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The synthesis of methoxy and keto mycolic acids containing methyl-*trans*-cyclopropanes

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ABSTRACT

The syntheses of a number of methoxy and keto-mycolic acids containing an α-methyl-*trans*-cyclopropane unit and with chain lengths identical to those reported in major homologues in natural samples are reported.

1. Introduction

Mycolic acids, e.g., **1** (Fig. 1), are characteristic components of the cells of mycobacteria and of a number of related organisms, such as *Rhodococcus* and *Nocardia*, some of which are pathogenic to animals and humans.[1–6] Their presence is thought to be linked to the characteristic resistance of many such organisms to most current antibiotics and other chemotherapeutic agents.[7]

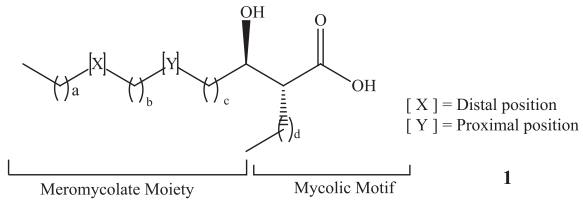

Fig. 1. A generalised mycolic acid structure.

The mycolic acids present as major constituents of the cell envelope of *Mycobacterium tuberculosis* can be divided into a number of main groups, including α-mycolic acids with two *cis*-cyclopropanes as X and Y, **2**, and methoxy-**3** and keto-mycolic acids **4** with one *cis*-cyclopropane.[5,6] In addition they include methoxy- and keto-mycolic acids in which there is a *trans*-cyclopropane substituted with a methyl group on the adjacent carbon distal from the hydroxy acid, **5** and **6** (Fig. 2). The stereocentres in the α and β-positions relative to the carboxylic group have both been found to be in the *R*-configuration for all the mycolic acids examined, irrespective of the other functional groups.[8–12]

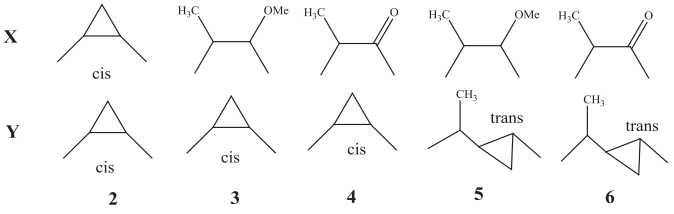

Fig. 2. Common combinations of X and Y in *M. tuberculosis* mycolic acids.

The presence of the β-hydroxy group and the relative configuration between it and the alkyl chain has been shown to be capable of altering the film molecular packing.[13,14] Moreover, the absolute configuration of these two chiral centres is necessary for efficient recognition by T cells and the generation of an immune response by the host organism against pathogenic mycobacteria;[15] the same is true for the anti-tumour properties of mycolic acid derivatives.[16] The balance of α-mycolic acids **2**, methoxy-**3** and **5** and

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keto-mycolic acids, such as **4** and **6** is characteristic of specific bacteria;[5,6] in each case, each class of mycolic acid is present as a mixture of homologues. In the case of *M. tuberculosis*, the exact role of each type in the pathogenesis of disease remains to be confirmed, but the oxygenated mycolic acids have a particular influence on macrophage growth; strains lacking ketomycolates have a reduced ability to grow within THP-1 cells.[17,18] Moreover, the absence of keto and methoxy-mycolates leads to attenuation of *M. tuberculosis* in mice;[19] the vaccine strain *Mycobacterium bovis* BCG-Pasteur lacks methoxy-mycolates.[20–22] *M. tuberculosis* controls host innate immune activation through cyclopropane modification of a glycolipid effector molecule.[23–25] Cyclopropane stereochemistry plays a key role in pathogenesis and immuno-modulatory function; thus a mutant strain lacking the ability to produce *trans*-cyclopropanes enhances the macrophage inflammatory response.[26] The stereochemistry was shown to directly affect the interaction with host cells to affect innate immune activation both positively and negatively. Such MAs have a particular effect on the cell wall and therefore on the sensitivity of mycobacterial species to hydrophobic antibiotics.[27] The genes responsible for *trans*-cyclopropanation have been examined,[27,28] and cyclopropanation has been theoretically modelled.[29]

We have recently reported the synthesis of an α-mycolate of type **2**,[30] and of methoxy-mycolates of type **3** with either absolute stereochemistry at the *cis*-cyclopropane or α-methyl-β-methoxy fragment.[31] We have also reported the synthesis of meromycolate fragments containing the α-methyl-*trans*-cyclopropane unit present in mycobacterial wax esters, again in a variety of stereochemistries, and provided evidence that the relative stereochemistry of methyl and cyclopropane is as shown in **7**, since the cyclopropane region of the ^{1}H NMR spectrum of this stereoisomer matches that of the natural material, whereas that of an isomer with the opposite relative stereochemistry of methyl group and cyclopropane is quite different.[32] Moreover, at least in the case of wax esters derived by enzymatic Baeyer–Villiger reaction of ketomycolates, the absolute stereochemistry of this sub-unit appears to be as shown in **7**.[33] There is evidence that in most cases the methoxy and methyl groups of mycolic acids **3** and **5** are *S*,*S* and that the α-methyl ketone of ketomycolates is *S*, though it is not clear whether the stereochemistry is important for biological effect.[9,34,35] We have briefly reported syntheses of protected ketomycolates containing both α-methyl-*trans*- and *cis*-cyclopropane fragments, **6** and **4** that can be adjusted to produce a variety of absolute stereochemistries and chain lengths.[36] However, deprotection of these led to epimerisation at the methyl-position adjacent to the ketone. More recently, we have reported in full the synthesis of keto-MA containing *cis*-cyclo-propanes.[37] We now report in full the synthesis of both keto- and methoxy-MA containing an α-methyl-*trans*-cyclopropane fragment **7** (Fig. 3).

