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Synthesis and properties of methyl 5-(1′*R*,2′*S*)-(2-octadecylcycloprop-1-yl)pentanoate and other ω-19 chiral cyclopropane fatty acids and esters related to mycobacterial mycolic acids

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Abstract

A 23–26-carbon chain length range of ω-19 (1′*R*,2′*S*) cyclopropane fatty acids, related to mycobacterial mycolic acids, has been prepared. The key cyclopropyl intermediate, (1′*R*,2′*S*)-(*Z*)-1-formyl-2-octadecylcyclopropane, underwent Wittig chemistry with various reagents to provide vinylic precursors, which were selectively reduced to the corresponding saturated ω-19 cyclopropane fatty acids or esters. The 24-carbon ω-19 cyclopropane ester was made by chain elongation of the 23-carbon ester. Saturated and unsaturated chiral cyclopropane acids and esters were assayed, using wall extracts of *Mycobacterium smegmatis*; the incorporation of ^{14}C-acetate was used to measure inhibition or stimulation of mycolic acid synthesis. Minor inhibition (2–3%) was shown by the 23- and 24-carbon saturated esters; all the other compounds were stimulants. The most effective (38–55%) stimulators of mycolate synthesis were the unsaturated esters with 23- and 26-carbons and the saturated and unsaturated 25-carbon acids.

Keywords: Fatty acids; Mycolic acids; Chiral cyclopropane; Biosynthetic inhibitors

1. Introduction

Tuberculosis remains the leading cause of death from infectious diseases (Enarson and Murray, 1994). It was estimated in 1991 that almost one-third of the world's population, or 1.7 billion people was infected. In recent years, tuberculosis infections among certain population groups have increased dramatically. This is partly due to a decline in living standards of these groups and to the increasing resistance of various strains to present antibiotic treatment. In addition to this is the coexistence of the disease within HIV

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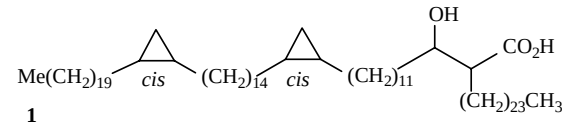
infected patients. Because of the ability of HIV to destroy the immune system this has emerged as the most significant risk factor for progression of dormant TB infection to the clinical disease. It has been estimated that from the beginning of the HIV pandemic until mid-1993, more than 5 million people worldwide had dual HIV and TB infection. This has highlighted the need for a better understanding of the nature of the disease and its control (Enarson and Murray, 1994).

Mycobacterium tuberculosis is the causative agent of tuberculosis and its pathogenicity depends on the presence of a number of very unusual lipid molecules. The high molecular weight 2-alkyl-3-hydroxy mycolic acids (e.g. **1**) are key structural components of *M. tuberculosis* (Minnikin, 1982, 1993) being essential for the integrity of the cell envelope. Mycolic acids show a wide variety of structural variations, including a range of homologues and functional units, such as methoxy and keto groups (Watanabe et al., 2001, 2002). All mycobacteria have so-called α-mycolates, which have no oxygen functions in addition to the 3-hydroxy acid unit. Structure **1** represents the α-mycolic acids from *M. tuberculosis*. Investigations into mycolic acid biosynthesis in *M. tuberculosis* by Takayama and Qureshi (1984) indicated that (*Z*)-tetracos-5-enoic acid (**2**) is a key initial intermediate. This acid **2** was shown to stimulate α-mycolic acid synthesis in extracts of the environmental agent, *Mycobacterium smegmatis* (Besra et al., 1993a; Wheeler et al., 1993a). (*Z*)-Tetracos-5-enoic acid (**2**) was considered to be formed by a Δ^5-desaturase from tetracosanoate and this was strongly supported by the inhibition of mycolate synthesis by the cyclopropene analogue **3** of **2** (Besra et al., 1993b; Wheeler et al., 1993b). Fatty acid desaturases had previously be shown to be inhibited by cyclopropene fatty acid derivatives (Johnson et al., 1967).



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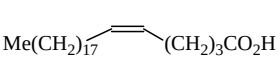

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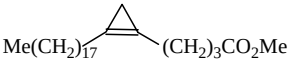

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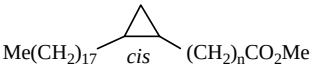
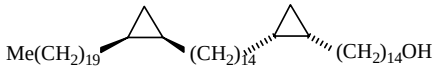


4 n = 3,4



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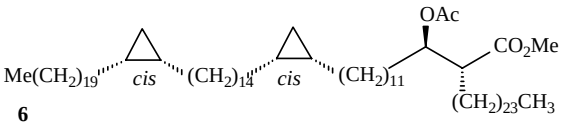


