Virulence Factors

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# Total Synthesis of the Triglycosyl Phenolic Glycolipid PGL-tb1 from *Mycobacterium tuberculosis***

*Santiago Barroso, Riccardo Castelli, Marc P. Baggelaar, Danny Geerdink, Bjorn ter Horst, Eva Casas-Arce, Herman S. Overkleeft, Gijsbert A. van der Marel, Jeroen D. C. Codée,* and Adriaan J. Minnaard**

*Mycobacterium tuberculosis* (M. tb) is one of the most important pathogens. Despite the availability of antibiotics and a vaccine (BCG), one third of the world's population is infected with M. tb, causing 8 million casualties and 1.5 million deaths yearly.[1] Synergy with HIV and the appearance of M. tb strains that are multi-drug resistant or hypervirulent, poses further threats.[2]

The search for novel drugs and more effective vaccines entered a new era with the publication of the genome sequence of M. tb H37Rv.[3] Based on this sequence, genes that code for enzymes involved in the critical steps of host–pathogen interaction were identified. Many of these enzymes are involved in the synthesis and transport of complex lipids, in particular phthiocerol dimycocerosates (DIM[4] or PDIM[5]) present in the outer layer of the M. tb cell envelope. Furthermore, several M. tb strains synthesize closely related phenolic glycolipids (PGL-tb1, Figure 1) in which the phthiocerol is connected to a glycosylated phenol. It has been shown that DIM/PDIMs are required for multiplication and persistence of M. tb in vivo.[6] Next to this, PGL-tb1 (**1**, Figure 1) is suspected to be involved in hypervirulence of specific M. tb strains.[7]

The interplay of M. tb with the human host is very complex, with PGL-tb1 as one of the most unusual virulence factors modulating its defense systems and causing disease. Thus, there is a great need for antigens that permit to distinguish between prior BCG vaccination and infection.

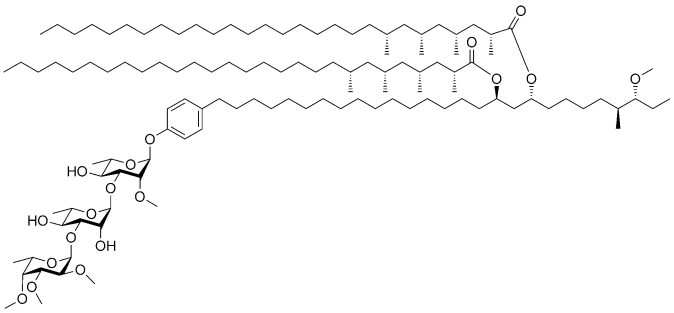


***Figure 1.*** PGL-tb1 (**1**).

Recently, an enzyme-linked immunosorbent assay (ELISA) based on PGL-tb1 has shown potential for the diagnosis of TB in HIV-infected patients.[8] Furthermore, a lipidomics platform has been established for chemotaxonomic analysis of M. tb.[9]

Thus, access to pure, chemically synthesized PGL-tb1 (**1**) has become crucial for reliable immunological studies. Preluded by the first synthesis of phthiocerol dimycocerosate PDIM A[10] we now report the first total synthesis of PGL-tb1.[11]

The size and complexity of PGL-tb1 is impressive. The parent phenylphthiocerol has four stereocenters and is esterified to two molecules of mycocerosic acid (**2**), a long-chain quadruple methyl-branched fatty acid. Through the phenol terminus, the aglycon is linked to a linear trisaccharide.

To get PGL-tb1 within reach, a strongly convergent synthetic strategy had to be designed (see Scheme 1). Esterification with mycocerosic acid was planned for a late stage because of its precious nature. Instead of the glycosylation of the phenol terminus with the trisaccharide construct, we chose to connect the trisaccharide in the form of a *para*-iodophenoxy-substituted glycan to a terminal alkyne through Sonogashira coupling. This strategy avoids a stereoselective glycosylation step with an activated trisaccharide in a late stage of the synthesis. The alkyne function should be fully reduced to the corresponding aliphatic fragment together with the removal of the benzyl protecting groups in the final step. An additional advantage is that the bifunctional "spacer" of the required length, equipped with a terminal alkyne, would be readily accessible from commercially available alkynol **5** in a few steps.