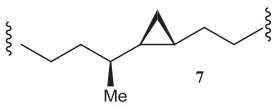
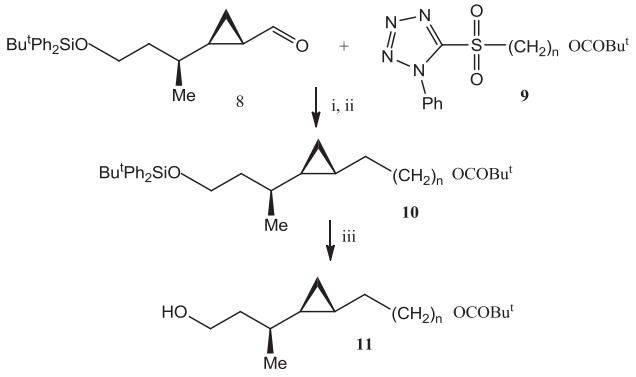


Fig. 3. A typical α-methyl-*trans*-cyclopropane fragment.

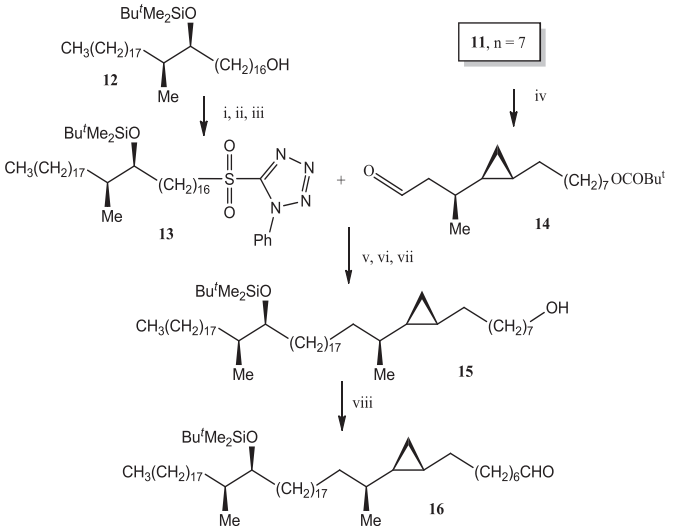
2. Results and discussion

As in other syntheses of mycolic acids, the molecules were constructed from three fragments, one the fragment containing group X, one group Y, the third the β-hydroxy acid part, and these were linked through modified Julia–Kocienski reactions, followed by hydrogenation of the derived mixture of *E*/*Z*-alkenes. The known cyclopropane **8**,[32,33] was first coupled to sulfone **9** (n=7) (prepared by a modification of a standard method; see Supplementary data) using lithium hexamethyldisilazide, then the derived mixture of alkenes was hydrogenated using 2,4,6-triisopropyl sulfonylhydrazide, to produce the chain extended ester **10** (n=7), $[\alpha]_D^{25}$ +7.61 (*c* 1.31, $CHCl_3$). This was deprotected to produce the alcohol **11** (n=7), $[\alpha]_D^{25}$ +16.05 (*c* 1.19, $CHCl_3$) (Scheme 1).



Scheme 1. (i) LiHMDS, THF, 69%; (ii) 2,4,6-triisopropyl sulfonylhydrazide, THF, 87%; (iii) tetra-*n*-butyl ammonium fluoride, THF, 91%.

The alcohol was then converted into aldehyde **14**, which was coupled in the next step to the sulfone **13**, prepared in three standard steps from alcohol **12**. Reaction between the two compounds in the presence of lithium hexamethyldisilazide led to a 70% yield of an *E*/*Z*-mixture of alkenes, which was hydrogenated using di-imide to produce the meromycolate fragment **15** after partial deprotection. Oxidation of **15** led to the aldehyde **16**, ready for the final coupling to produce the complete carbon skeleton of the mycolic acid (Scheme 2).



Scheme 2. (i) *N*-Bromosuccinimide, PPh_3, CH_2Cl_2, 91%; (ii) 1-phenyl-1*H*-tetrazol-5-thiol, K_2CO_3, acetone, THF, 95%; (iii) H_2O_2, $Mo_7O_{24}(NH_4)_6{\cdot}4H_2O$, IMS, THF, 82%. (iv) PCC, CH_2Cl_2, 91%; (v) LiHMDS, THF, 70%; (vi) KOOCN=NCOOK, AcOH/MeOH/THF, 75%; (vii) $LiAlH_4$, THF, 82%; (viii) PCC, CH_2Cl_2, 86%.

The final coupling required the sulfone **20**, prepared, as described earlier for different chain lengths, from aldehyde **18** (Scheme 3).

Coupling of **16** and **20** with base, followed by saturation of the derived alkenes led to the protected mycolic acid **21** (Scheme 4). Deprotection using HF-pyridine and pyridine, followed by lithium hydroxide produced the hydroxymycolic acid **23**, the first example of a synthetic hydroxy-MA containing a *trans*-cyclopropane.