Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 2165–2169

# The first synthesis of single enantiomers of ketomycolic acids

Gani Koza and Mark S. Baird*

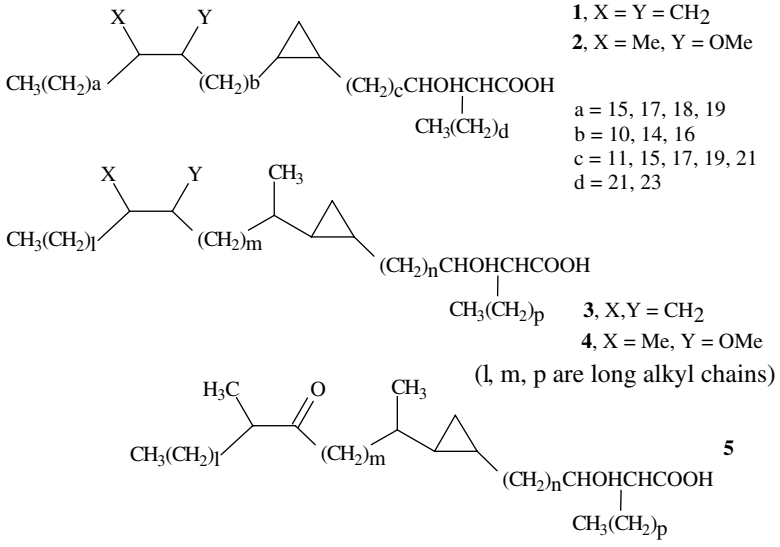
*School of Chemistry, University of Wales, Bangor, Gwynedd LL 57 2 UW, UK*

Received 28 November 2006; revised 11 January 2007; accepted 18 January 2007
Available online 24 January 2007

**Abstract**—We report the synthesis of single enantiomers of two protected ketomycolic acids, one containing a *cis*-cyclopropane the other an α-methyl-*trans*-cyclopropane, and of related hydroxy-mycolic acids.


Mycolic acids, for example **1**–**5** (Scheme 1), are major constituents of the cell envelope of *Mycobacterium tuberculosis* and other mycobacteria, some of which are pathogenic to animals and humans.[1–4] Their presence is thought to be linked to the characteristic resistance of these organisms to most current antibiotics and other chemotherapeutic agents.[5]

The two 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.[6–10] The presence of the hydroxyl group and the relative configuration between it and the alkyl chain has been demonstrated to be capable of altering the film molecular packing.[11,12] 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;[13] the same is also true for the antitumour properties of mycolic acid derivatives.[14] The balance of α-mycolic acids **1** and **3**, methoxy- **2** and **4** and ketomycolic acids such as **5** is