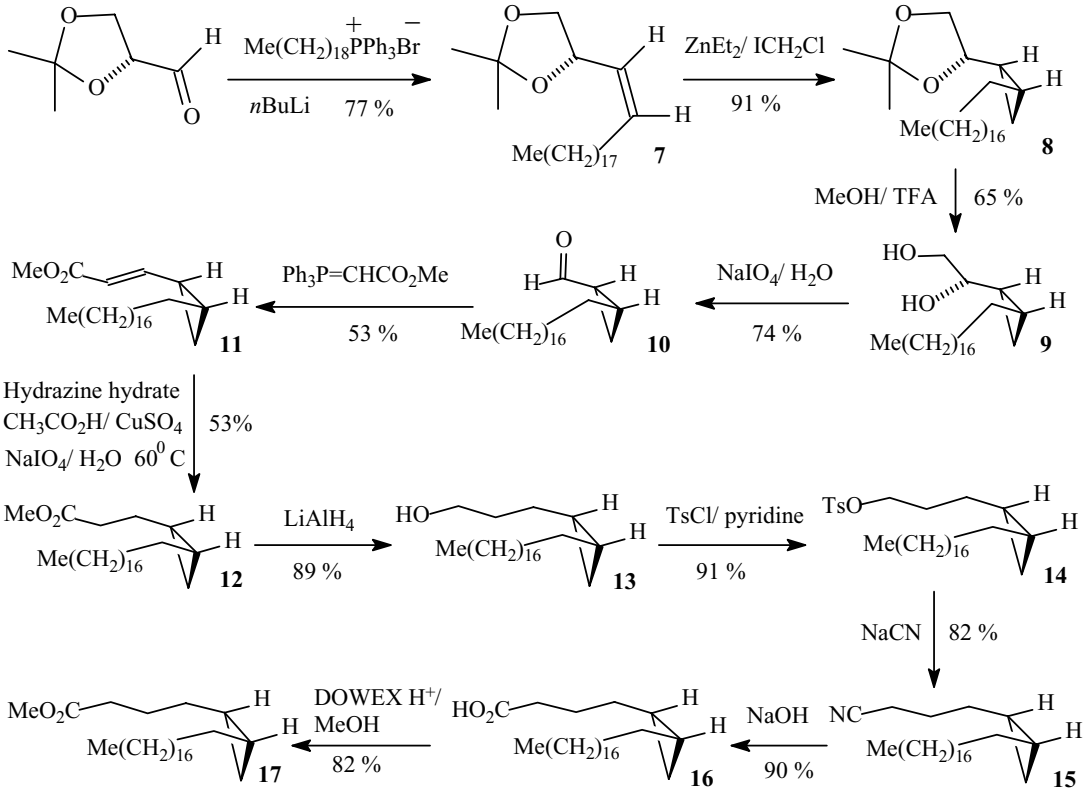
6

Racemic *cis*-cyclopropane acids **4**, related to α-mycolates have been synthesised and shown to be weak inhibitors of α-mycolate synthesis in *M. smegmatis* (Hartmann, 1993; Hartmann et al., 1994; Coxon et al., 2003a). Recently, parallel routes to chiral long-chain cyclopropane fatty acids, such as lactobacillic acid, have been developed (Coxon et al., 1999, 2003b). One of these routes has been used to synthesise a so-called meromycolate portion **5** (Al Dulayymi et al., 2000) and a derivative of an α-mycolic acid enantiomer **6** (Al Dulayymi et al., 2003). This report details the synthesis of the key chiral intermediate **10** (Scheme 1) and its conversion into the chiral *cis*-cyclopropane fatty acids and esters (**11**, **12**, **16**, **17**, **18**–**25**) (Schemes 1 and 2), which were tested against mycolate-synthesising extracts of *M. smegmatis*. The synthesis of some of these long-chain cyclopropane esters has been outlined in a preliminary communication (Coxon et al., 1999).

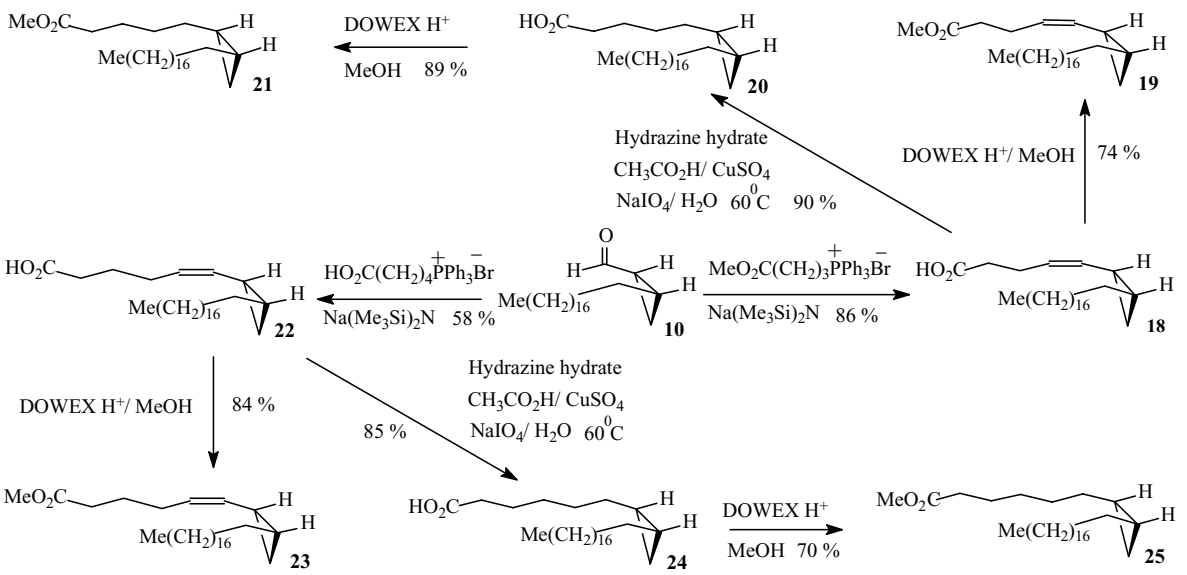
2. Experimental

2.1. Materials and methods

^{1}H and ^{13}C NMR spectra were recorded on Bucker AC 200, AC 250 CP/MAS or JEOL λ500 NMR spectrometers in $CDCl_3$. IR spectra were recorded using a Nicolet FTIR PCIR or Perkin-Elmer 1600 FTIR Infrared spectrophotometer as liquid films. Electron impact (EI) mass spectra (MS) were recorded using either a Kratos MS 80RF of Micromass Auto Spec M spectrometer. Gas chromatography (GC) analysis was carried out on a Perkin-Elmer 8410 gas chromatograph. Optical rotations were achieved using



Scheme 1.



Scheme 2.

an Optical Activity POLAAR 2001 automatic polarimeter. Melting points (uncorrected) were carried out on a Kofler hot stage apparatus. Thin layer chromatography was performed using glass backed plates precoated with silica gel F254 of layer thickness 0.25 ml and Fluka aluminium backed TLC cards with fluorescent indicator 254 nm with a silica gel thickness of 0.2 mm. Flash column chromatography was performed at medium pressure using Fluka 60738 silica gel 60. Starting materials and chemical reagents were all purchased from Aldrich, Fluka or Lancaster Synthesis. Organic solutions were dried over magnesium sulfate. Petrol refers to petroleum ether bp 40–60 °C.

