57 in 14% yield over these 18 steps. With 57 in hand the thioester was converted into the corresponding methyl ketone 58. Baeyer-Villiger oxidation followed by hydrolysis of the formed ester provided the desired chain length of hydroxyphthioceranic acid. The obtained alcohol was oxidized and esterified whereafter the hydroxyl group was deprotected, giving 59. At this stage, comparison with natural hydroxyphthioceranic acid showed the stereochemistry of the hydroxyl function to be incorrect and therefore the alcohol functionality in 59 was inverted by a Mitsunobu reaction using $p$-nitrobenzoic acid. The obtained ester was transesterified using MeOH and catalytic NaCN, affording hydroxyphthioceranic acid methyl ester 60 in 85% over the two steps.
A critical view at our first generation hydroxyphthioceranic acid synthesis, reveals that it lacks the flexibility to also obtain phthioceric acid from this route. This is a consequence of the early introduction of the latent chiral hydroxyl moiety in 52 (Scheme 9). This downside was addressed in a second generation synthesis in which the chiral hydroxyl moiety was installed after the iterative conjugate addition sequence, thereby allowing the preparation of phthioceric acid as well (Scheme 10). After the iterative conjugate addition sequence, installing seven of the desired methyl groups, 61 was converted into allylic bromide 62 using standard chemistry. This compound was treated with 7 mol% of CuBr•SMe₂ and 8 mol% of (R,R)-taniaphos L₃ in presence of MeMgBr. The allylic alkylation provided octamethyl alkene 63 in 88% yield with an excellent diastereomeric excess of >95%. The alkene functionality served as an excellent scaffold for the introduction of the hydroxyl stereogenic center. Initial attempts with the Sharpless asymmetric dihydroxylation led to the desired product, albeit with an unsatisfying diastereomeric excess of 70%. We therefore set out to install the dihydroxy functionality via asymmetric diborylation, which after oxidation gives the desired product. Such methodology has been developed by the Morken laboratory in which bis(pinacolato)diboron (B₂pin₂) is added across the double bond catalyzed by Pt₃dba and taddol-based ligand L₄. The diborylation/oxidation smoothly furnished desired diol 64 in 98% yield with an diastereomeric excess exceeding 95%. With all the stereocenters set, a straightforward five step sequence was employed to install the aliphatic side chain, benzyl protecting group and carboxylic acid moiety to furnish protected hydroxyphthioceranic acid 65 in 38% over the steps.
With hydroxyphthioceranic acid successfully crafted, unification with the trehalose core was left to complete the total synthesis of $\text{Ac}_2\text{SGL}$ (Scheme 11). Trehalose was first protected and acylated in such a way that only the C3-position remained available. 67 was then used in a Yamaguchi esterification with hydroxyphthioceranic acid 65 to give 75 in 76% yield. Removal of the bis(diisopropylsilyl)ether under standard conditions smoothly provided diol 69 which was regioselectively sulfated using 2,2,2-trichloroethyl sulfuryl imidazolium salt 70 as reported by Taylor and co-workers.\cite{42} The approach reported by Taylor was preferred because the 2,2,2-trichloroethyl group can be removed under hydrogenolysis conditions, identical to those planned for the removal of the benzylidene acetals. Introduction of the protected sulfate group furnished 71 in 61% yield which after hydrogenolysis with $\text{Pd(OH)}_2/\text{C}$ and ammonium formate under an hydrogen atmosphere provided us, after 39 steps, with $\text{Ac}_2\text{SGL}$. All analytic data were in agreement with those of the natural material and additional biological studies confirmed the successful synthesis of $\text{Ac}_2\text{SGL}$.

