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Catalytic Asymmetric Synthesis of Mycolipenic and Mycolipanolic Acid

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The first asymmetric synthesis of mycolipenic acid and mycolipanolic acid by using an improved iterative procedure involving catalytic asymmetric conjugate addition of MeMgBr as the key step is described. Mycolipenic and mycolipanolic acid are obtained in 11 steps with perfect stereocontrol, and both acids are identical to their counterparts from natural sources.

Introduction

Mycobacterium tuberculosis (*M. tuberculosis*), the bacterium responsible for the disease tuberculosis is still one of the most lethal pathogens causing an annual death rate of nearly two million people worldwide.[1] The cell wall of the bacterium contains very complex lipids exclusively found in pathogenic mycobacteria.[2] Pentaacyltrehalose (PAT, **1**) and diacyltrehalose (DAT, **2**) are examples of cell-wall lipids that are only found in virulent strains of *M. tuberculosis* (Figure 1).[3b] Besides the fact that these lipids can potentially be used as biomarkers, they are also known to elicit an immune response in human, which makes them candidates for vaccination studies.[3–6]

PAT (**1**) and DAT (**2**) are two trehalose-based glycolipids esterified with mycolipenic (**3**) and mycolipanolic acid (**4**), respectively (Figure 2). Mycolipenic acid, the major acyl residue found in PAT and some forms of DAT, has shown to be a potent inhibitor of leukocyte migration (pointing at an immune response), in vitro.[3b,7] It has also been shown that **3** acts as a B-cell antigen.[4,8,12]

Mycolipenic acid (phthienoic acid, **3**) and mycolipanolic acid (**4**) are both considered to be produced by polyketide synthase Pks2 (polyketide synthase 2), a member of the same enzyme class involved in the synthesis of phthioceranic and hydroxyphthioceranic acid.[3a,10] This accounts for the stereochemical relationship of the methyl substituents, which is all-*syn* and of the (*S*) configuration at the stereocenters, identical to phthioceranic acid and hydroxyphthioceranic acid.[11] In addition, the biosynthetic pathway of mycolipenic and mycolipanolic acid are closely related, as both are most likely derived from the same precursor.

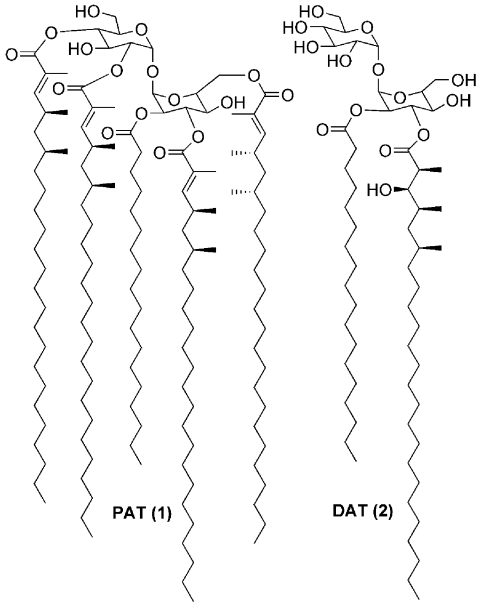

Figure 1. Penta- and diacyltrehalose PAT (**1**) and DAT (**2**) from *M. tuberculosis*.

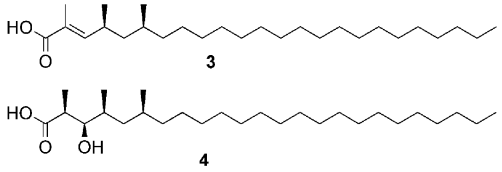

Figure 2. Methyl-branched acids mycolipenic (**3**) and mycolipanolic acid (**4**).

The dehydratase unit of Pks2 can eliminate water from mycolipanolic acid resulting in α,β-unsaturated mycolipenic acid.

Mycolipenic acid (**3**) was first isolated and characterized in the 1950s by Polgar.[12,13] The compound was synthesized in 1958 by a lengthy route, comprising a kinetic resolution,

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which confirmed the stereochemistry to be all-(*S*) and the double bond to have (*E*) stereochemistry.[13] Mycolipenic acid has been synthesized as a racemate by Minnikin et al. in 1992.[9]

Mycolipanolic acid (**4**) was isolated and characterized by degradation studies in 1968 by Polgar.[14] A racemic total synthesis of mycolipanolic acid (as a mixture of diastereomers) has been reported by Minnikin in 1996.[15]