The right-hand side of phenylphthiocerol was planned to be prepared by using our asymmetric conjugate addition–

[*] Dr. S. Barroso, M. P. Baggelaar, D. Geerdink, Dr. B. ter Horst, Dr. E. Casas-Arce, Prof. A. J. Minnaard
Stratingh Institute for Chemistry
University of Groningen
Nijenborgh 7, 9747 AG, Groningen (The Netherlands)
E-mail: a.j.minnaard@rug.nl
Homepage: http://www.rug.nl/fmns-research/stratingh
Dr. R. Castelli, Prof. H. S. Overkleeft, Prof. G. A. van der Marel, Dr. J. D. C. Codée
Leiden Institute of Chemistry, Gorlaeus Laboratories
Leiden University
P.O. Box 9502, Leiden (The Netherlands)
E-mail: jcodee@chem.leidenuniv.nl
Homepage: http://biosyn.lic.leidenuniv.nl/

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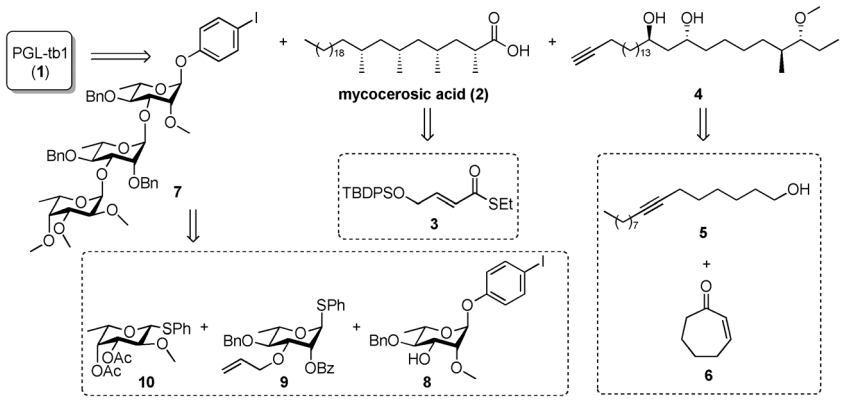


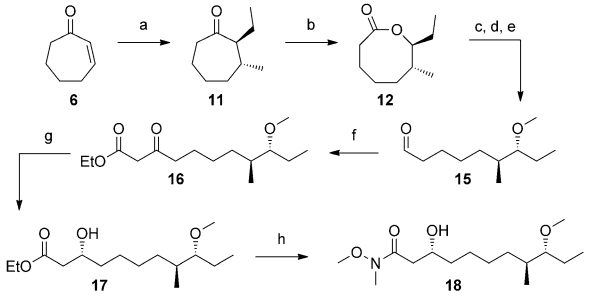

***Scheme 1.*** Retrosynthetic analysis of **1**.

alkylation strategy (see below). The advantage of this approach compared to an asymmetric aldol reaction is that it avoids extensive functional group manipulation to extend this fragment to the required length. The subsequent transformation of this building block to the corresponding β-keto ester, followed by $Ru^{II}$-catalyzed asymmetric hydrogenation, would provide the right-hand side of phenylphthiocerol, which is suitable to connect to the alkyne iodide. After coupling both fragments, the β-hydroxy ketone thus obtained should then be converted into the 1,3-*anti* diol.

The target trisaccharide should be accessible in two distinct glycosylation events between *p*-iodophenol rhamnoside **8**, rhamnosyl thioglycoside **9**, and fucosyl thioglycoside **10**. To achieve α-selective glycosylations, a benzoate ester was chosen as a temporary protecting group for rhamnoside donor **9**, while acetate esters were installed on fucosyl donor **10** to tune its reactivity[12] and to be finally replaced by methyl ethers, as present in the final product. Although the trisaccharide portion had been prepared before,[13] we devised a different approach that centered around the *p*-iodophenol moiety, relying on its pivotal role both for the Sonogashira cross-coupling reaction with alkyne **4** and as anomeric protection for the reducing end of the growing trisaccharide. The construction of the two glycosidic bonds profited from the *N*-thiophenyl-ε-caprolactam/$Tf_2O$ combination to activate the thioglycoside donors.[14] This recently developed method was chosen for its orthogonality to the allyl ether protection in the 3-O position of the central rhamnose unit; which is readily installed by means of a $Bu_2SnO$-mediated regioselective allylation.[15,16]