2.2. Synthesis

2.2.1. (1Z,4′S)-1-(2′,2′-Dimethyl-1′,3′-dioxolan-4′-yl)-1-icosene (7)

n-Nonadecyltriphenylphosphonium bromide (Al Dulayymi et al., 2000) (36.0 g, 59.2 mmol) was suspended in anhydrous tetrahydrofuran (THF; 200 ml) and stirred under nitrogen at −78 °C. *n*-Butyllithium (77.0 ml, 86.0 mmol) was added and the reaction warmed to ambient temperature for 10 min before being recooled to −78 °C, when 2,3-*O*-isopropylidene-D-glyceraldehyde (7.0 g, 53.8 mmol) in THF (10 ml) was added. The reaction was stirred between −10 and 0 °C for 2 h, until no starting material remained by TLC. Saturated aq. ammonium chloride solution (20 ml) was then added. The organic phase was extracted with dichloromethane (3 × 50 ml), washed with water (3 × 50 ml), dried and concentrated under vacuum. The crude residue was purified by column chromatography, using petrol and diethyl ether (5:2), to yield the product **7** (15.7 g, 77%) as a white solid. Mp: 35–37 °C; $[\alpha]_D^{24} = +67.5^\circ$ (*c*: 16.5 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3030, 2975, 2945, 2832, 1620, 1043, 9134, 780 cm^{-1}; δ_H: 5.65 (d, CH, 1H, $J = 10.0$ Hz), 5.41 (t, CH, 1H, $J = 8.6$ Hz), 4.85 (br, q, CH, 1H, $J = 8.1$ Hz), 4.05 (dd, CH, 1H, $J = 8.0$, 6.1 Hz), 3.52 (t, CH, 1H, $J = 8.1$ Hz), 2.08 (m, CH_2, 2H), 1.43 (s, Me, 3H), 1.41 (s, Me, 3H), 1.26 (s, $C_{16}H_{32}$, 32H), 0.89 (br, t, CH_3, 3H, $J = 5.6$ Hz); δ_C: 135.2 (CH), 126.8 (CH), 109.2 (OCO), 66.8 (CO), 67.2 (CO), 31.9, 31.8, 31.4, 31.0, 30.8, 30.6, 30.5, 30.1, 29.8, 29.7, 29.2, 27.4, 26.8, 26.0, 22.7, 14.1. Found M^+: 380.3648. Calculated for $C_{25}H_{48}O_2$: 380.3654.

2.2.2. (1R,2S,4′S)-1-(2′,2′-Dimethyl-1′,3′-dioxolan-4′-yl)-2-octadecylcyclopropane (8)

Diethyl zinc (67.0 ml, 73.6 mmol) followed by chloroiodomethane (26.0 g, 147.2 mmol) was added to compound **7** (14.0 g, 36.8 mmol) stirred in anhydrous 1,2-dichloroethane (100 ml) at −30 °C under nitrogen. The solution was warmed to 0 °C and stirred for 1 h whilst being monitored by TLC, then quenched with saturated aq. ammonium chloride solution (40 ml), the organic phase extracted with chloroform (2 × 60 ml) and the combined organic phases washed with water (3 × 50 ml) and dried. The organic phase was concentrated under vacuum and the impure compound was purified by flash chromatography, using petrol and diethyl ether (5:1), as eluent to yield the product **8** (13.2 g, 91%) as a pale yellow residue. Mp: 36–38 °C; $[\alpha]_D^{24} = +6.8^\circ$ (*c*: 0.46 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3031, 2945, 2826, 1227, 929 cm^{-1}; δ_H: 4.07 (br, q, CH, 1H, $J = 5.2$ Hz), 3.61 (m, 2 × CH, 2H), 1.44 (s, Me, 3H), 1.35 (s, Me, 3H), 1.25 (s, $C_{16}H_{32}$, 32H), 1.15 (m, CH_2, 2H), 0.87 (br, t, 3 × CH, CH_3, 6H, $J = 6.0$ Hz), 0.24 (m, CH, 1H); δ_C: 108.3, 78.2, 69.6, 31.4, 30.7, 29.1, 28.5, 28.3, 27.5, 27.1, 26.9, 26.4, 23.2, 22.7, 17.5, 16.4, 9.8. Found M^+: 394.3794. Calculated for $C_{26}H_{50}O_2$: 394.3810.

2.2.3. (1R,2S,1′S)-1-(1′,2′-Dihydroxyeth-1′-yl)-2-octadecylcyclopropane (9)

Trifluoroacetic acid (TFA) (80 ml, 80%) in methanol (20 ml, 20%) was added to a solution of **8** (11.0 g, 27.9 mmol) in methanol (100 ml) at 0 °C. The solution was stirred for 2 h, the TFA and methanol were removed under vacuum and the residue was purified by column chromatography, eluting with petrol and diethyl ether (1:1), to yield **9** (6.2 g, 65%) as a white solid. Mp: 32–34 °C; $[\alpha]_D^{24} = +22.4^\circ$ (*c*: 1.05 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3428 (br), 3056, 2989, 2970, 1252 cm^{-1}; δ_H: 3.76 (m, CH, 1H), 3.50 (m, CH, 1H), 3.32 (m, CH, 1H), 2.06 (br, s, 2 × OH, 2H), 1.25 (s, $C_{16}H_{32}$, 32H), 0.92 (m, CH_2, 2H), 0.89 (br, t, CH_3, 3H, $J = 6.1$ Hz), 0.87 (m, 3 × CH, 3H), 0.11 (m, CH, 1H); δ_C: 73.3 (COH), 67.1 (COH), 31.9, 30.0, 29.7, 29.6, 29.1, 27.8, 22.8, 20.4, 19.8,

19.6, 19.4, 19.1, 18.4, 16.8, 15.7, 14.1. Found M^+: 354.3496. Calculated for $C_{23}H_{46}O_2$: 354.3497.

2.2.4. (1R,2S)-1-Formyl-2-octadecylcyclopropane (10)

Sodium periodate (4.5 g, 225 mmol) in distilled water (100 ml) was added to a solution of **9** (6.0 g, 17.5 mmol) in methanol (200 ml) cooled to 0 °C. The mixture was stirred for 20 min until TLC indicated the completion of the reaction and then quenched with saturated aq. ammonium chloride (20 ml). The organic phase was extracted with ethyl acetate (2 × 50 ml), dried, concentrated under vacuum and purified by column chromatography using petrol and diethyl ether (2:1), to yield the product **10** (4.2 g, 74%) as a pale yellow residue. Mp: 33–34 °C; $[\alpha]_D^{24} = +15.6°$ (*c*: 0.73 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3071, 2986, 2970, 1648, 1239 cm^{-1}; δ_H: 9.35 (d, CHO, 1H, $J = 5.6$ Hz), 1.49 (m, CH_2, 2H), 1.35 (s, $C_{16}H_{32}$, 4 × CH, 36H), 0.86 (br, t, CH_3, 3H, $J = 6.0$ Hz); δ_C: 179.7 (CHO), 68.4, 32.8, 30.1, 29.6, 29.2, 29.0, 26.9, 24.1, 22.9, 27.4, 23.4, 23.1, 22.8, 17.8, 14.4, 14.0. Found M^+: 322.3239. Calculated for $C_{22}H_{42}O$: 322.3235.