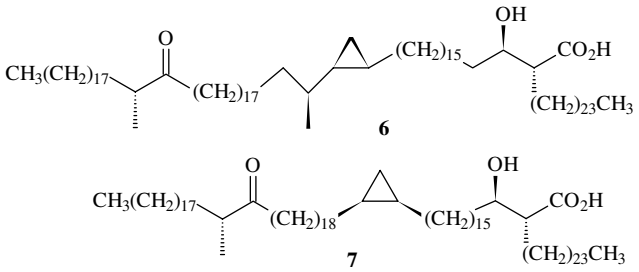



**Scheme 1.**

* Corresponding author. E-mail: m.baird@bangor.ac.uk

0040-4039/$ - see front matter 
doi:10.1016/j.tetlet.2007.01.100

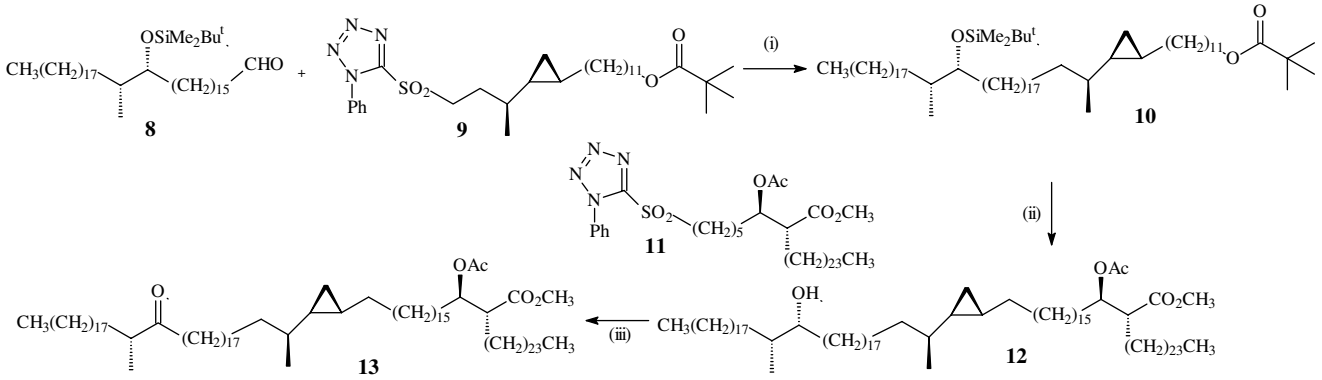
characteristic of specific bacteria;[4,5] in each case, each type of mycolic acid is present as a mixture of homologues. In the case of *M. tuberculosis*, the exact role of each type in the pathogenesis of disease remains to be confirmed, but the oxygenated mycolic acids have a particular influence on macrophage growth; strains lacking ketomycolates have a reduced ability to grow within THP-1 cells.[15,16] Moreover, the absence of keto and methoxymycolates leads to attenuation of *M. tuberculosis* in mice; the vaccine strain *Mycobacterium bovis* BCG-Pasteur lacks methoxy-mycolates.[17] It was shown recently that 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 induced macrophage inflammatory response.[18] The stereochemistry was shown to affect host innate immune responses both positively and negatively. We have recently reported the synthesis of an α-mycolate of type **1**,[19] and of methoxymycolates of type **2** with either absolute stereochemistry at the *cis*-cyclopropane or α-methyl-β-methoxy fragment.[20] We have also reported the synthesis of meromycolate fragments containing the α-methyl-*trans*-cyclopropane unit, again in a variety of stereochemistries, and provided evidence that the relative stereochemistry of methyl and cyclopropane is as shown in **6**, and that, at least in the case of wax esters derived by enzymatic Baeyer–Villiger reaction of ketomycolates, the absolute stereochemistry of this sub-unit is also as shown in **6**.[21] There is evidence that in some cases the methoxy and methyl groups of mycolic acids **2** and **4** 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.[7,22b] We now report a synthesis of ketomycolates containing both α-methyl-*trans*- and *cis*-cyclopropane fragments, **6** and **7**, that can be adjusted to produce a variety of absolute stereochemistries and chain lengths (Scheme 2). The method in each case involves linking three separate intermediates containing each set of chiral centres. The α-methylketone is introduced through either **8**[23] (or its enantiomer),[20] and the *R*,*R*-hydroxy acid through a sulfone **11**.[24] The α-methyl-*trans*-cyclopropane is introduced as sulfone **9** or one of its diastereomers, and the *cis*-cyclopropane as **23** or its enantiomer.[20,21]