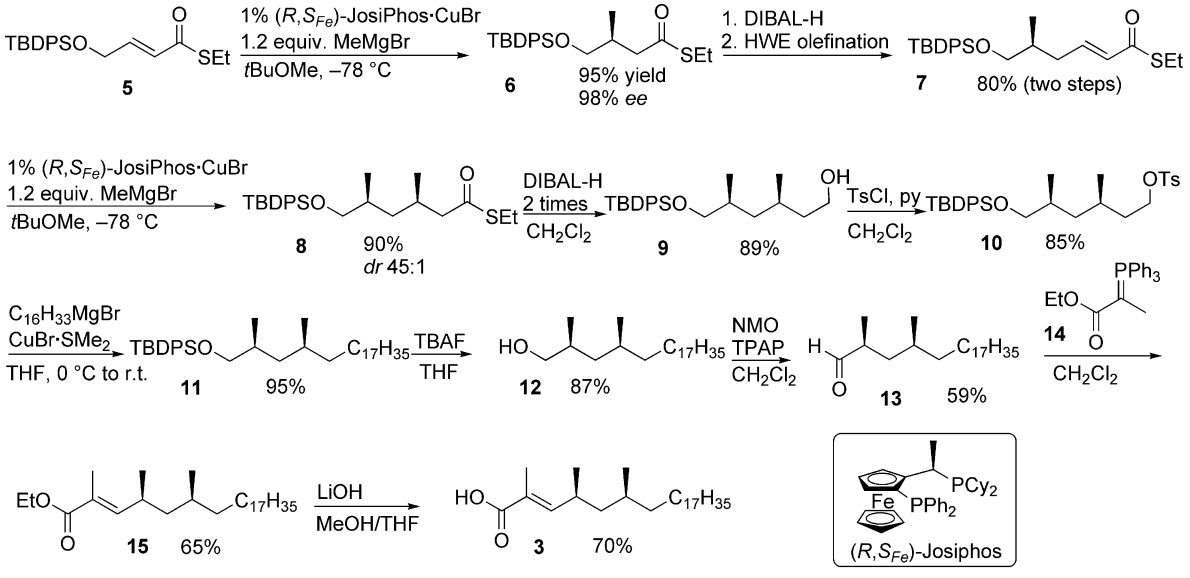
Because only pathogenic *M. tuberculosis* contains **1** and **2**, isolation of these lipids and acids from *M. tuberculosis* is undesirable. Besides the danger of working with virulent *M. tuberculosis*, the extremely slow growth of the bacteria (cell division every 22–24 h[16] compared to 20 min for *E. coli*) makes it difficult to obtain sufficient quantities for biological studies. Moreover, most of the glycolipids occur as mixtures of analogues, which differ in the number of acyl chains on trehalose, the number of methyl substituents and the chain length. To study the biological activity of PAT (**1**) and DAT (**2**) it is important to obtain pure and fully characterized material, and therefore a synthetic approach is highly desirable. Nevertheless, as these lipids contain multiple stereocenters, their efficient and enantioselective preparation is far from trivial. As part of our ongoing research on the cell-wall constituents of *M. tuberculosis*[11,17] comprising the synthesis of PAT (**1**) and DAT (**2**), we therefore decided to embark on the enantioselective synthesis of mycolipenic (**3**) and mycolipanolic acid (**4**).

Results and Discussion

We started the synthesis of mycolipenic acid with a careful optimization of our iterative copper-catalyzed asymmetric 1,4-addition protocol we reported previously.[11,18] Starting from **5** and using 1 mol-% of (R,S_{Fe})-Josiphos/CuBr as the catalyst in combination with 1.2 equiv. of MeMgBr, we obtained compound **6** as the (*S*) enantiomer in excellent yield (95%) and enantiomeric excess (*ee* = 98%).

In our previous reported reduction/olefination sequence, a Fukuyama reduction with Pd/C and Et_3SiH was applied, followed by Wittig olefination. This typically resulted in a yield of 70% over two steps.[11] It turned out, however, that reduction of thioester **6** with DIBAL-H followed by olefination with Horner–Wadsworth–Emmons (HWE) reagent $(EtO)_2P(O)CH_2COSEt$, afforded unsaturated thioester **7** in 80% yield over two steps (typically 80–90%). This improved sequence makes the methodology, as the steps are iterative, considerably more efficient.