2.2.5. (1′E,1R,2S)-1-(3′-Methoxycarbonyleth-2′-en-1′-yl)-2-octadecylcyclopropane (11)

Carbomethoxymethylenetriphenylphosphorane (400 mg, 3.1 mmol) was added to a solution of **10** (1.0 g, 3.1 mmol) in methanol (50 ml) and stirred at ambient temperature overnight. The reaction was quenched with saturated aq. ammonium chloride (10 ml), the organic phase extracted with dichloromethane (2 × 30 ml), the combined organic phases washed with water (2 × 30 ml) and dried. The crude product was purified by flash chromatography, using petrol and diethyl ether (5:2), to yield **11** as a white solid (62 mg, 53%). Mp: 41–42 °C; $[\alpha]_D^{24} = +34.7°$ (*c*: 0.37 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3053, 2981, 2947, 1652 cm^{-1}; δ_H: 6.71 (dd, CH, 1H, $J = 15.3$, 10.5 Hz), 5.90 (d, CH, 1H, 15.3 Hz), 3.70 (s, MeO, 3H), 1.57 (m, CH_2, 2H), 1.21 (s, $C_{16}H_{32}$, 3 × CH, 35H), 0.82 (br, t, CH_3, 3H, $J = 6.0$ Hz), 0.51 (m, CH, 1H); δ_C: 170.3 (COOMe), 133.8 (CH), 131.9 (CH), 53.8, 51.2, 31.9, 31.7, 30.9, 29.7, 29.3, 29.2, 25.3, 22.7, 22.0, 21.8, 20.3, 19.5, 16.7, 15.7, 15.0, 14.1. Found M^+: 378.3507. Calculated for $C_{25}H_{46}O_2$: 378.3497.

2.2.6. (1R,2S)-1-(2′-Methoxycarbonyleth-1′-yl)-2-octadecylcyclopropane (12)

Sodium periodate (90 mg, 9.3 mmol) in water (10 ml) was added dropwise via a dropping funnel over 2 h to a stirred mixture of **11** (350 mg, 0.93 mmol), hydrazine hydrate (0.65 ml), ethanoic acid (0.5 ml) and saturated aq. copper sulfate solution (0.5 ml) in isopropanol (20 ml). The reaction was stirred for 3 days before being quenched with saturated aq. ammonium chloride (20 ml). The organic phase was extracted with dichloromethane (2 × 50 ml), washed with water (2 × 20 ml) and dried. The organic phase was reduced under vacuum and the residue purified by column chromatography, eluting with petrol and ethyl acetate (8:1), to yield **12** (150 mg, 42%) as a low melting solid. Mp: 35–36 °C; $[\alpha]_D^{24} = +34.7°$ (*c*: 0.37 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3052, 2980, 2971, 1745, 1205 cm^{-1}; δ_H: 3.61 (s, Me, 3H), 2.27 (t, CH_2, 2H, $J = 7.2$ Hz), 1.82 (m, CH_2, 2H), 1.34 (m, CH_2, 2H), 1.28 (s, $C_{16}H_{32}$, 32H), 0.92 (br, t, CH_3, 3H, $J = 5.6$ Hz), 0.72 (m, 3 × CH, 3H), −0.32 (q, CH, 1H, $J = 4.6$ Hz); δ_C: 51.5 (OMe), 34.7, 32.0, 30.2, 29.7, 29.4, 29.2, 29.1, 29.0, 28.9, 28.7, 28.6, 24.1, 22.7, 16.0, 15.3, 14.1, 10.8. Found M^+: 380.3656. Calculated for $C_{25}H_{48}O_2$: 380.3654.

2.2.7. (1R,2S)-1-(3′-Hydroxypropyl)-2-octadecylcyclopropane (13)

Lithium aluminium hydride (260 mg, 6.3 mmol) in anhydrous THF (30 ml) was added dropwise over 15 min to **12** (1.1 g, 2.89 mmol) in THF (100 ml). The solution was stirred at reflux temperature for 1 h before being quenched with 10% aq. sodium sulfite solution (20 ml), allowed to cool, filtered through magnesium sulfate and reduced under vacuum. The residue was purified by column chromatography, eluting with petrol and diethyl ether (2:1), to yield **13** (950 mg, 94%) as a white solid. Mp: 33–34 °C; $[\alpha]_D^{24} = +11.6°$ (*c*: 0.22 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3412 (br), 3062, 2980, 2954, 1208 cm^{-1}; δ_H: 3.44 (t, CH_2, 2H, $J = 6.6$ Hz), 2.85 (s, OH, 1H), 1.62 (m, CH_2, 2H), 1.42 (m, 2 × CH_2, 4H), 1.08 (s, $C_{16}H_{32}$, 32H), 0.76 (br, t, CH_3, 3H, $J = 5.6$ Hz), 0.48 (m, 3 × CH, 3H), −0.41 (m, CH, 1H); δ_C: 62.9 (COH), 33.2, 31.8, 30.1, 29.6, 29.3, 28.6, 25.2, 24.9, 24.3, 24.1, 23.4, 23.0, 22.6, 15.8, 15.4, 14.0, 10.9. Found M^+: 352.3701. Calculated for $C_{24}H_{48}O$: 352.3705.

*2.2.8. (1R,2S)-2-Octadecyl-1-[[(3′-tosyl)oxy]-propyl]cyclopropane (**14**)*

To a stirred solution of **13** (900 mg, 2.55 mmol) in pyridine (50 ml) at 0 °C: was added tosyl chloride (730 mg, 3.83 eq.). The mixture was allowed to warm to ambient temperature and stirred for 2 h until TLC analysis showed no starting material. Saturated aq. ammonium chloride (20 ml) was added to the solution and the organic phase extracted with chloroform (3 × 30 ml). The combined organic phases were dried and reduced under vacuum. The crude mixture was purified by column chromatography, eluting with petrol and diethyl ether (5:2), to yield **14** (800 mg, 62%) as a white solid. Mp: 39–41 °C; $[\alpha]_D^{24} = +8.7^\circ$ (*c*: 1.3 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3110, 3092, 3062, 2998, 2978, 1410, 1380, 1252 cm^{-1}; δ_H: 7.64 (m, 2 × CH, 2H), 7.19 (m, 2 × CH, 2H), 3.95 (t, CH_2, 2H, $J = 7.4$ Hz), 2.25 (s, Me, 3H), 1.59 (t, CH_2, 2H, $J = 7.4$ Hz), 1.48 (m, 2 × CH_2, 4H), 1.10 (m, $C_{16}H_{32}$, 32H), 0.89 (br, t, CH_3, 3H, $J = 5.6$ Hz), 0.46 (m, 3 × CH, 3H), −0.32 (m, CH, 1H); δ_C: 144.8, 129.7, 127.9, 70.9 (COTs), 31.9, 30.1, 29.7, 28.6, 24.5, 22.7, 21.6, 15.7, 14.9, 10.9; MS: no parent ion found.

