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The synthesis of a single enantiomer of a major α-mycolic acid of *Mycobacterium tuberculosis*

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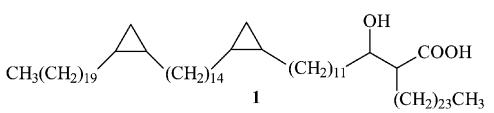
We report a synthesis of a single enantiomer of a dicyclopropane containing mycolic acid from *Mycobacterium tuberculosis*; this method can be simply varied to modify the chain lengths or the absolute stereochemistry of either cyclopropane.

Tuberculosis, caused by *Mycobacterium tuberculosis*, kills some 3 m people each year. It has been estimated that between a quarter and a half of the world population is infected with the organism, though in most cases this does not lead to the development of the disease. Many other mycobacteria are either obligate or opportunistic pathogens.[1] The cell walls of these bacteria show unusually low permeability, a factor linked to their resistance to therapeutic agents; they are highly complex but include esters of long-chain (*R,R*)-β-hydroxy-acids (R′CH(OH)CH(CO_2H)R″), mycolic acids, commonly containing *cis*-cyclopropanes, α-methyl-*trans*-cyclopropanes, α-methyl-β-keto- and -methoxy groups in the R′-chain;[2–6] an example is **1**, the major mycolic acid of *M. tuberculosis*, reported by Minnikin and Polgar.[2] which could be used, *e.g.*, in developing early assays of disease. Bacteria also contain trehalose 6,6′-dimycolates, 'cord factors', that are not wall bound,[1,7] and show many biological properties—*e.g.* granuloma forming activity.[8] Thus the detection of antibodies against cord factor produced in the serum of patients with pulmonary tuberculosis is clinically useful in the rapid serio-diagnosis of the disease.[9] The synthesis of single stereoisomers of individual mycolic acids may lead to a fuller understanding of their biosynthesis and of the structures of bacterial cell walls and to cord-factor antibodies derived from discrete mycolic acids characteristic of individual bacteria. Syntheses of the 'meromycolic acid' **2** as a mixture of four isomeric di-*cis*-cyclopropanes,[10] and of a single enantiomer of compound **3** have been reported.[11] We now report the synthesis of a single enantiomer **4** of the mycolic acid **1**.[2] The stereochemistry of the cyclopropanes A and B is that which could have a common biosynthetic precursor with some *S*-methyl-branched mycolates.[12] The synthetic method used can, however, be varied to allow the synthesis of either absolute stereochemistry of each cyclopropane.



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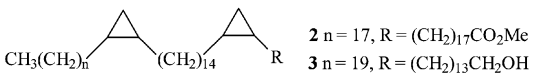
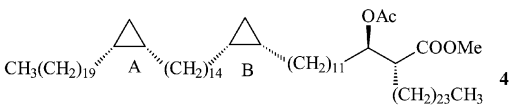


2 n = 17, R = $(CH_2)_{17}CO_2Me$
3 n = 19, R = $(CH_2)_{13}CH_2OH$



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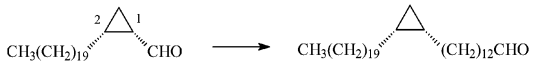
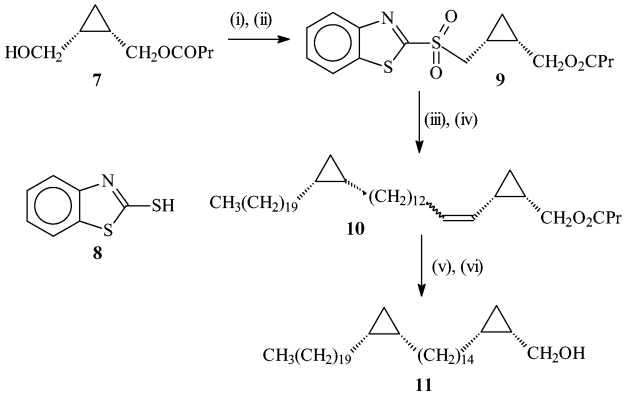
The (1*S*,2*R*)-aldehyde **5**[13] was converted into aldehyde **6** by reaction with 1-carbomethoxy-12-triphenylphosphoniumdodecane iodide and base, reduction to the corresponding alcohol, hydrogenation of the alkene using diimide, and then oxidation to the aldehyde (Scheme 1).



5 **6**

Scheme 1 *Reagents and conditions*: (i) $MeO_2C(CH_2)_{11}PPh_3I$, MeONa, DMF; (ii) $LiAlH_4$, THF; (iii) N_2H_4, $NaIO_4$, AcOH, $CuSO_4$, i-PrOH; (iv) PCC, CH_2Cl_2.

The ester **7**[13] was converted into sulfone **9** by reaction with the thiazole **8**, triphenylphosphine and diethyl azodicarboxylate then oxidation of the derived thioether.[14] This was treated with aldehyde **6** in a Julia reaction,[14] to give a mixture of *E*- and *Z*-alkenes **10**. Reduction of the derived ester to the corresponding alcohol using lithium aluminium hydride followed by hydrogenation of the alkene, again using diimide gave a single enantiomer of alcohol **11** (Scheme 2).



Scheme 2 *Reagents and conditions*: (i) (**8**) PPh_3, DEAD; (ii) H_2O_2 or MCPBA, CH_2Cl_2; (iii) LiBSA; (iv) (**6**), (v) $LiAlH_4$); (vi) NH_2NH_2, $NaIO_4$, $CuSO_4$, AcOH, i-PrOH.