**Scheme 2.**

Thus, a protected analogue **13** of a single enantiomer of *trans*-ketomycolic acid **6** was prepared by coupling aldehyde **8**, with sulfone **9** and base in a modified Kocienski–Julia reaction, followed by saturation of the *E*/*Z*-mixture of alkenes produced with di-imide to give ester **10** (Scheme 3). Conversion of this into an aldehyde, a second Kocienski–Julia reaction using **11**, saturation and then deprotection of the silyl ether gave compound **12**, $[\alpha]_D^{21}$ +10.0 (*c* 0.83, $CHCl_3$).[25] This represents the first synthetic hydroxymycolic acid, although the *R*-hydroxy-*R*-methyl stereochemistry is probably the reverse of that in nature. Although hydroxymycolic acids are not widely reported, they are thought to be on the biosynthetic pathway to methoxymycolic acids,[16,22a] and have been detected in *M. tuberculosis*, *Mycobacterium smegmatis* and *M. bovis* BCG Pasteur and Glaxo.[22b] The specific rotation of **12** is more positive than natural hydroxymycolic acid mixtures from *M. bovis* BCG or *M. smegmatis*; this may be explained based on the approximate additivity of molecular rotations, the *S*,*S*–CH($CH_3$)–CHOH– fragment being reported to contribute −43 to $M_R$.[22b] Finally, alcohol **12** was oxidized to the protected acid **13**, $[\alpha]_D^{20}$ +3.13 (*c* 0.96, $CHCl_3$);[26] this value was considerably less positive than that reported for the natural ketomycolates of *M. bovis* BCG,[22b] again supporting the fact that the α-methyl ketone is *S*- in the natural sample.[27]

The $^1$H NMR of **13** was identical to the major component of a mixture of natural ketomycolic acid methyl esters isolated from MTB,[28] after acetylation of the alcohol (the minor component was mainly *cis*-cyclopro-



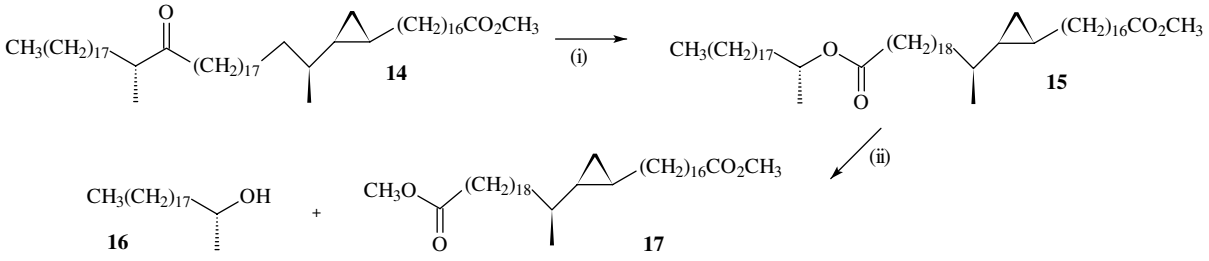

**Scheme 3.** Reagents and conditions (i) (a), $LiN(SiMe_3)_2$, THF (80%); (b), 2,4,6-triisopropylbenzenesulfonyl hydrazide (TPBSH), THF (84%); (ii) (a), $LiAlH_4$, THF (97%); (b), PCC, $CH_2Cl_2$ (98%); (c), $LiN(SiMe_3)_2$, (**11**), THF (52%); (d), dipotassium azodicarboxylate, MeOH, $CH_3COOH$, THF (91%); (e), HF·pyridine, pyridine, THF (92%); (iii) PCC, $CH_2Cl_2$ (100%).

pane-containing ketomycolic acids); there was also a complete correspondence between the $^{13}C$ NMR peaks seen for **13** and those for the major isomer in the natural sample; in particular the cyclopropane $CH_2$-carbon appeared at $\delta$ 10.49 for both. Moreover, the MALDI-MS[29] was identical to the major component of the natural sample. To establish that the oxidation of alcohol **12** to ketone **13** had not induced epimerization adjacent to the ketone, a Baeyer–Villiger oxidation of model ketone **14** was carried out (Scheme 4).

