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The first synthesis of epoxy-mycolic acids

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ABSTRACT

We report the synthesis of single enantiomers of two epoxy-mycolic acids containing an α-methyl-*trans*-alkene.

Mycolic acids (MAs), **1** (Fig. 1), are the major constituents of the cell envelope of *Mycobacterium tuberculosis* and other mycobacteria, some of which are pathogenic to animals and humans.[1–3] Their presence is thought to be linked to the resistance of these organisms to most current antibiotics and other chemotherapeutic agents.[4]

The two stereocentres at 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. In each case 'a–d' represent long alkyl chains, and generally each *Mycobacterium* contains a mixture of several homologues. In the common classes of MA, the proximal group is often a cyclopropane or an alkene and the distal group is a cyclopropane (α-MA), an α-methoxy-β-methyl fragment (methoxy-MA) or an α-keto-β-methyl fragment (keto-MA).[2,3] In 1981, Daffé et al. reported the identification of a new kind of mycolic acid in *Mycobacterium fortuitum* containing an α-methyl epoxy-group at the distal position and a *cis*-alkene at the proximal position (**2**, R = H) (Scheme 1).[5] Minnikin et al. described the presence of similar molecules in *M. fortuitum*, *Mycobacterium farcinogenes* and *Mycobacterium senegalense*; in these cases the major isomers had a *cis*-alkene at the proximal position, but there was a minor (around 30% by NMR spectroscopy) component (**2**, R = Me) containing an α-methyl-*trans*-alkene at the proximal position; the major isomer of the latter contained 78 carbons.[6–8] Epoxy MAs are also present in *Mycobacterium chitae*,[8] *Mycobacterium giae*,[8] *Mycobacterium peregrinum*,[7–9] *Mycobacterium porcinum* and *M. senegalense*,[10] and in a mutant strain of *M. tuberculosis*.[11] Epoxy-mycolic acids have also been detected directly by MALDI-TOF mass spectrometry; the major isomers containing a *trans*-alkene at the proximal position are reported to be C_{78} and C_{80} molecules.[12,13] Studies using *Mycobacterium smegmatis* have identified the function of the protein MSMEG0913 in adding the methyl branch adjacent to both an alkene and a cyclopropane at the proximal position of epoxy-mycolates to produce the *trans*-homologues.[13] The relative and absolute stereochemistry of epoxy-mycolates containing a proximal *cis*- or α-methyl-*trans*-alkene has been probed by two methods (Scheme 1).[14] Firstly, opening of the epoxide **2** by acetolysis followed by saponification and oxidative cleavage of both the derived 1,2-diol and the alkene led to three products, including (*R*)-ester **3**. Secondly, reductive ring-opening of the epoxide **2** followed by oxidative cleavage of the proximal alkene, saponification and

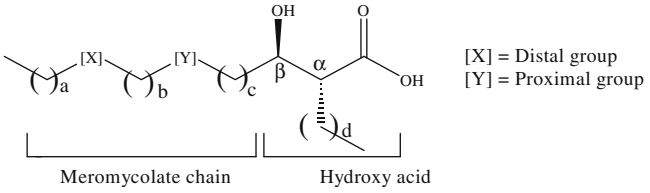
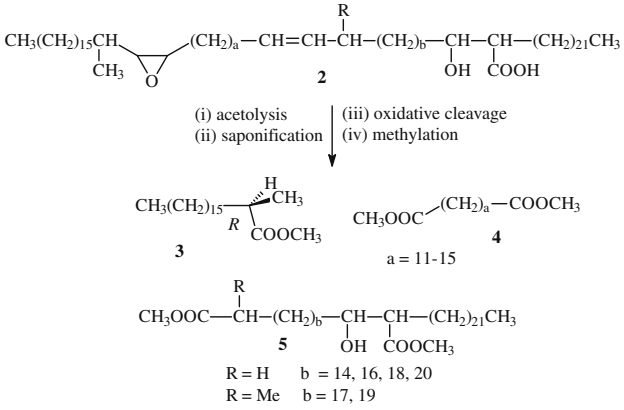


Figure 1.