Syntheses of a shorter chain dialkyl mycolic acid containing no cyclopropane ring as the *R,R*-isomer has been reported earlier.[15,16] We have adopted a modified approach for the synthesis of **4**. Ring opening of the epoxide **12**[17] was used to produce a single enantiomer of the mono-protected diol **13**. This was transformed in four steps into the diol **14**. The diol was protected as a mono-silyl ether and then alkylated,[18] to give the hydroxy ester **15** using a method that has been applied to shorter chain alkyl mycolic acids.[16] Protection of the secondary alcohol, deprotection of the primary alcohol and oxidation led to the aldehyde **16**. Reaction of alcohol **11** with the thiazole **8**, triphenylphosphine and diethyl azodicarboxylate then oxidation of the derived thioether as before gave the sulfone **17**. Reaction of this with the aldehyde **16** and base in a Julia reaction led to a mixture of *Z*- and *E*- alkenes **18**; hydrogenation with diimide gave the saturated dicyclopropane **4**,[19] containing the complete carbon skeleton of the natural α-mycolic acid (Scheme 3).

The ^{1}H and ^{13}C NMR spectra of **4** were essentially identical to those of a sample extracted from *M. tuberculosis* and then protected ($[\alpha]_D$ = +3.7),[21] which is a mixture of homologues in

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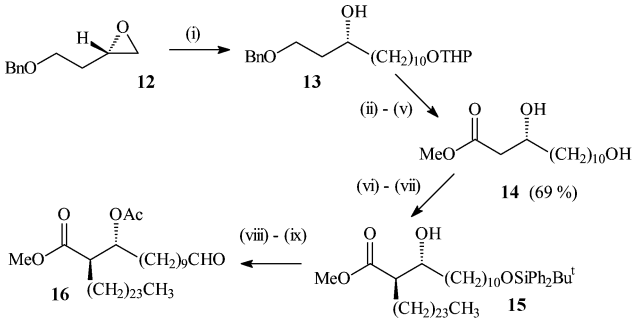
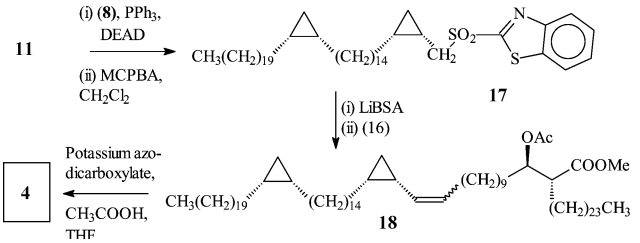


Scheme 3 *Reagents and conditions*: (i) $BrMg(CH_2)_9OTHP$, CuI, 2 h, −30 °C (86%); (ii) Bu^tSiMe_2Cl, imidazole, DMF (93%); (iii) H_2, Pd/C, MeOH (84%); (iv) $NaIO_4$, $RuCl_3{\cdot}H_2O$, CH_3CN, H_2O, CCl_4; (v) MeOH, H_2SO_4; (vi) Bu^tPh_2SiCl, DMAP, Et_3N (84%); (vii) LDA; (viii) $CH_3(CH_2)_{23}I$, HMPA (31%); (ix) Ac_2O, pyridine (86%); (v) F^- (75%); (vi) PCC (95%).



which **4** predominates. Studies to further compare the natural and synthetic samples are under way.

Variation of the chain lengths and absolute stereochemistry of the cyclopropanes **5** and **9** can be used to provide a simple and flexible approach to any *cis*-dicyclopropane mycolic acid.

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13 Aldehyde (**5**) was prepared from (**7**) by a sequence very similar to that reported for the preparation of (*R*,*R*)-1-hydroxymethyl-2-[(*Z*)-hex-1-en-1-yl]cyclopropane from the enantiomer of (**7**), followed by diimide reduction and PCC oxidation.[20] Compound (**7**) was prepared from *cis*-dicyclopropane-1,2-dimethanol, vinyl butyrate and porcine pancreatic lipase (G. D. Coxon, S. Knobl, E. Roberts, M. S. Baird, J. R. Al Dulayymi, G. S. Besra, P. J. Brennan and D. E. Minnikin, *Tetrahedron Lett.*, 1999, 6689; we regret that this was incorrectly reported in that paper. The enantiomer of (**7**) was in fact converted into natural lactobacillic acid, whereas (**7**) gave its enantiomer).

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19 Ester **4** (Found: C; 81.5, H; 12.9; $(M + NH_4)^+$: 1211.2339. $C_{81}H_{156}O_4$ requires: C; 81.47, H; 13.17; $(M + NH_4)^+$: 1211.2347) showed δ_H (500 MHz): 5.04 (1H, ddd, J 4.2, 7, 11.3), 3.64 (3H, s), 2.58 (1H, ddd, *J* 4.2, 6.7, 10.7 Hz), 1.99 (3H, s) 1.55-1 (134H, br s), 0.82 (6H, t, *J* 6.45 Hz), 0.57 (4H, m), 0.49 (2H, dt, J 4, 8 Hz), −0.4 (2H, br q, *J* 5.5 Hz); δ_C: 173.6, 170.3, 74.1−, 51.5−, 49.6−, 31.9+, 31.7+, 30.2+, 29.7 (very broad, +), 29.5+, 29.4+, 29.3+, 28.7+, 28.1+, 27.45+, 24.96+, 22.6+, 21−, 15.75−, 14.1−, 10.9+ [+ = CH_2 , − = CH, CH_3] ; ν_{max}: 2921, 2849, 1736, cm^{-1}; $[\alpha]^{22}_D$ = +4.2 (*c* = 0.735, $CHCl_3$). Thanks are due to the EPSRC MS Service in Swansea for obtaining the molecular mass of **4**.

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21 We thank Professor D.E. Minnikin for supplying a sample of the material originally extracted for ref. 2.