*2.2.9. (1R,2S)-1-(3′-Cyanopropyl)-2-octadecylcyclopropane (**15**)*

To a slurry of sodium cyanide (73 mg, 1.52 mmol) in DMSO (10 ml), stirred at 90 °C, was added to **14** (700 mg, 1.38 mmol). The solution was warmed to 120 °C and stirred for 2 h before being cooled to 0 °C. Water (10 ml) was added, the organic phase was extracted with chloroform (3 × 20 ml), dried and reduced under vacuum. The crude mixture was purified by column chromatography, eluting with petrol and diethyl ether (2:1), to yield **15** (480 mg, 84%) as a white solid. Mp: 43–44 °C; $[\alpha]_D^{24} = 112^\circ$ (*c*: 2.24 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3054, 3898, 2010, 1410, 1251 cm^{-1}; δ_H: 2.18 (t, CH_2, 2H, $J = 6.7$ Hz), 1.57 (t, CH_2, 2H, $J = 6.5$ Hz), 1.32 (m, 2 × CH_2, 4H), 1.08 (s, $C_{16}H_{32}$, 32H), 0.61 (br, t, CH_3, 3H, $J = 5.6$ Hz), 0.46 (m, 3 × CH, 3H), −0.42 (m, CH, 1H); δ_C: 31.9, 30.1, 29.7, 29.4, 28.7, 27.6, 26.8, 26.5, 26.2, 26.0, 25.3, 25.0, 24.3, 22.5, 21.7, 16.9, 15.7, 14.7, 14.2, 10.9. Found M^+: 361.3706. Calculated for $C_{25}H_{47}N$: 361.3708.

*2.2.10. (1R,2S)-1-(3′-Carboxypropyl)-2-octadecylcyclopropane (**16**)*

To a stirred solution of **15** (400 mg, 1.12 mmol) in ethanol (20 ml) was added sodium hydroxide (600 mg, 8.7 mmol). The mixture was stirred at reflux for 3 h before being cooled to 0 °C, and water (10 ml) added. The organic phase was extracted with dichloromethane (2 × 30 ml), dried and reduced under vacuum. Purification by column chromatography, eluting with petrol and diethyl ether (2:1), gave the product as a white solid **16** (310 mg, 73%). Mp: 31–32 °C; $[\alpha]_D^{24} = 9.4^\circ$ (*c*: 1.7 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3820 (br), 3051, 2994, 2891, 1412 cm^{-1}; δ_H: 2.20 (t, CH_2, 2H, $J = 7.3$ Hz), 1.52 (p, CH_2, 2H, 7.3 Hz), 1.42 (m, 2 × CH_2, 2H), 1.08 (s, $C_{17}H_{34}$, 34H), 0.86 (br, t, CH_3, 3H, $J = 5.6$ Hz), 0.43 (m, 3 × CH, 3H), −0.48 (m, CH, 1H); δ_C: 179.2 (COOH), 33.7, 31.9, 30.2, 29.7, 29.4, 28.7, 28.1, 27.2, 26.4, 25.2, 23.8, 22.9, 22.7, 15.7, 15.2, 14.1, 10.9. Found M^+: 380.3642. Calculated for $C_{25}H_{48}O_2$: 380.3654.

*2.2.11. (1R,2S)-1-(3′-Methoxycarbonylprop-1′-yl)-2-octadecylcyclopropane (**17**)*

To a stirred solution of **16** (200 mg, 0.52 mmol) in dry methanol (30 ml) was added DOWEX® 50WX2-100 ion-exchange resin (0.2 g). The solution was stirred at reflux for 3 h, allowed to cool to ambient temperature and filtered. The filtrate was reduced under vacuum and purified by column chromatography, using petrol and diethyl ether (5:2), to yield **17** as a pale yellow solid (120 mg, 54%). Mp: 29–30 °C; $[\alpha]_D^{24} = +8.7^\circ$ (*c*: 2.12 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3060, 2996, 2894, 1410, 1230 cm^{-1}; δ_H 3.47 (s, OMe, 3H), 2.16 (t, CH_2, 2H, $J = 7.4$ Hz), 1.57 (p, CH_2, 2H, $J = 7.4$ Hz), 1.44 (m, 2 × CH_2, 2H), 1.11 (s, $C_{16}H_{32}$, CH_2, 34H), 0.85 (br, t, CH_3, 3H, $J = 5.6$ Hz), −0.41 (m, 3 × CH, 3H), −0.41 (m, CH, 1H); δ_C: 176.2 (COOMe), 51.4 (OMe), 34.0, 31.9, 30.2, 29.7, 29.4, 28.7, 28.2, 27.2, 26.4, 25.5, 23.8, 23.7, 23.5, 23.2, 23.0, 22.9, 22.7, 15.7, 15.3, 14.1, 10.9. Found M^+: 394.3805. Calculated for $C_{26}H_{50}O_2$: 394.3810.