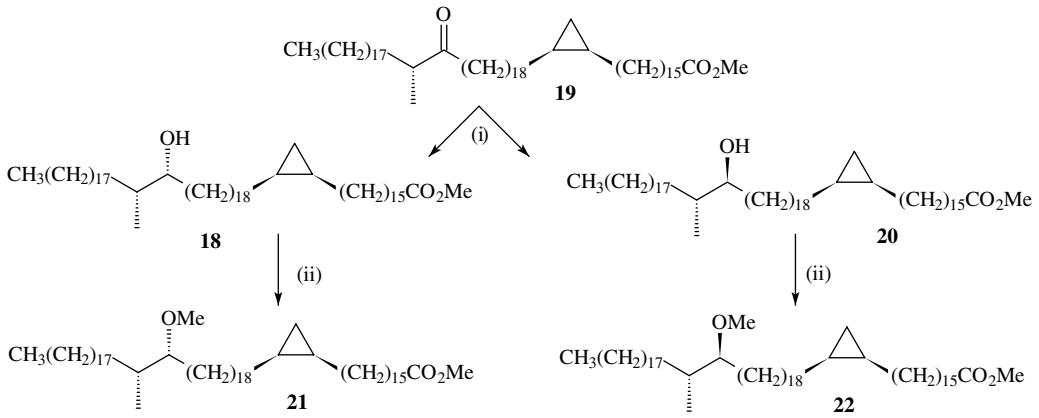
Hydrolysis of ester **15** gave alcohol *R*-**16**, $[\alpha]_D^{23}$ −3.8 (*c* 0.47, $CHCl_3$), the specific rotation of which was in agreement with that reported and (after re-esterification) ester **17** of a synthetic mero-wax dicarboxylic acid.[30] This established both that no epimerization had occurred in the oxidation to produce the ketone and that, at least in this model, the supposed enzymatic Baeyer–Villiger oxidation could be reproduced chemically and occurred with the retention of stereochemistry at the migrating centre. Assuming that the enzymatic oxidation of natural ketomycolic acid also occurs with retention of stereochemistry, the latter can also be assigned as *S* at the α-methyl ketone, as the alcohol produced is *S*-**16** $[\alpha]_D$ +3.5 or *S*-2-octadecanol $[\alpha]_D$ +5.7.[30] The synthesis of the *S*-isomer of ketomycolic acid, using the above method and the known enantiomer of **11**, is underway.

A second model was used to establish the relative stereochemistries of the methyl and methoxy groups in methoxymycolates. Thus oxidation of alcohol **18**[31] led to ketone **19**; reduction of this with sodium borohydride led to an inseparable mixture of alcohols **18** and **20**, which could be distinguished by the positions of the hydrogens adjacent to the alcohol at $\delta$ 3.50 and 3.43 and the attached carbon at $\delta$ 76.1 or 75.2, respectively.[32] The mixture was methylated to give **21** and **22**. NMR analysis of the mixture showed that the two isomers gave distinct patterns for the methoxy group at $\delta$ 3.36 and 3.35 and for the β-methyl-group at 0.85 and 0.87. Pairs of signals for the two isomers were also seen in the $^{13}C$ NMR at $\delta$ 85.5/85.4 and 57.7/57.3. That for **21** was identical to the pattern for a natural methoxymycolic acid (Scheme 5).