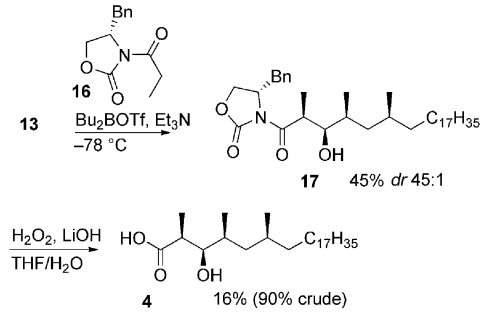
The second asymmetric 1,4-addition reaction was performed under the same conditions as the first addition; *syn* product **8** was obtained in 90% yield with an excellent *syn*/*anti* ratio of 97:3. Thioester **8** was subsequently reduced to alcohol **9** with DIBAL-H in 89%. Tosylation of alcohol **9** to give **10** (85%), followed by a copper-catalyzed Grignard cross coupling with $C_{16}H_{33}MgBr$ resulted in silyl ether **11** (95%). Subsequent deprotection with tetrabutylammonium fluoride (TBAF) in THF resulted in alcohol **12** in 87% yield. Alcohol **12** was oxidized to aldehyde **13** under neutral conditions with *N*-methylmorpholine oxide (NMO) and catalytic tetrapropylammonium perruthenate (TPAP) in 90% yield.[19] No epimerization of the α-methyl stereocenter was observed during this transformation. Aldehyde **13** was treated with Wittig reagent **14**, which resulted in the corresponding olefin with an (*E*)/(*Z*) ratio of 9:1; the desired (*E*) isomer **15** was isolated in 65% yield.[20] This reagent has been used previously in the synthesis of polymethylated fatty acids.[21] In the final step, ethyl ester **15** was hydrolyzed in a water/THF mixture with LiOH, and mycolipenic acid (**3**) was obtained in 85% yield (Scheme 1). The optical rotation of the synthetic material {[a] = +16.4 (c = 1.96, $CHCl_3$)} matches well with that reported in literature for the natural product isolated from *M. tuberculosis* ([a] = +19).[13] The methyl ester of mycolipenic acid was subsequently prepared by treatment of **3** with (trimethylsilyl)diazomethane in methanol, and its optical rotation {[a]



Scheme 1. Total synthesis of mycolipenic acid (**3**).

= +14.5 (c = 0.47, $CHCl_3$)} corresponds well with the values of [a] = +14.7 and +16.8 found in the literature for the methyl ester.[13,22]

For the synthesis of mycolipanolic acid we envisioned an Evans aldol reaction by using the boron enolate of enantiopure oxazolidone **16** with aldehyde **13** for the introduction of the *syn*-hydroxymethyl unit.[23,24] To prevent over-oxidation or epimerization at the α-stereocenter, aldehyde **13** was prepared freshly for the Evans aldol reaction (Scheme 2). After the aldol reaction with **16** and workup, **17** was isolated in a moderate 45% yield with perfect stereocontrol.



Scheme 2. Evans aldol reaction for the introduction of the *syn*-vicinal hydroxymethyl motif in mycolipanolic acid (**4**).

Although a very small amount of a diastereomer of **17** was observed in the ^{13}C NMR spectrum (see Supporting Information), this is most likely the minor diastereomer already present in **13** as a result of the two enantioselective 1,4-addition reactions (*syn*/*anti* ratio of 97:3).[11b] Removal of the chiral auxiliary with H_2O_2 and LiOH yielded the desired mycolipanolic acid **4** in 90% crude yield. Unfortunately, we lost material during chromatography, and only 16% of pure **4** was isolated. Later it was found that purification on silica with pentane/diethyl ether (5:1) containing 1% acetic acid gave perfect separation, and no problems were observed during the isolation process. A sample of **4** was converted into the corresponding methyl ester by using excess (trimethylsilyl)diazomethane in MeOH to compare the optical rotation to the literature value of the natural product. The optical rotation of the methyl ester of synthetic **4** {[a] = –7.0 (c = 0.2, $CHCl_3$)} is in perfect agreement with the literature value of [a] = –7.19.[14]

Conclusions

Mycolipenic and mycolipanolic acid, two methyl-branched fatty acids from *M. tuberculosis*, have been successfully synthesized by using an improved iterative protocol for asymmetric conjugate addition of MeMgBr. Both compounds have been prepared for the first time as a single enantiomer. Mycolipenic acid was obtained with an overall yield of 5% in 11 steps with an average of 84% yield per step. The closely related mycolipanolic acid was synthesized enantioselectively with an overall yield of 2% in 11 steps with an average yield of 75% per step. Both acids are identical to their counterparts from natural sources and are now available for the synthesis of PAT (**1**) and DAT (**2**), which is currently under investigation in our laboratories.

Supporting Information (see footnote on the first page of this article): Detailed experimental procedures, ^1H and ^{13}C NMR spectroscopic and analytical data of all compounds in Schemes 1 and 2.

Acknowledgments

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