Scheme 1.

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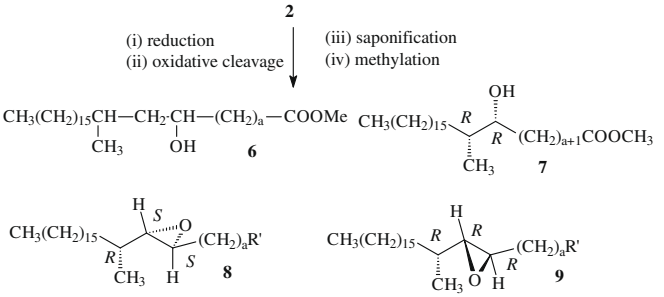


Scheme 2.

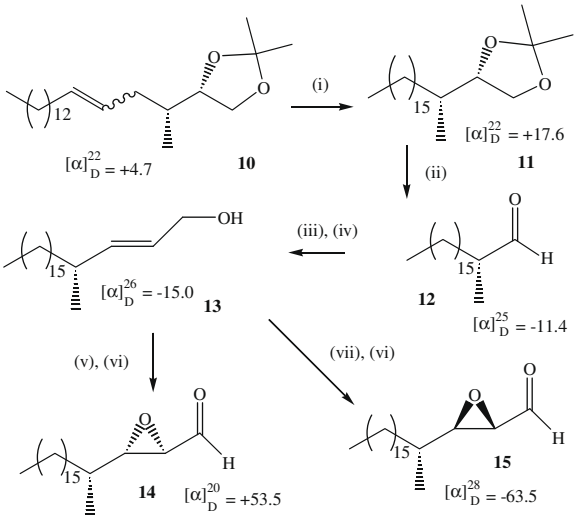
methylation led to the two acids **6** and **7** (Scheme 2). The latter was shown to have *R,R*-stereochemistry by comparison to a model compound. On this basis the authors assigned all the stereocentres in the epoxy-mycolic acid as *R*.[14]

However, it seems clear in fact that the result actually suggests that the epoxy fragment is *R,S,S* as in **8** rather than *R,R,R* as in **9**, the priorities in the epoxide being different from those in the ring-opened alcohol.

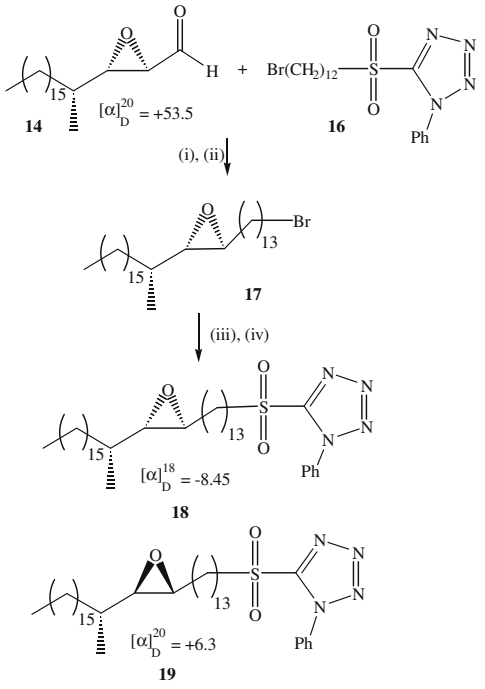
We have recently reported the syntheses of an α-mycolic acid[15] and of methoxymycolic acids with absolute stereochemistry either at the *cis*-cyclo-propane or at α-methyl-β-methoxy fragment.[16] We have also reported the synthesis of meromycolate fragments containing the α-methyl-*trans*-cyclo-propane unit,[17] and of hydroxy- and ketomycolic acids containing an (*R*)-α-methyl-*trans*-alkene unit.[18] We now report the synthesis of two stereoisomeric epoxy-mycolic acids **28** and **29** containing an (*R*)-α-methyl-*trans*-alkene at the proximal position; these have the chain lengths reported for one mycolic acid in *M. fortuitum*,[19] and the total carbon number is consistent with those observed for one component of a mixture from *M. smegmatis* by MALDI mass spectrometry.[12,13]

The *trans*-epoxide unit was obtained from the acetal **10**, itself derived from D-mannitol. Hydrogenation and cleavage of the acetal led to aldehyde **12** which could be converted into alcohol **13** by standard reactions (Scheme 3). Asymmetric epoxidation led to the two diastereoisomers **14** and **15**.