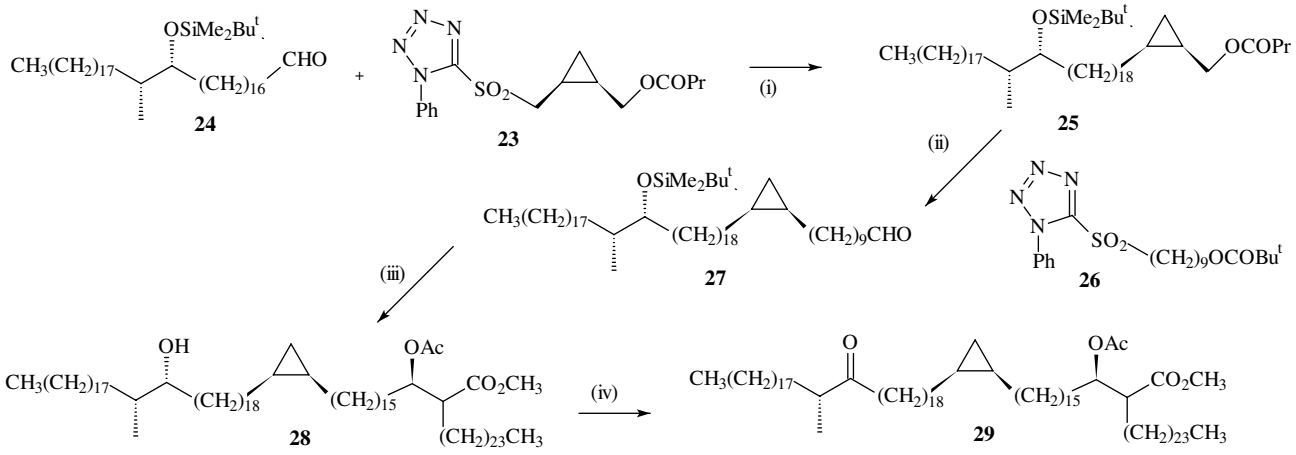
In the same way, *cis*-cyclopropane **23**[23] was coupled to **24**[19] in the presence of base, then the resultant mixture of *E*/*Z* alkenes saturated with di-imide to give **25**, de-protected, oxidized and chain extended with **26** to give **27**. A second coupling to **11** with base, saturation of the alkene and removal of the silyl protecting group led to **28**, $[\alpha]_D^{20}$ +9.3 (*c* 1.07, $CHCl_3$). This was oxidized to ketone **29**,[33] which gave a molecular ion on MALDI-MS identical to that of one component of a mixture of ketomycolic acids, corresponding to a major homologue containing a *cis*-cyclopropane.[29] The $^1H$ NMR showed cyclopropane signals identical to those of the minor components in the natural sample; the $^{13}C$ NMR was largely identical to that of the natural sample and the cyclopropane methylene group appeared at $\delta$ 10.93, identical to the signal for




**Scheme 4.** Reagents and conditions (i) *m*-CPBA, $NaHCO_3$, $CH_2Cl_2$ (63%); (ii) (a), KOH, MeOH, water, THF; (b), dil.$H_2SO_4$; (c), $CH_2N_2$, ether (**16** 67%, **17**, 45%).




**Scheme 5.** Reagents and conditions (i) $NaBH_4$, THF (92%); (ii) NaH, $CH_3I$, THF (80%).



**Scheme 6.** Reagents and conditions (i) (a), $LiN(SiMe_3)_2$, THF (91%); (b), TPBSH, THF (70%); (ii) (a), $LiAlH_4$, THF (97%); (b), PCC, $CH_2Cl_2$(100%); (c), $LiN(SiMe_3)_2$, (**26**), THF (87%); (d), TPBSH, THF (93%); (e), $LiAlH_4$, THF (69%); (f), PCC, $CH_2Cl_2$ (95%) (iii) (a), $LiN(SiMe_3)_2$, (**11**), THF (56%); (b), dipotassium azodicarboxylate, MeOH, $CH_3COOH$, THF (88%); (c), HF·pyridine, pyridine, THF (94%); (iv) PCC, $CH_2Cl_2$ (100%).

this group in the minor components of the natural mixture (Scheme 6).

The availability of ketomycolates **13** and **29**,[34] and the corresponding deprotected hydroxy acids, coupled with the synthesis of the corresponding *S*,*S*-hydroxy and *S*-ketoacids using the known enantiomer of **8** will allow the specific factors affecting the biological properties of these molecules to be determined.