To date our laboratory reported the only complete total synthesis of $\text{Ac}_2\text{SGL}$. The hydroxyphthioceranic acid side chain on the other hand has been synthesized several times.\cite{43} In 2012, Pfaltz and Schneider communicated a total synthesis in which chiral methyl groups were largely installed via iridium-catalyzed substrate controlled asymmetric hydrogenation reactions.\cite{43a} The Aggarwal laboratory reported their hydroxyphthioceranic acid total synthesis in 2014, based on their in-house developed stereoselective lithiation–borylation–protodeboronation sequence.\cite{43b} More recently the same group showed elegant use of this methodology in an iterative fashion by an “assembly-line synthesis” of hydroxyphthioceranic acid.\cite{43c} The most recent synthesis came from the hands of Negishi and co-workers who employed their developed Zr-catalyzed asymmetric carboalumination of alkenes to install the chiral methyl groups.\cite{43d}
1.7 Sulfolipid-1

Shortly after our synthesis of Ac₂SGL we set out to construct the even more complex molecule sulfolipid-1.\[44\] Whereas Ac₂SGL was isolated in 2004, sulfolipid-1 is known for over 40 years since its isolation and structure elucidation, by meticulous degradation studies in 1970 by Goren.\[45\] Sulfolipid-1 is a prominent cell wall constituent of *Mycobacterium tuberculosis* and was postulated to be a virulence factor. Studies with knockout mutants unable to produce sulfolipid-1 supported this hypothesis, indicating a role in host-pathogen interactions by mediating between a cationic human antimicrobial peptide and the bacterium.\[46\]

For the synthesis of sulfolipid-1 we used our previously gained knowledge in the Ac₂SGL total synthesis.\[36\] Yamaguchi esterification of protected trehalose 67 with phthioceranic acid gave 72 with a satisfying yield of 76% (Scheme 12). Installation of the two hydroxyphtioceranic side chains required the regioselective ring-opening of the benzylidene acetals to liberate the free 6-OH and 6'-OH groups while maintaining benzyl protecting groups on the C4 and C4' hydroxyl moieties. Several reductive ring-opening strategies were studied, among them CoCl₂, Cu(OTf)₂, and TMSOTf in combination with BH₃•THF.\[40\] With cobalt the reaction did not give any conversion, whereas Cu(OTf)₂ and TMSOTf afforded 73, albeit with low yields. However, when TMSOTf was used in combination with 12 equivalents of BH₃•THF, 73 was obtained in
a satisfactory 59% yield. Whether this result can be attributed to the kinetics of the desired reaction, or to the quenching of adventitious water, is still not clear.

Scheme 12. The Geerdink and Minnaard synthesis of sulfolipid-1.

The free alcohol moieties were successfully doubly acylated with hydroxyphthioceranic acid 65 using EDC as the coupling agent (71% yield), since the Yamaguchi esterification only gave rise to monoacylated product. Desilylation then set the stage for the sulfation à la Taylor. This time, however, sulfation did not merely affect the C2'-OH position, as in the Ac2SGL synthesis, but also gave rise to sulfation at the 3'-OH position. This observation was attributed to the reduced rigidity of desilylated 74 (compared to 68, Scheme 11) due to opening of the benzylidene acetal. However, with sulfated product in hand the final hydrogenolysis was initially performed under conditions described for the Ac2SGL synthesis. Since atmospheric H2 pressure did not lead to any conversion the pressure was increased to 250 psi, which again, did not lead to full conversion. Further enhancement of the pressure to 500 psi did lead to sulfolipid-1, however, in a low yield of 15%. To account for the high pressure needed it was hypothesized that the long-tailed lipids induced significant steric hindrance, making it difficult for the heterogeneous palladium catalyst to reach the reaction center. As a result of these drastic hydrogenolysis conditions, desulfation of the quite labile sulfate group occurred in part, as confirmed by isolation of the desulfated product next to the
desired product. All in all, 40 years after its isolation we managed to describe the full total synthesis of sulfolipid-1 in 46 steps, thereby confirming the structure of this impressive molecule.