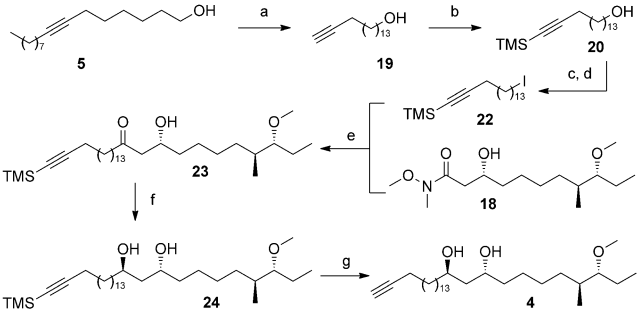
The construction of the aglycone started with a copper/phosphoramidite-catalyzed asymmetric conjugate addition of $Me_2Zn$ to **6** (Scheme 2). The resulting enolate was alkylated in situ with EtI to afford **11** in high yield and excellent stereoselectivity. A subsequent Baeyer–Villiger oxidation of **11** gave the desired lactone **12** in high regioselectivity though with a moderate 60% yield. Efforts to improve this yield were unsuccessful. Lactone **12** was subjected to methanolysis and subsequent O-methylation of the resulting hydroxyester. DIBAL-H reduction afforded the desired aldehyde **15**.

Efficient transformation of **15** into β-ketoester **16** was achieved by treatment with ethyl diazoacetate employing $NbCl_5$ as catalyst.[17] This transformation set the stage for the introduction of the third stereocenter. Thus, β-ketoester **16** was treated with (*R*)-[{RuCl(tol-binap)}$_2$(μ-Cl)$_3$][$NH_2Me_2$] (1 mol%; tol-binap = 2,2′-bis(di-*p*-tolylphosphanyl)-1,1′-binaphthyl) and $H_2$ at 20 bar.[18] Gratifyingly, this gave hydroxyester **17** in good yield and more than 99% *de*. To finalize the building block, **17** was transformed into its Weinreb amide **18** by treatment with $AlMe_3$ and *N*,*O*-dimethylhydroxylamine, which gave higher yields than using the corresponding lithium amide.




***Scheme 2.*** Reagents and conditions: a) $Cu(OTf)_2$ (0.5 mol%), (*S*,*R*,*R*)-phosphoramidite (1 mol%), $Me_2Zn$, toluene, −25 °C. Then EtI, HMPA, 0 °C, 83%, > 20:1 *trans*/*cis*, 95% *ee* (for *trans*); b) *m*-CPBA, $CH_2Cl_2$, reflux, 60%; c) $K_2CO_3$, MeOH, RT; d) NaH, MeI, DMF, RT, 92%; e) DIBAL-H, $Et_2O$, −84 °C, 86%; f) ethyl diazoacetate, $NbCl_5$ (5 mol%), $CH_2Cl_2$, 86%; g) (*R*)-[{RuCl(tol-binap)}$_2$(μ-Cl)$_3$][$NH_2Me_2$] (1 mol%), 20 bar $H_2$, EtOH, RT, 76%, *de* > 99.5%; h) $AlMe_3$, MeNH(OMe)·HCl, THF, 73%. HMPA = hexamethyl phosphoramide, *m*-CPBA = *m*-chloroperbenzoic acid, DIBAL-H = diisobutylaluminum hydride.