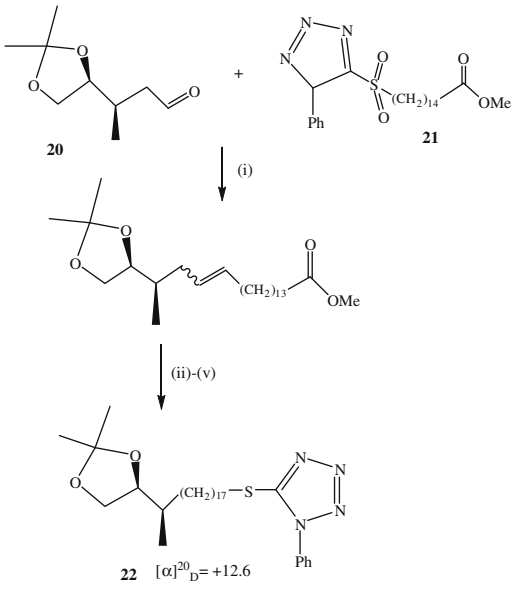
The aldehyde **14** was chain extended by reaction with the sulfone **16** and base, followed by saturation of the derived alkene using di-imide. The resulting bromide **17**[20] was then converted



Scheme 3. Reagents and conditions: (i) H_2, Pd/C, ethanol (92%); (ii) HIO_4 (77%); (iii) $Ph_3P{=}CHCOOMe$, toluene (65%); (iv) DIBAL, CH_2Cl_2 (95%); (v) L-(+)-diethyl tartrate, Ti(O*i*-Pr)$_4$, *t*-BuOOH, CH_2Cl_2, −20 °C (75%); (vi) PCC, CH_2Cl_2 [82% (**14**), 75% (**15**)]; (vii) D-(−)-diethyl tartrate, Ti(O*i*-Pr)$_4$, *t*-BuOOH, CH_2Cl_2, −20 °C (67%).



Scheme 4. Reagents and conditions: (i) LiBSA, THF, −10 °C (71%); (ii) $KO_2CN{=}N\text{-}CO_2K$, AcOH, THF, MeOH (93%); (iii) 1-phenyl-1*H*-tetrazole-5-thiol, acetone, K_2CO_3 (82%); (iv) H_2O_2, $Mo_7O_{24}(NH_4)_6{\cdot}H_2O$, IMS, THF (67%).



Scheme 5. Reagents and conditions: (i) LiBSA, −10 °C (61%); (ii) H_2, Pd/C, IMS, THF (93%); (iii) $LiAlH_4$, THF (91%); (iv) NBS, Ph_3P, CH_2Cl_2 (96%); (v) 1-phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone (90%).

into the corresponding sulfone **18**. In the same manner, diastereoisomer **15** was converted into **19** (Scheme 4).