## References and notes


1. Minnikin, D. E. In *The biology of the mycobacteria*; Standford, J., Ed.; Academic Press: San Diego, 1982; pp 95–184.
2. Barry, C. E.; Lee, R. E.; Mdluli, K.; Sampson, A. E.; Schoeder, B. G.; Slayden, R. A.; Yuan, Y. *Prog. Lipid Res.* **1998**, *37*, 143–179.
3. Watanabe, M.; Aoyagi, Y.; Ridell, M.; Minnikin, D. E. *Microbiology* **2001**, *147*, 1825–1837.
4. Watanabe, M.; Aoyagi, Y.; Mitome, H.; Fujita, T.; Naoki, H.; Ridell, M.; Minnikin, D. E. *Microbiology* **2002**, *148*, 1881–1902.
5. Heath, R. J.; White, S. W.; Rock, C. O. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 695–703.
6. Minnikin, D. E.; Polgar, N. *J. Chem. Soc., Chem. Commun.* **1966**, *18*, 648–649; Minnikin, D. E.; Polgar, N. *Tetrahedron Lett.* **1966**, 2643–2647.
7. Asselineau, C.; Tocanne, G.; Tocanne, J. F. *Bull. Soc. Chim. Fr.* **1970**, *4*, 1455–1459; Asselineau, C.; Asselineau, J. *Bull. Soc. Chim. Fr.* **1966**, 1992–1999; Tocanne, J. F.; Asselineau, C. *Bull. Soc. Chim. Fr.* **1968**, 4519–4525; Lacave, C.; Lanéelle, M.-A.; Daffé, M.; Montrozier, H.; Rols, M.-P.; Asselineau, C. *Eur. J. Biochem.* **1987**, *163*, 369–378.
8. Lacave, C.; Lanéelle, M.-A.; Montrozier, H.; Rols, M.-P.; Asselineau, C. *Eur. J. Biochem.* **1987**, *163*, 369–378.
9. Daffé, M.; Lanéelle, M. A.; Guillen, L. V. *Eur. J. Biochem.* **1988**, *177*, 339–340.
10. Daffé, M.; Lanéelle, M. A.; Lacave, C. *Res. Microbiol.* **1991**, *142*, 397–403.
11. Durand, H.; Welby, M.; Lanéelle, G.; Tocanne, J.-F. *Eur. J. Biochem.* **1979**, *93*, 103–112.
12. Durand, H.; Gillois, M.; Tocanne, J.-F.; Lanéelle, G. *Eur. J. Biochem.* **1979**, *94*, 109–118.
13. Moody, D. B.; Guy, M. R.; Grant, E.; Cheng, T.-Y.; Brenner, M. B.; Besra, G. S.; Porcelli, S. A. *J. Exp. Med.* **2000**, *192*, 965–976.
14. Watanabe, R.; Yoo, Y. C.; Hata, K.; Mitobe, M.; Koike, Y.; Nishizawa, M.; Garcia, D. M.; Hiroshi, Y.; Nobuchi, I.; Yamada, H.; Azuma, I. *Vaccine* **1999**, *17*, 1484–1492.
15. Riley, L. W. *J. Clin. Invest.* **2006**, *116*, 1475–1478.