*2.2.12. (1′Z,1R,2S)-1-(4′-Carboxybut-2′-en-1′-yl)-2-octadecylcyclopropane (**18**)*

Sodium bis(trimethylsilyl) amide (3.1 ml, 3.6 mmol) was added to a suspension of carbomethoxypropyl triphenylphosphonium bromide (1.51 g, 3.41 mmol)

in anhydrous THF (20 ml), stirring under nitrogen at ambient temperature. The mixture was stirred for a further 10 min, cooled to −78 °C and **10** (100 mg, 1.0 eq., 3.1 mmol) in THF (20 ml) added. The reaction was stirred for 2 h at ambient temperature before being quenched with saturated aq. ammonium chloride (20 ml). The organic phase was extracted with dichloromethane (30 ml), washed with water (2 × 50 ml), dried and reduced under vacuum. The crude product was purified by column chromatography, eluting with petrol and diethyl ether (5:2), to yield **18** as a white low melting solid (62 mg, 86%). Mp: 38–40 °C; $[\alpha]_D^{24} = +21.9^\circ$ (*c*: 0.43 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3400 (br), 3058, 3035, 3035, 3015, 2990, 2920, 1687 cm^{-1}; δ_H: 5.38 (dt, CH, 1H, $J = 10.8$, 6.5 Hz), 5.12 (t, CH, 1H, $J = 10.8$ Hz), 2.46 (m, 2 × CH_2, 4H), 1.52 (br, m, CH_2, 2H), 1.22 (s, $C_{16}H_{32}$, 32H), 0.84 (br, t, CH_3, 3H, $J = 5.6$ Hz), 0.83 (m, 3 × CH, 3H), 0.12 (m, CH, 1H); δ_C: 179.2 (COOH), 131.8 (CH), 127.3 (CH), 34.4, 32.2, 29.9, 29.8, 29.7, 29.6, 25.3, 25.1, 24.9, 24.7, 24.6, 23.1, 22.9, 22.6, 18.9, 15.4, 14.4, 14.3, 14.2. Found M^+: 392.3812. Calculated for $C_{26}H_{48}O_2$: 392.3810.

*2.2.13. (1′Z,1R,2S)-1-(4′-Methoxycarbonylbut-2′-en-1′-yl)-2-octadecylcyclopropane (**19**)*

To a stirred solution of **18** (30 mg, 0.076 mmol) in dry methanol (15 ml) was added DOWEX® 50WX2-100 ion-exchange resin (0.1 g). The solution was stirred at reflux for 2 h, allowed to cool to ambient temperature and filtered. The filtrate was reduced under vacuum and purified by column chromatography, using petrol and diethyl ether (5:2), to yield **19** as a white solid (23 mg, 74%). Mp: 38–40 °C; $[\alpha]_D^{24} = +9.2^\circ$ (*c*: 0.06 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3129, 3052, 2996, 2991, 2983, 1730 cm^{-1}; δ_H: 5.38 (dt, CH, 1H, $J = 10.6$, 6.6 Hz), 5.12 (t, CH, 1H, $J = 10.6$ Hz), 3.65 (s, OMe, 3H), 2.21 (m, 2 × CH_2, 4H), 1.42 (m, CH_2, 2H), 1.10 (s, $C_{16}H_{32}$, 32H), 0.91 (br, t, CH_3, 3 × CH, 6H), 0.06 (m, CH, 1H); δ_C: 131.3 (CH), 127.4 (CH), 51.5 (OMe), 34.2, 31.9, 29.7, 29.6, 29.5, 29.4, 23.2, 22.7, 18.7, 16.0, 15.6, 14.5, 14.1. Found M^+: 408.3766. Calculated for $C_{27}H_{50}O_2$: 408.3767.

*2.2.14. (1R,2S)-1-(4′-Carboxybut-1′-yl)-2-octadecylcyclopropane (**20**)*

Sodium periodate (1.2 g, 245 mmol) in water (10 ml) was added dropwise via a dropping funnel over 2 h to a stirred mixture of **18** (20 mg, 0.051 mmol), hydrazine hydrate (0.65 ml), ethanoic acid (0.5 ml) and saturated aq. copper sulfate solution (0.5 ml) dissolved in isopropanol (20 ml). The reaction was stirred for 3 days before being quenched with saturated aq. ammonium chloride (10 ml). The organic phase was extracted with dichloromethane (2 × 50 ml), washed with water (2 × 20 ml), dried and reduced under vacuum. The residue was purified by column chromatography, eluting with petrol and ethyl acetate (8:1), to yield **20** (18 mg, 90%) as a white low melting solid. Mp: 36–38 °C; $[\alpha]_D^{24} = +1.6^\circ$ (*c*: 0.5 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3510 (br), 3071, 2985, 2953, 2916, 1719 cm^{-1}; δ_H: 2.29 (t, CH_2, 2H, $J = 7.2$ Hz), 1.61 (m, CH_2, 2H), 1.48 (3 × CH_2, 6H), 1.18 (s, $C_{16}H_{32}$, 32H), 0.91 (br, t, CH_3, 3H, $J = 5.6$ Hz), 0.58 (m, 3 × CH, 3H), −0.34 (m, CH, 1H); δ_C: 180.3 (COOH), 34.2, 32.0, 30.2, 29.7, 29.4, 28.7, 28.3, 24.7, 24.2, 23.5, 22.7, 15.8, 15.5, 14.1, 10.9. Found M^+: 394.3793. Calculated for $C_{26}H_{50}O_2$: 394.3810.

*2.2.15. (1R,2S)-1-(4′-Methoxycarbonylbut-1′-yl)-2-octadecylcyclopropane (**21**)*

DOWEX® 50WX2-100 ion-exchange resin (0.2 g) was added to a stirred solution of **20** (10 mg, 0.025 mmol) in dry methanol (15 ml), stirred at reflux for 4 h, allowed to cool to ambient temperature and filtered. The filtrate was reduced under vacuum and purified by column chromatography, eluting with petrol and diethyl ether (5:2), to yield a white solid, **21** (9 mg, 89%). Mp: 39–40 °C; $[\alpha]_D^{24} = +7.1^\circ$ (*c*: 0.7 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 2953, 2920, 2851, 1742 cm^{-1}; δ_H: 3.61 (s, Me, 3H), 2.21 (t, CH_2, 2H, $J = 7.2$ Hz), 1.45–1.24 (m, 3 × CH_2, 6H), 1.18 (m, $C_{16}H_{32}$, CH_2, 34H), 0.89 (t, CH_3, 3H, $J = 5.6$ Hz), 0.56 (m, 3 × CH, 3H), −0.34 (m, CH, 1H); δ_C: 185.0 (COOMe), 52.1 (OMe), 34.1, 31.9, 30.2, 29.7, 29.4, 28.7, 24.5, 24.2, 24.1, 23.5, 23.4, 23.0, 22.8, 22.6, 19.4, 19.3, 16.1, 12.2, 10.1. Found M^+: 408.3955. Calculated for $C_{27}H_{52}O_2$: 408.3967.