1.8 A tuberculostearic acid containing glycerophospholipid

Phosphatidylethanolamines (cephalins) form a class of glycerophospholipids which play an important role in the structure and function of cellular membranes. In collaboration with the Moody laboratory, a new and abundant glycerophospholipid was isolated from the virulent *M. tuberculosis* strain, H37Rv. Such natural products can be used for identification of specific mycobacterial species and are therefore potentially useful as chemical markers in diagnostic tests for tuberculosis infection. Identification of such products is therefore of utmost importance.

Initial efforts to elucidate the chemical composition of the natural isolate led to the identification of cephalin, palmitoyl, and tuberculostearoyl fragments. Decisive insight into the connectivity of the fatty acids to the cephalin core could not be obtained from mass-spectrometry data, and since the isolated quantities were too low for NMR studies, this hampered full characterization of the natural product. To find out whether the palmitoyl, and tuberculostearoyl fragments were connected to the primary and secondary alcohol in the glycerol core, or *vice versa*, a total synthesis of both isomers was embarked upon to answer this question (Scheme 13, *only one product shown*).

The total synthesis started with a cross-metathesis reaction between thioacrylate and terminal alkene to provide α,β-unsaturated thioester. As a novel substrate for the well-developed asymmetric conjugate addition reaction, a yield of 91% with an enantiomeric excess of 90% could be achieved in the construction of methyl branched 77. Reduction of the thioester via a Fukuyama reduction followed by a Wittig olefination then provided alkene in 92% over the two steps. The alkene was then reduced by an in-house developed Flavin-catalyzed reduction to, after hydrolysis of the isopropyl ester, afford tuberculostearic acid. Coupling of the latter with commercially available (R)-benzyl glycidyl ether using Et₄NBr as the catalyst, gave a smooth opening of the epoxide to yield alcohol 81. Acylation with palmitic acid and debenzylolation gave then furnished alcohol 82, without significant acyl migration of the palmitic acid. Installation of the phosphatidylethanolamine was achieved by reaction of 82 with phosphoramidite reagent 83. Oxidation of the intermediate product gave, after hydrogenolysis of the Bn and Cbz protecting groups, the desired product 84.
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Comparison of the mass spectral data of the natural isolate with 1-O-TBSA-2-O-palmitoyl-sn-phospholipid 84 and its regioisomeric counterpart, that is 1-O-palmitoyl-2-O-TBSA-sn-phospholipid (structure not shown), established the natural product to be 84. The synthesis of the phospholipid not only proved to be helpful in elucidation of the structure, but also provided material to function as an LC-MS standard for the development as a chemical marker.

A revised synthesis of phospholipid 84 was reported in 2013 during our studies into the stereoselective synthesis of glycerol-based lipids (Scheme 14). The previous synthesis of tuberculostearic acid was re-evaluated, providing a two-step shorter route. To achieve this, nonanal 85 was olefinated by means of an Wittig reaction with ylide 86 in the presence of LiCl using “on water” conditions, constructing unsaturated thioester 87 in 92% yield with an excellent E:Z ratio exceeding 95:5. Introduction of the methyl group was achieved in 90% ee and 90% yield. Reduction of the thioester, olefination with the free acid(1) and reduction of the formed double bond using the flavin catalyst, provided tuberculostearic acid in the shortest synthesis to date.

Construction of the glycidyl part was this time accomplished starting from silyl protected glycidol 89 by treatment with Co-salen catalyst C2, Hünig’s base and tuberculostearic acid. The reaction cleanly provided the acylated product and could be acylated in the same pot with palmitic acid under Steglich esterification conditions. The
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diacyl TBDMS protected glycerol 90 was then deprotected using BF$_3$•CH$_2$CN to the free alcohol without migration of the palmitoyl functionality. Further elaboration to install the phosphate group, as previously described, then afforded phospholipid 84.

Scheme 14. A revised total synthesis of diacyl glycerol based phospholipid 84.