Synthesis of the required alkyne iodide **22** started with commercially available alkynol **5** (Scheme 3). A Zipper reaction converted **5** into terminal alkyne **19** (61% yield), which was TMS-protected and transformed into iodide **22** in two steps.[19]

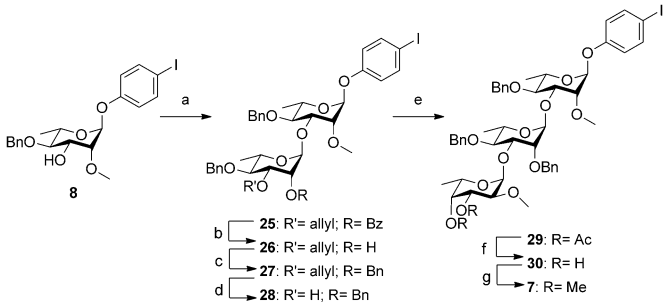



***Scheme 3.*** Reagents and conditions: a) NaH, 1,3-diaminopropane, 70 °C, 61%; b) *n*BuLi, TMSCl, THF, −40 °C to RT; c) *p*-TsCl, pyridine, $CHCl_3$, RT, 87%; d) NaI, acetone, RT, 86%; e) **22**, *t*BuLi, $Et_2O$, −84 °C, 2 h, then amide **18**, 81%; f) $NH_4BH(OAc)_3$, MeCN, AcOH, THF, −25 °C, 76%; g) MeOH, $K_2CO_3$, RT, quant.; TMS = trimethylsilyl, *p*-TsCl = *p*-toluenesulfonyl chloride.

The addition of **22**, as its corresponding Li reagent, to **18** turned out to be not trivial.[20] Initially, a stepwise procedure with initial deprotonation of the hydroxy group in **18** by strong bases suffered from various problems, depending on the base. Therefore it was finally decided to use an excess of lithiated **22** both for the deprotonation and the addition. Extensive optimization showed that using **22** and *t*BuLi in a 1:1.6 ratio for the lithiation prevented the formation of an inseparable *tert*-butyl ketone and gave the maximum yield of the desired hydroxyketone **23**, a satisfying 81%. *Anti*-1,3-diol **24** was obtained stereoselectively in good yields using $NH_4BH(OAc)_3$ according to the Evans protocol.[21] Removal of the TMS group led quantitatively to the required terminal alkyne **4**.

Synthesis of trisaccharide **7** (Scheme 4) commenced with connecting rhamnosides **8** and **9** by exposure to an equimolar amount of *N*-thiophenyl-ε-caprolactam and $Tf_2O$ in a TTBP-buffered solution, without the requirement of preactivation.