*2.2.16. (1′Z,1R,2S)-1-(5′-Carboxypent-1′-yl)-2-octadecylcyclopropane (**22**)*

To a stirred suspension of 4-carboxybutyltriphenylphosphonium bromide (0.38 g, 3.41 mmol) in anhydrous THF (40 ml), under an atmosphere of nitrogen at ambient temperature was added sodium bis(trimethylsilyl) amide (3.1 ml, 2.0 eq., 6.2 mmol).

The mixture was stirred for a further 10 min cooled to −78 °C and **10** dissolved in THF (10 ml) (100 mg, 3.1 mmol) was added. The reaction was stirred for 3 h at ambient temperature before being quenched with saturated aq. ammonium chloride (30 ml). The organic phase was extracted with dichloromethane (30 ml), washed with water (2 × 40 ml), dried and reduced under vacuum. The crude product was then purified by column chromatography, eluting with petrol and diethyl ether (5:2), to yield **22** as a white low melting solid (72 mg, 58%). Mp: 42–43 °C; $[\alpha]_D^{24} = +56.2°$ (*c*: 0.8 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3400–3100 (br), 3057, 3013, 2988, 2953, 2920, 1705 cm^{-1}; δ_H: 6.53 (dd, CH, 1H, $J = 15.6$, 10.2 Hz), 6.19 (d, CH, 1H, $J = 15.6$ Hz), 2.41 (t, CH_2, 2H, $J = 7.5$ Hz), 2.21 (p, CH_2, 2H, $J = 7.5$ Hz), 1.50 (t, CH_2, 2H, $J = 8.4$ Hz), 1.34 (m, CH_2, 2H), 1.22 (s, $C_{16}H_{32}$, 32H), 0.89 (br, t, CH_3, 3H, $J = 5.6$ Hz), 0.88 (m, 3 × CH, 3H), 0.57 (q, CH, 1H, $J = 5.9$ Hz); δ_C: 207.1 (COOH), 150.7 (CH), 130.1 (CH), 32.1, 31.1, 29.8, 29.7, 29.6, 29.5, 29.3, 27.2, 27.1, 23.1, 22.8, 22.3, 20.0, 16.2, 15.7,14.2, 14.1; MS: no parent ion found.

2.2.17. *(1′Z,1R,2S)-1-(5′-Methoxycarbonylpent-2′-en-1′-yl)-2-octadecylcyclopropane (**23**)*

To a stirred solution of **22** (30 mg, 0.074 mmol) in dry methanol (15 ml) was added DOWEX® 50WX2-100 ion-exchange resin (0.3 g). The solution was stirred at reflux for 2 h, cooled to ambient temperature and filtered. The filtrate was reduced under vacuum and purified by chromatography, eluting with petrol and diethyl ether (5:2), to yield a white low melting solid **23** (26 mg, 84%). Mp: 43–45 °C; $[\alpha]_D^{24} = +7.8°$ (*c*: 0.1 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3067, 3023, 2987, 1756, 1270 cm^{-1}; δ_H: 5.22 (dt, CH, 1H, $J = 15.2$, 10.1 Hz), 4.99 (t, CH, 1H, $J = 15.0$ Hz), 3.49 (s, OMe, 3H), 2.24 (t, CH_2, 2H, $J = 7.5$ Hz), 1.52–1.41 (m, 2 × CH_2, 4H), 1.19 (s, $C_{16}H_{32}$, CH_2, 34H), 0.89 (br, t, CH_3, 3 × CH, 6H, $J = 5.6$ Hz), 0.01 (m, CH, 1H); δ_C: 179.4 (COOMe), 130.9 (CH), 128.5 (CH), 51.5 (OMe), 33.5, 31.9, 29.7, 29.6, 29.4, 26.9, 25.0, 24.3, 24.2, 24.0, 23.7, 23.4, 22.7, 18.6, 14.2, 14.1. Found M^+: 418.3813. Calculated for $C_{28}H_{52}O_2$ 418.3810.

2.2.18. *(1R,2S)-1-(5′-Carboxypent-1′-yl)-2-octadecylcyclopropane (**24**)*

Sodium periodate (1.2 g, 0.5 mmol) in water (15 ml) was added dropwise over 2 h to a stirred mixture of **22** (20 mg, 0.05 mmol), hydrazine hydrate (0.65 ml), ethanoic acid (0.5 ml) and saturated aq. copper sulfate solution (0.5 ml) in isopropanol (40 ml). The reaction was stirred for 2 days before being quenched with saturated aq. ammonium chloride (25 ml). The mixture was extracted with dichloromethane (2 × 50 ml), washed with water (2 × 20 ml) and dried. The organic phase was reduced under vacuum and the residue purified by column chromatography, eluting with petrol and ethyl acetate (8:1), to yield **24** (17 mg, 85%) as a pale yellow solid. Mp: 41–43 °C; $[\alpha]_D^{24} = +2.9°$ (*c*: 0.3 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3450–3120 (br), 3068, 2986, 2953, 2916, 1733 cm^{-1}; δ_H: 2.25 (m, CH_2, 2H), 1.58 (m, 2 × CH_2, 4H), s, ($C_{16}H_{36}$, C_3H_6, 38H), 0.92 (br, t, CH_3, 3H, $J = 5.6$ Hz), 0.57 (m, 3 × CH, 3H), −0.31 (m, CH, 1H); δ_C: 178.0 (COOH), 31.9, 30.2, 29.7, 29.4, 28.8, 28.5, 25.3, 24.8, 24.6, 24.2, 23.8, 22.7, 15.8, 15.6, 14.2, 10.9; MS: no parent ion found.

