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Asymmetric Total Synthesis of PDIM A: A Virulence Factor of *Mycobacterium tuberculosis***

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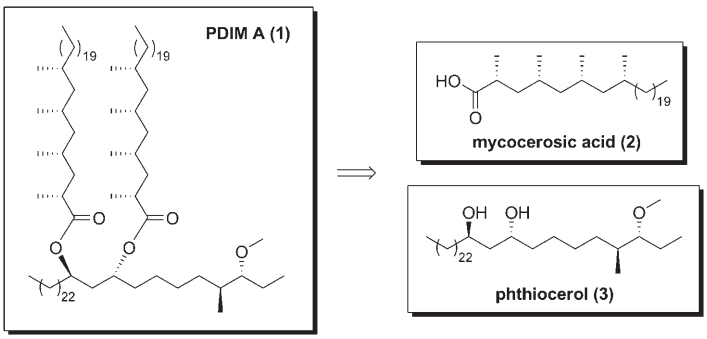
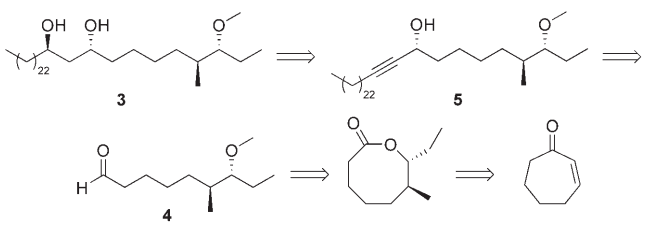
Tuberculosis is the leading cause of death in the world resulting from a single bacterial infection, killing 1.6 million people annually.[1] *Mycobacterium tuberculosis* possesses a complex cell envelope, which is considered one of the major determinants of virulence. An interesting feature of this envelope is its extraordinary high lipid content comprising 40% of its dry weight.[2] In addition, this impermeable barrier imparts resistance against hostile environments as well as therapeutic agents and plays an active role in modulating the host immune response.[3] One of these lipids is phthiocerol dimycocerosate A, PDIM A (**1**, see below), a wax which contains two tetramethyl substituted saturated acids (mycocerosic acid, **2**) esterified to phthiocerol (**3**). Over the years, the cluster of genes responsible for PDIM A biosynthesis has been studied in detail.[4] Cox et al.[5] and Camacho et al.[6] independently reported in 1999 that *Mycobacterium tuberculosis* mutants that are PDIM A defective show severely attenuated virulence. These findings strongly suggest a role for this lipid as an important virulence determinant.



To carry out more detailed immunological studies it is essential to have access to pure PDIM A. Culturing of *M. tuberculosis* and purification of PDIM A from the lipidic fraction is, however, prohibitively difficult. This complex lipid has been isolated as a mixture of long-chain 1,3-diols esterified with different long-chain multimethyl-branched fatty acids.[7] The synthesis of PDIM A is a major challenge, due to the presence of 12 stereocenters and its entirely acyclic nature, but would allow immunological studies not blurred by other cell wall components.

The structure and absolute configuration of mycocerosic acid and phthiocerol were proposed by Polgar and Smith in 1963 and by Maskens and Polgar in 1973,[8,9] respectively. More recent studies, involving MALDI-TOF and ^{1}H NMR analysis, supported this overall structural assignment,[7] but rigorous confirmation by chemical synthesis is lacking.[10] We recently reported the first catalytic asymmetric synthesis of mycocerosic acid[11] and now we present the first asymmetric total synthesis of phthiocerol and its esterification with mycocerosic acid to provide PDIM A.

As outlined in the retrosynthetic analysis given in Scheme 1, we were looking for an efficient approach to prepare the vicinal *anti*-methoxy methyl unit which is present in, among other natural products, various mycobacterial lipids.[2] A tandem catalytic conjugate addition, alkylation sequence on cycloalkenones is known to give predominantly the *trans* product.[12] Carrying out this conjugate addition enantioselectively, in conjuction with a regio- and stereospe-



Scheme 1. Retrosynthetic analysis of phthiocerol.

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[**] PDIM A = phthiocerol dimycocerosate A.