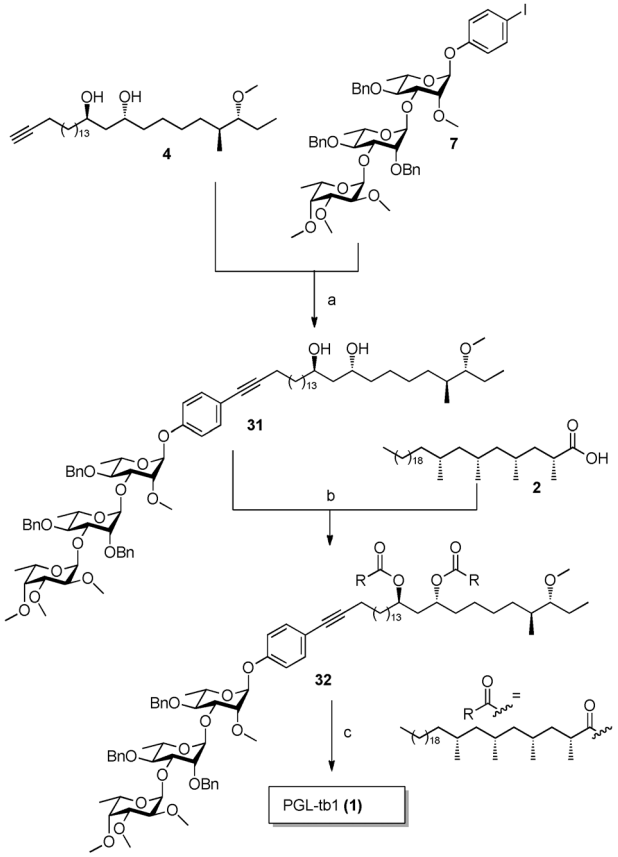



***Scheme 4.*** Reagents and conditions: a) **9**, *N*-thiophenyl-ε-caprolactam, TTBP, $Tf_2O$, $CH_2Cl_2$, −25 °C to 20 °C, 4 h, 71%; b) MeONa, MeOH, 20 °C, 16 h; c) BnBr, NaH, DMF, 0 °C to 20 °C, 2 h, 87% two steps; d) $PdCl_2$ (5 mol%), MeOH, 20 °C, 16 h, 82%; e) **10**, *N*-thiophenyl-ε-caprolactam, TTBP, $Tf_2O$, $CH_2Cl_2$, −25 °C to 20 °C, 4 h, 52%; f) MeONa, MeOH, 20 °C, 16 h; g) MeI, NaH, DMF, 0 °C to 20 °C, 16 h, 85% two steps. TTBP = 2,4,6-tri-*tert*-butyl pyrimidine, $Tf_2O$ = trifluoromethanesulfonic anhydride.

Full stereochemical control to the desired α-configuration was ensured by the 2-*O*-benzoate ester in **9**. The ester was then replaced by a benzyl group in two steps. The allyl ether moiety in disaccharide **27** was then selectively excised by catalytic $PdCl_2$ in MeOH.[22] The obtained disaccharide acceptor **28** was welded together with fucose donor **10** under similar conditions as for the previous glycosylation, yielding trisaccharide **29** in moderate yield and complete α-selectivity. After catalytic transesterification, the hydroxy groups of crude **30** were finally methylated by treatment with an excess of sodium hydride and methyl iodide to furnish **7** in good yield.

We earlier reported a stereoselective preparation of mycocerosic acid in 12% yield over 15 steps, relying on the copper-catalyzed conjugate addition of Grignard reagents to α,β-unsaturated thioesters.[23] However, the iterative protocol as optimized for the synthesis of mycolipenic acid was used, by employing DIBALH for the reduction of thioesters and Horner–Wadsworth–Emmons instead of Wittig olefinations.[24]

With the required building blocks **2**, **7**, and **4** in hand, we could enter the convergent part of the synthesis, depicted in Scheme 5. A key step, Sonogashira reaction between the alkyne **4** and iodophenyl trisaccharide **7**, gave after optimization the desired **31** in 51% yield, along with 35% of recovered starting material. Esterification of the diol moiety in **31** with mycocerosic acid **2** was accomplished using EDC and DMAP, to afford diester **32** in a 72% yield.




***Scheme 5.*** Reagents and conditions: a) $[PdCl_2(PPh_3)_2]$ (5 mol%), $PPh_3$ (5 mol%), CuI (10 mol%), $Et_3N$, 40 °C, 51% (36% **4** recovered); b) EDC·HCl, DMAP, $CDCl_3$, 72%; c) Pd/C, 1 bar $H_2$, EtOAc, EtOH, RT, 86%. EDC·HCl = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, DMAP = 4-(dimethylamino)pyridine, TMS = trimethylsilyl.

We were very pleased to see that the total synthesis of **1** could be concluded efficiently by full reduction of the triple bond and hydrogenolysis of the benzyl ethers with $H_2$ and Pd/C. Full analysis of the resulting compound by NMR spectroscopy, MS, and optical rotation showed **1** to be identical with PGL-tb1 isolated from M. tb.[11]

In summary, the first total synthesis of PGL-tb1 has been accomplished. For mycocerosic acid and the aglycon phthiocerol, Cu-catalyzed asymmetric conjugate additions of MeMgBr and $Me_2Zn$ were used as the key approach, respectively. The synthesis of the iodophenol-functionalized trisaccharide profited from the fully stereoselective glycosylation with rhamnose and fucose thioglycosides using *N*-thiophenyl-ε-caprolactam and $Tf_2O$. Connection of the building blocks by Sonogashira cross-coupling, esterification of the

mycocerosic acid units, and one-step deprotection completed the synthesis. The so-prepared PGL-tb1 is currently applied in a recently established lipidomics platform of M. tb and its availability is expected to advance the development of efficient diagnostic tools for the detection of hypervirulent strains of M. tb and contribute to the understanding of the role of PGL-tb1 in virulence.

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