1.9 Outline of this thesis

As evident from this chapter, the Minnaard lab has a longstanding interest in the asymmetric total synthesis of (glyco)lipids, and preferably those exhibiting some sort of biological activity. This already led to fruitful collaborations over the past years in which the biological relevance of several Mycobacterium tuberculosis produced lipids have been investigated. These synthetic efforts not only helped to assess bioactivity, but also led to structure-function analysis and assistance in structural assignment of natural isolates. In addition to natural product synthesis, the laboratory also develops new synthetic methodology with an emphasis on asymmetric catalysis.

This dissertation describes my efforts within natural product synthesis and the development of new asymmetric synthesis methodology. Application of the latter in total synthesis, biological studies of synthesized natural products, and the use of synthetic material in structure elucidation are all presented in this thesis.

In chapter 2, research on the stereochemical assignment of saturated oligoisoprenoids is described. Using the β-mannosyl phosphomycoketide side chain as a model, we have demonstrated that the relative stereochemistry of the 1,5-methyl array can be elucidated.
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by comparison of a Traficante processed high field $^{13}$C (175 MHz) or $^1$H-NMR (700 MHz) spectrum, with that of a predicted set of spectra of all possible stereoisomers. The results in this chapter show that the developed method is capable of assessing diastereopurity, and is potentially useful for the structural assignment of saturated oligoisoprenoid bearing natural products, a feature previously absent in the literature.

The content of chapter 3 involves the investigation into a, previously unknown, “lipid” of Mycobacterium tuberculosis. Now known as 1-tuberculosinyl adenosine (1-TbAd), the molecule was not only found to be specific for pathogenic Mtb, but also a highly abundant lipid of which its production is encoded by the virulent associate Rv3377c-Rv3378c locus. In addition, its development into a chemical marker, for a diagnostic test for tuberculosis infection, is reported. This investigation also led to the discovery of a rearranged version of 1-TbAd, called $N^6$-TbAd.

Chapter 4 describes the first enantioselective and diastereoselective total synthesis of 1-TbAd and $N^6$-TbAd. Containing a cyclohexene motive, an asymmetric Diels-Alder cycloaddition is at the heart of the synthesis. Besides successful completion of the synthesis on a multi-gram scale, an extensive overview of the development of a highly stereoselective Diels-Alder reaction is outlined. Furthermore, the synthetic material allows further studies of TbAd as virulence factors.

In chapter 5, the development of the asymmetric palladium-catalyzed conjugate addition of ortho-substituted arylboronic acids to 3-methyl cyclic enones, creating benzylic all carbon quaternary stereocenters, is communicated. Despite previous reports of the conjugate addition of arylboronic acids to 3-substituted cyclic enones, ortho-substituted arylboronic acids were not tolerated in these studies, proving only trace amounts of products, or as one case showed, modest enantioselectivity. The developed methodology was applied in the shortest synthesis of herbertenediol, enokipodin A and enokipodin B to date.

With the asymmetric total synthesis of herbertenediol reported (chapter 5), the synthesis of its dehydrodimer mastigophorene A serves the central theme of chapter 6. Mastigophorene A, containing a benzylic quaternary stereocenter, also bears axial chirality in the form of a tetra-ortho-substituted biaryl axis. With the advent of lithium cross-coupling methodology, developed by the Feringa laboratory, the biaryl axis was introduced with a newly discovered palladium-catalyzed homo-coupling of aryl bromides using lithium reagents. The chiral biaryl axis was constructed with high diastereoselectivity arising from an unexpected point-to-axial chirality transfer.

Chapter 7, provides an overview of our synthetic efforts on the asymmetric total synthesis of taiwaniaquinoid family members. This class of natural products contains a benzylic all-carbon quaternary stereocenter, which is planned to be introduced on a highly unusual substituted 3-methyl cycloheptenone derivative. The conjugate addition
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is planned to be followed by a rearrangement cascade, of which preliminary data is provided, to build the tricyclic \([6,5,6]-abeo\)-abietane core-structure.

1.10 References


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