16. Yuan, Y.; Qi, Z.; Crane, D. D.; Barry, C. E. *Mol. Microbiol.* **1998**, *29*, 1449–1458.
17. Dubnau, E.; Chan, J.; Raynaud, C.; Mohan, V. P.; Laneelle, M. A.; Yu, K. M.; Quemard, A.; Smith, I.; Daffe, M. *Mol. Microbiol.* **2000**, *36*, 630–637.
18. Rao, V.; Gao, F.; Chen, B.; Jacobs, W. R.; Glickman, M. S. *J. Clin. Immunol.* **2006**, *116*, 1660–1666.
19. Al Dulayymi, J. R.; Baird, M. S.; Roberts, E. *J. Chem. Soc., Chem. Commun.* **2003**, 228–229; Al Dulayymi, J. R.; Baird, M. S.; Roberts, E. *Tetrahedron* **2005**, *61*, 11939–11951.
20. Al Dulayymi, J. R.; Baird, M. S.; Roberts, E.; Deysel, M.; Verschoor, J.; *Tetrahedron*, in press.
21. Al Dulayymi, J. R.; Baird, M. S.; Roberts, E.; Minnikin, D. E. *Tetrahedron* **2006**, *62*, 11867–11880; Al-Dulayymi, J. R.; Baird, M. S.; Mohammed, H.; Roberts, E.; Clegg, W. *Tetrahedron* **2006**, *62*, 4851–4862.
22. (a) Behr, M. A.; Schroeder, B. G.; Brinkman, J. N.; Slayden, R. A.; Barry, C. E. *J. Bact.* **2000**, *182*, 3394–3399; (b) Quémard, A.; Lanéelle, M. A.; Marrak-chi, H.; Promé, D.; Daffé, M. *Eur. J. Biochem.* **1997**, *250*, 758–763; Dubnau, E.; Lanéelle, M. A.; Soares, S.; Benichou, A.; Vaz, T.; Promé, D.; Promé, J.-C.; Daffé, M.; Quémard, A. *Mol. Microbiol.* **1997**, *23*, 313–322.
23. Prepared by methods described earlier.[20]
24. Prepared by methods described earlier: Toschi, G.; Baird, M. S. *Tetrahedron* **2006**, *62*, 3221–3227.
25. $\nu_{max}/cm^{-1}$: 1739; $\delta_H$ (500 MHz, $CDCl_3$): 5.09 (1H, ddd, *J* 4.1, 7.3, 8.2 Hz), 3.68 (3H, s), 3.52–3.49 (1H, m), 2.62 (1H, ddd, *J* 4.4, 6.9, 10.7 Hz), 2.04 (3H, s), 1.67–1.14 (150H, v br m), 0.90 (3H, d, *J* 6.6 Hz), 0.89 (6H, t, *J* 6.9 Hz), 0.86 (3H, d, *J* 6.9 Hz), 0.71–0.64 (1H, m), 0.48–0.42 (1H, m), 0.22–0.09 (3H, m); $\delta_C$ (125 MHz, $CDCl_3$): 173.6, 170.3, 75.2(−), 74.1(−), 51.5(−), 49.6(−), 38.2(−), 38.1(−), 37.4(+), 34.5(+), 34.5(+), 33.4(+), 31.9(+), 31.7(+), 30.1(+), 30.0(+), 29.72(+), 29.70(+, v br), 29.65(+), 29.6(+), 29.5(+), 29.44(+), 29.39(+), 29.36, 28.1(+),