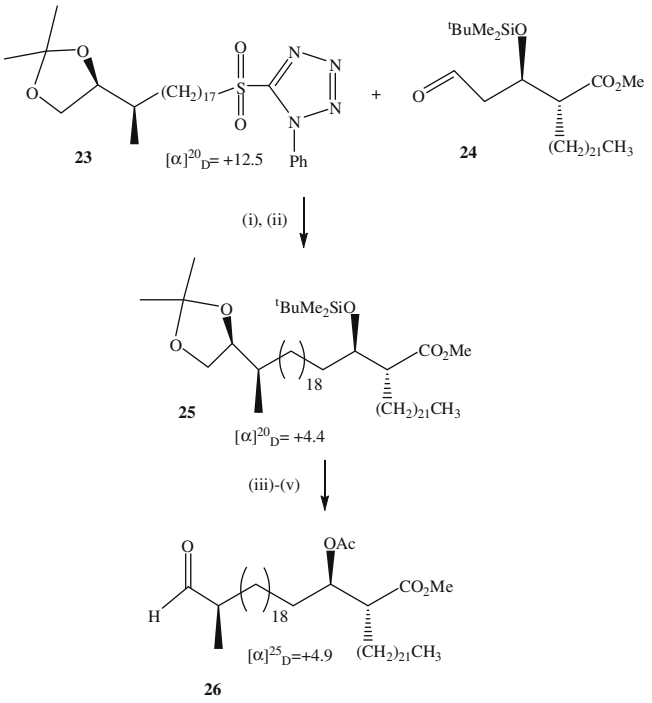
The α-methyl-*trans*-alkene unit was obtained by chain extension of the aldehyde **20**, prepared as described earlier,[18] with the sulfone **21** and base, followed by saturation of the derived mixture of alkenes and then conversion into the sulfide **22** by standard methods (Scheme 5).

The reaction of the sulfone **23**, derived by the oxidation of **22**, with aldehyde **24**[18,21] and base, followed by saturation of the alkenes produced, led to the acetal **25**. This could be transformed

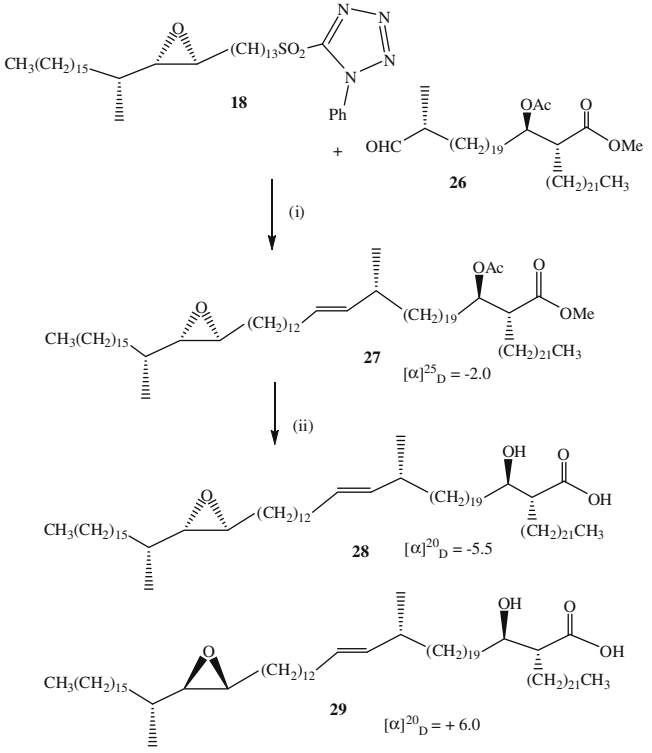
into aldehyde **26** by changing the protecting group to acetate followed by oxidative cleavage (Scheme 6).

Finally, coupling of **26** and **18** in the presence of base led to the protected epoxy-mycolic acid **27**, using the method described earlier.[18] Compound **27** could be deprotected to **28** (Scheme 7). In the same way **29** was obtained from **19**. Compound **28** showed signals for the two epoxide hydrogens at δ 2.73 (1H, dt, *J* 2.2, 5.4 Hz) and 2.43 (1H, dd, *J* 2.2, 7.3 Hz) and for the two methyl signals at δ 1.0 and 0.94. The carbons of the epoxide appeared at δ 63.99 and 59.00 in the ^{13}C NMR spectrum.[22] Epoxy-mycolates present in *M. smegmatis* are reported to show a double doublet at δ 2.43 and a broad triplet at δ 2.72 and methyl signals at δ 1.02 and 0.92.[23] An earlier paper reports the chemical shifts as δ 2.39 and 2.695, with the signal for the methyl adjacent to the epoxide appearing at δ 0.98 and that adjacent to the alkene at δ 0.92; the epoxide carbons are reported to appear at δ 63.7 and 58.6.[6] Compound **29** showed similar spectra to those of **28** but showed differences in detailed chemical shifts;[24] thus the epoxide carbons appeared at δ 63.91 and 57.60 and the methyl group signals corresponding to those mentioned above occurred at δ 0.94 and 0.92.