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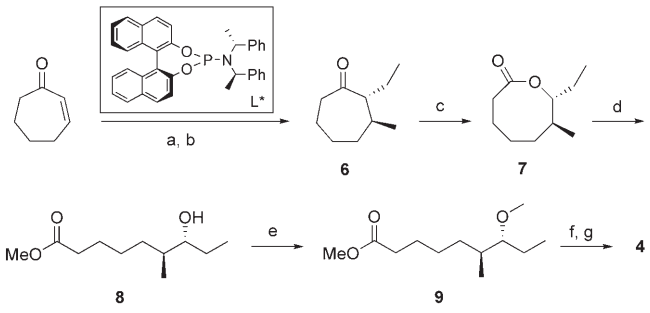
cific Baeyer–Villiger reaction, would lead directly to the desired stereochemistry. Ring opening and methylation of the hydroxy function, followed by reduction of the ester moiety to the corresponding aldehyde, would complete the synthesis of building block **4**. To ensure the correct chain length, cycloheptenone should be the starting enone. For the construction of the *anti*-1,3 diol unit we planned to rely on two recently developed catalytic reactions, the asymmetric alkyne addition from Carreira et al.[13] on **4** and the regioselective ruthenium-catalyzed hydrosilylation from the Trost group[14] on **5**. Subsequent oxidation, followed by stereoselective reduction of the resulting hydroxy ketone would lead to phthiocerol. Double esterification with mycocerosic acid would complete the synthesis of PDIM A.

The construction of building block **4** (Scheme 2) started with the copper/phosphoramidite-catalyzed asymmetric conjugate addition of Me_2Zn to cycloheptenone, followed by in

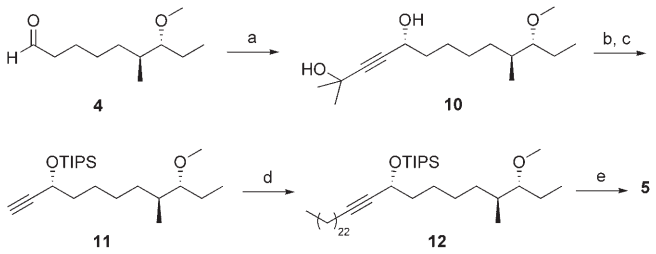


Scheme 2. Catalytic asymmetric synthesis of fragment **4**. a) $Cu(OTf)_2$, (*S*,*R*,*R*)-L*, Me_2Zn, toluene, −25 °C; b) HMPA, EtI, 0 °C, 83 % (over two steps), >20:1 *trans*/*cis*, 95 % *ee* (for *trans*); c) *m*CPBA, CH_2Cl_2, Δ, 60 %; d) K_2CO_3, MeOH, RT, 90 %; e) NaH, MeI, DMF, 40 °C, 92 %; f) DIBAL-H, THF, −78 °C, 95 %; g) Dess–Martin reagent, CH_2Cl_2, RT, 92 %. HMPA = hexamethyl phosphoramide, DIBAL-H = diisobutylaluminum hydride.

situ ethylation.[12] Ketone **6** was isolated in high yield and with excellent *trans* selectivity (>20:1) and *ee* (95 %).[15] Baeyer–Villiger oxidation using excess m-chloroperoxybenzoic acid (*m*CPBA) followed by treatment of the resulting lactone **7** with K_2CO_3 in MeOH led to the formation of the linear product **8**. To prepare aldehyde **4**, the hydroxy group of **8** was converted into its methyl ether, and the ester moiety of **9** was reduced.

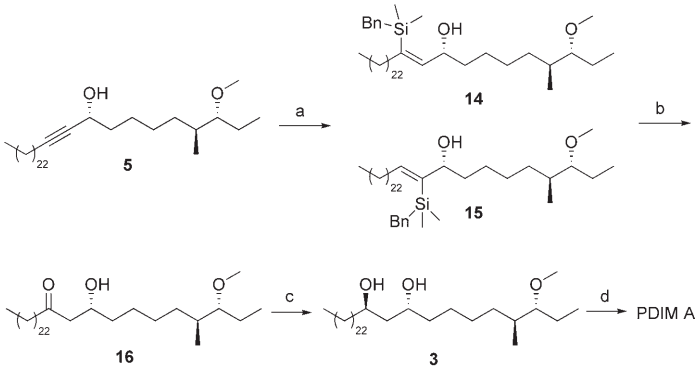
Enantioselective addition of 2-methyl-3-butyn-2-ol to aldehyde **4** in the presence of $Zn(OTf)_2$, Et_3N, and (+)-*N*-methylephedrine[13] allowed the formation of propargylic alcohol **10** (Scheme 3) with excellent selectivity (95 % *de*).[16] The hydroxy group in **10** was protected as a silyl ether, and the alkyne moiety deprotected under basic conditions to afford **11**. Alkylation of the corresponding alkynyllithium compound using $CH_3(CH_2)_{22}Br$ in the presence of NaI afforded the protected propargylic alcohol **12**.[17] Finally, treatment with tetrabutylammonium fluoride (TBAF) led to the formation of building block **5**.