27.5(+), 27.4(+), 27.3(+), 26.3(+), 16.1(−), 25.0(+), 22.7(+), 21.0(−), 19.7(−), 18.6(−), 14.1(−), 13.6(−), 10.5(+) [+ = $CH_2$, − = CH, $CH_3$].

26. $\nu_{max}/cm^{-1}$: 1737; $\delta_H$ (500 MHz, $CDCl_3$): 5.10 (1H, br dt, *J* 4.0, 8.1 Hz), 3.69 (3H, s), 2.62 (1H, ddd, *J* 4.4, 7.0, 10.7 Hz), 2.51 (1H, sext, *J* 6.7 Hz), 2.43 (1H, dt, *J* 14.6, 7.6 Hz), 2.40 (1H, dt, *J* 14.6, 7.6 Hz), 2.03 (3H, s), 1.65–1.17 (146H, v br m), 1.05 (3H, d, *J* 7.0 Hz), 0.90 (3H, d, *J* 7.0 Hz), 0.89 (6H, t, *J* 6.8 Hz), 0.70–0.65 (1H, m), 0.48–0.42 (1H, m), 0.22–0.09 (3H, m); $\delta_C$ (125 MHz, $CDCl_3$): 215.1, 173.6, 170.3, 74.1(−), 51.5(−), 49.6(−), 46.3(−), 41.1(+), 38.1(−), 37.4(+), 34.5(+), 33.0(+), 31.9(+), 31.7(+), 30.1(+), 29.72(+), 29.70(+, v br), 29.67(+), 29.65(+), 29.60(+), 29.57(+), 29.55(+), 29.51(+), 29.48(+), 29.46(+), 29.44(+), 29.39(+), 29.35(+), 28.1(+), 27.5(+), 27.4(+), 27.3 (+), 26.1(−), 25.0(+), 23.72(+), 22.68(+), 21.0(−), 19.7(−), 18.6(−), 16.4(−), 14.1(−), 10.5(+).
27. Deprotection of the acetate and methyl ester groups of **13** using LiOH, THF, MeOH, $H_2O$ led to the free hydroxy acid **6**, but with a specific rotation of +5.3. This is consistent with partial or complete racemisation at the α–methyl-ketone and with the value reported for a natural sample obtained by digestion of cells with base at elevated temperature, which again is presumed to have epimerised adjacent to the ketone.[6]
28. We thank Proffessor D.E. Minnikin (Univ. of Birmingham) for providing a sample of natural ketomycolic acid.
29. Thanks are due to Mr. B. Grail, School of Biological Sciences, Bangor for running these spectra.
30. (a) Anderson, R. J.; Creighton, M. M.; Peck, R. L. *J. Biol. Chem.* **1940**, *132*, 675–693; (b) Geiger, W. B.; Anderson, R. J. *J. Biol. Chem.* **1939**, *131*, 539–548; (c) Minnikin, D. E.; Dobson, G.; Goodfellow, M.; Draper, P.; Magnusson, M. *J. Gen. Microbiol.* **1985**, *131*, 2013.
31. Prepared by a method similar to that described below for **24**.
32. The reduction of natural homologous mixtures of keto- to hydroxymycolic acids has been described. The EI-MS is reported to be similar to the natural hydroxymycolic acids, but no NMR data are provided to indicate whether a mixture of epimers was observed.[22b]
33. $\nu_{max}/cm^{-1}$: 1740, 1708; $\delta_H$ (500 MHz, $CDCl_3$): 5.09 (1H, ddd, *J* 4.0, 7.0, 8.1 Hz), 3.69 (3H, s), 2.62 (1H, ddd, *J* 4.4, 6.9, 10.7 Hz), 2.50 (1H, sext, *J* 6.8 Hz), 2.43 (1H, dt, *J* 14.7, 7.3 Hz), 2.40 (1H, dt, *J* 14.7, 7.3 Hz), 2.03 (3H, s), 1.68–1.14 (144H, m, v br), 1.05 (3H, d, *J* 7.0 Hz), 0.89 (6H, t, *J* 7.0 Hz), 0.68–0.64 (2H, m), 0.57 (1H, br dt, *J* 4.1, 8.2 Hz), −0.32 (1H, br q, *J* 5.0 Hz); $\delta_C$ (125 MHz, $CDCl_3$): 215.1, 173.6, 170.3, 74.1(−), 51.5(−), 49.6(−), 46.3(−), 41.1(+), 33.1(+), 31.9(+), 31.7(+), 30.2(+), 29.8(+), 29.70(+, v br), 29.65(+), 29.63(+), 29.60(+), 29.56(+), 29.51(+), 29.49(+), 29.46(+), 29.44(+), 29.40(+), 29.36(+), 28.7(+), 28.1(+), 27.5(+), 27.3(+), 25.0(+), 23.7(+), 22.7(+), 21.0(−), 16.4(−), 15.8(−), 14.1(−), 10.9(+).
34. Compound **29** ([α] +3.0 (*c* 0.7, $CHCl_3$)) was de-protected,[27] to the free β-hydroxyacid **7**, but with [α] +4.4 (*c* 1.02, $CHCl_3$), again consistent with partial or complete racemisation at the α-methylketone.