This method produces an epoxy-MA **28** with the same chain lengths as those reported for one natural example, and molecular rotations for fragments which are consistent with those reported. Moreover, by simple variation the method can be adjusted to provide an epoxy-MA of any necessary chain length and varying absolute stereochemistry.



Scheme 6. Reagents and conditions: (i) LiBSA, THF, −10 °C (89%); (ii) H_2, Pd/C, IMS, THF (95%); (iii) HF·pyridine, pyridine, THF (80%); (iv) Ac_2O, pyridine, toluene (98%); (v) HIO_4, Et_2O (64%).



Scheme 7. Reagents and conditions: (i) KBSA, 1,2-dimethoxyethane, −5 °C (26%); (ii) LiOH, THF, H_2O, MeOH, 45 °C (70%).

Acknowledgement

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22. Compound **28**, mp = 71–73 °C, $[\alpha]_D^{20}$ −5.5, (*c* 0.74, $CHCl_3$). Found $[M+Na]^+$: 1204.1912; $C_{80}H_{156}NaO_4$ requires: 1204.1896. δ_H (500 MHz, $CDCl_3$): 5.33 (1H, td, *J* 6.3, 15.1 Hz), 5.24 (1H, dd, *J* 7.6, 15.1 Hz), 3.74–3.70 (1H, m), 2.73 (1H, dt, *J* 2.2, 5.4 Hz), 2.49–2.45 (1H, m), 2.43 (1H, dd, *J* 2.2, 7.3 Hz), 2.11–2.01 (2H, m),

1.96 (2H, q, *J* 6.7 Hz), 1.78–1.06 (134H, br m), 1.0 (3H, d, *J* 6.3 Hz), 0.94 (3H, d, *J* 6.7 Hz), 0.89 (6H, t, *J* 6.6 Hz); δ_C (125 MHz, $CDCl_3$): 178.70, 136.46, 128.41, 72.13, 63.99, 59.00, 50.72, 37.24, 36.69, 36.02, 35.54, 33.77, 32.58, 32.24, 31.92, 29.86, 29.77, 29.70, 29.58, 29.53, 29.50, 29.42, 29.36, 29.14, 27.34, 27.21, 26.07, 25.71, 22.68, 20.95, 17.28, 14.11; ν_{max}: 3368, 2922, 2851, 1686, 1463, 1048 cm^{-1}.

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24. Compound **29**, mp: 54–55 °C, $[\alpha]_D^{20}$ +6.0 (*c* 0.52, $CHCl_3$). Found $[M+Na]^+$: 1204.1843; $C_{80}H_{156}NaO_4$ requires: 1204.1896. δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, *J* 6.6, 15.1 Hz), 5.24 (1H, dd, *J* 7.6, 15.2 Hz), 3.77–3.69 (1H, m), 2.69–2.67 (1H, m), 2.49–2.45 (2H, m), 2.05–2.02 (2H, m), 1.96 (2H, q, *J* 6.6 Hz), 1.79–1.03 (134H, br m), 0.94 (3H, d, *J* 6.7 Hz), 0.92 (3H, d, *J* 6.6 Hz), 0.88 (6H, t, *J* 6.1 Hz); δ_C (125 MHz, $CDCl_3$): 178.19, 136.45, 128.41, 72.11, 63.91, 57.60, 50.63, 37.23, 36.68, 35.81, 35.55, 34.59, 32.57, 32.18, 31.92, 29.93, 29.77, 29.65, 29.59, 29.57, 29.52, 29.50, 29.44, 29.41, 29.35, 29.23, 29.12, 27.32, 26.88, 26.13, 25.71, 22.67, 20.95, 15.93, 14.10; ν_{max}: 3392, 2924, 2853, 1748, 1464, 1070 cm^{-1}.