We were pleased to observe regioselective hydrosilylation of propargylic alcohol **5** with benzyldimethylsilane



Scheme 3. Asymmetric synthesis of fragment **5**. a) $Zn(OTf)_2$, Et_3N, (+)-*N*-methylephedrine, 2-methyl-3-butyn-2-ol, toluene, RT, 78 %, 95 % *de*; b) TIPSOTf, 2,4-lutidine, CH_2Cl_2, 0 °C, 95 %; c) NaH, toluene, Δ, 96 %; d) *n*BuLi, THF, −78 °C, then $CH_3(CH_2)_{22}Br$, NaI, THF, Δ, 87 %; e) TBAF, THF, 0 °C, 92 %. TIPS = triisopropylsilyl.

(BDMSH) catalyzed by $[Cp^*Ru(MeCN)_3]PF_6$, following the protocol described by Trost (Scheme 4). It displays this reaction as a versatile method in natural product synthesis.[14,18] It afforded a mixture of benzyldimethyl silanes **14** and **15** with a ratio 4:1. Treatment with TBAF followed by a Fleming–Tamao oxidation,[19] using $KHCO_3$ and H_2O_2, resulted in the formation of the corresponding hydroxy ketones, which could be separated by column chromatography affording **16** as a pure isomer.



Scheme 4. Catalytic asymmetric synthesis of phthiocerol and coupling with mycocerosic acid. a) BDMSH, $[Cp^*Ru(MeCN)_3]PF_6$, CH_2Cl_2, RT, 86 %, **14**/**15** ratio 4:1; b) TBAF, THF, 0 °C, then $KHCO_3$, H_2O_2, RT, 63 %; c) $Me_4N(CH_3CO_2)_3BH$, THF/AcOH, RT, 90 %, *anti*/*syn* 88:12; d) mycocerosic acid, DCC, DMAP, CH_2Cl_2, RT, 63 %.

To produce selectively the *anti*-1,3-diol, reduction of **16** was carried out with tetramethylammonium triacetoxyborohydride (Scheme 4).[20] A solvent mixture of acetic acid and THF was required to ensure sufficient solubility, and led to an excellent yield and *anti*/*syn* selectivity (88:12). Both diols were separated by column chromatography. The *syn*- and *anti*-1,3-diols show distinct differences in their ^{13}C NMR spectra,[21] which were used to assign their relative configuration.[22] The optical rotation of *anti*-**3**, $[\alpha]_D^{22} = -4.5°$ ($c = 0.4$, $CHCl_3$), is consistent with the literature value for phthiocerol ($[\alpha]_D = -4.5°$, $CHCl_3$).[9]

Double esterification of phthiocerol with mycocerosic acid, prepared following the protocol recently disclosed

from our laboratories,[11] in the presence of dicyclohexyl carbodiimide (DCC) and 4-dimethylaminopyridine (DMAP)[23] gave PDIM A in 63% yield (Scheme 4). MALDI-TOF and ^{1}H NMR analysis of the final product matched with the ones reported in the literature for PDIM A isolated from *Mycobacterium tuberculosis* (see Supporting Information).[7]

In summary, we have achieved the first asymmetric synthesis of phthiocerol in 15 steps and 5.6% overall yield by applying three efficient catalytic transformations. Enantiopure PDIM A has been prepared from phthiocerol and mycocerosic acid, thereby confirming its structure. The access to these mycobacterial cell wall lipids in pure form will be used to establish their role as virulence factors of *Mycobacterium tuberculosis.*

Acknowledgement

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Keywords: conjugate addition · alkyne addition · asymmetric synthesis · hydrosilylation · *mycobacterium tuberculosis*

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[15] GC analysis of (3*S*)-methylcycloheptanone showed 95% *ee* (see Supporting Information). After trapping the enolate, the c*is* isomer was not visible by ^{1}H NMR analysis, but it appeared in trace amounts in ^{13}C NMR (see Supporting Information).

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[22] It is reported (ref. [21]) that, for a pair of diastereoisomers, the signals of the α-hydroxy carbons appear at higher field for the *anti*-diols than for the *syn*-diols. For *anti*-**18** these signals appear at $\delta =$ 69.3 and 69.4 ppm and for *syn*-**18**, at $\delta = 73.2$ and 73.3 ppm (see Supporting Information).

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