2.2.19. *(1R,2S)-1-(5′-Methoxycarbonypent-1′-yl)-2-octadecylcyclopropane (**25**)*

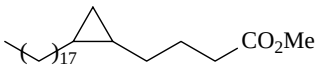
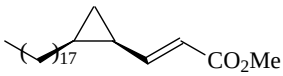
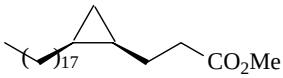
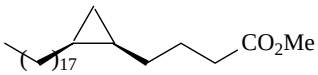
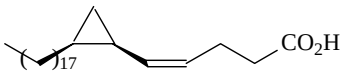
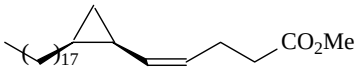
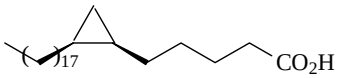
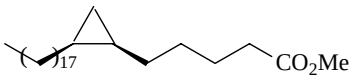
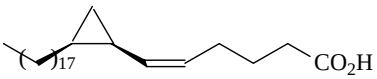
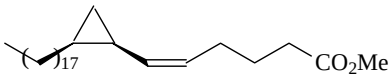
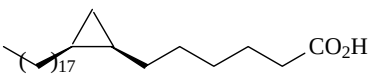
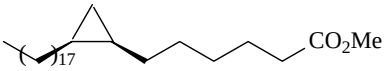
To a stirred solution of **24** (10 mg, 0.025 mmol) in dry methanol (15 ml) was added DOWEX® 50WX2-100 ion-exchange resin (0.3 g), stirred at reflux for 5 h, allowed to cool to ambient temperature and filtered and the filtrate evaporated. The residue was purified by column chromatography, using petrol and diethyl ether (5:2) to yield **25** (7 mg, 70%) as a white solid. Mp: 43–44 °C; $[\alpha]_D^{24} = +1.6°$ (*c*: 0.06 mg ml^{-1}, $CHCl_3$); ν_{max} (film): 3058, 2988, 2822, 2853, 1743 cm^{-1}; δ_H: 60 (s, Me, 3H), 2.21 (t, CH_2, 2H, $J = 7.2$ Hz), 1.25 (m, 2 × CH_2, 4H), 1.20 (m, $C_{16}H_{32}$, C_3H_6, 38H), 0.88 (br, t, CH_3, 3H, 5.6 Hz), 0.52 (m, 3 × CH, 3H), −0.33 (m, CH, 1H); δ_C: 179.4 (COOMe), 51.4 (OMe), 34.2, 31.9, 30.2, 29.7, 29.4, 29.2, 28.7, 28.5, 25.0, 22.7, 15.8, 15.7, 15.6, 14.1, 10.9. Found M^+: 422.4137. Calculated for $C_{28}H_{54}O_2$: 422. 4123.

2.3. Biological testing

M. smegmatis mc^2 cells were used to prepare a cell-wall fraction, with mycolate synthesising ability (Wheeler et al., 1993a,b). The "P60" wall fraction was obtained from broken cells by Percoll-density gradient centrifugation. For each low water assay, performed in triplicate, a mixture of P60 (130 μl),

hexane (900 μl) and inhibitor/stimulator (200 μg ml^{-1} in hexane) solution (100 μl) concentration was pre incubated at 37 °C for 30 min. Uniformly labelled 1 μCi ^{14}C-acetate (2.11 GBq mmol^{-1}) (10 μl) and a pH 5.0 buffer, consisting of di-potassium hydrogen orthophosphate 0.5 M, and potassium hydrogen carbonate 0.1 M (14 μl) were then added to give a total volume of 1154 μl; each assay was incubated for 1 h. The reaction was quenched with aqueous tetrabutylammonium hydroxide (15%, 900 μl), and the hexane evaporated. After heating at 100 °C for 18 h, dichloromethane (2 ml) and iodomethane (200 μl) were added, and each assay agitated for 45 min. The aqueous phase was separated and the organic phase washed with hydrochloric acid (1 M, 1.0 ml) and water (2 × 1 ml). The organic phase was evaporated and the residue extracted with ether (2 × 1 ml). Mycolic and fatty acid methyl esters were isolated from this extract using preparative TLC with petrol and acetone (95:5) as eluent and diphenyl hexatriene for

Table 1
Effect on the incorporation of ^{14}C-acetate into mycolic acids, in extracts of *M. smegmatis*, of long-chain cyclopropane compounds (tested at 20 μg/1154 μl)

Structure number	Cyclopropane compound	Counts per min (cpm)	% Inhibition	Chain length
Control	None	75755	0	–
RacC24		88633	−17 ± 5	24
11		104876	−38 ± 37	23
12		73995	+2 ± 1	23
17		78027	+3 ± 2	24
18		106041	−40 ± 5	25
19		85232	−13 ± 20	25
20		117562	−55 ± 17	25
21		78586	−4 ± 5	25
22		91161	−20 ± 7	26
23		106889	−41 ± 13	26
24		83942	−11 ± 20	26
25		88815	−18 ± 5	26

visualisation under long wave UV light. The radioactivity of the methyl esters was then determined by scintillation counting. Control assays were carried out in the absence of inhibitor/stimulator to provide baseline information against which to assess inhibitory or stimulatory behaviour (Table 1).

3. Results and discussion

The key chiral intermediate **10** was prepared from 2,3-*O*-isopropylidene-D-glyceraldehyde (Jackson, 1988) as outlined in Scheme 1. Nonadecyl triphenylphosphonium bromide (Al Dulayymi et al., 2000) reacted, in a Wittig reaction, with 2,3-*O*-isopropylidene-D-glyceraldehyde to give the *Z* alkene **7**, in 100% de as indicated by ^{1}H and ^{13}C NMR. Enantioselective formation of the cyclopropane ring, according to Morikawa et al. (1994) and Zhao et al. (1995), was achieved, using a modified Simmons–Smith reaction (Furakawa et al., 1966; Nishimura et al., 1969). This involved the stereoselective addition of diethyl zinc and chloroiodomethane to the alkene **7** to yield the *cis*-cyclopropane **8**. Deprotection of the isopropylidene ring of **8** with TFA in methanol gave the diol **9** and oxidative cleavage with sodium(*meta*)periodate then gave the key formylcyclopropane intermediate **10** (Scheme 1). It was expected that Wittig chemistry on **10**, using the appropriate phosphonium salts would enable the addition of 3-, 4-, 5- and 6-carbon carboxy side chains to provide the corresponding chiral cyclopropane acids. This approach was successful for the synthesis of three of the acids or esters (**12**, **21**, **25**), as outlined in Schemes 1 and 2. It was found necessary to prepare the remaining acid **17** by chain extension of **12** (Scheme 1).

Reaction of the aldehyde **10** with carbomethoxymethylenetriphenylphosphorane yielded the *E*-alkene **11** (Scheme 1). Reduction of the alkene **11**, using hydrazine hydrate, sodium(*meta*)periodate, copper(II) sulfate and ethanoic acid at 60 °C for 24 h yielded the saturated ester **12**. The phosphonium salt, prepared from ethyl 3-bromopropanoate, failed to react with **10** to provide a route to the ester **17** (Scheme 1). Therefore, reduction of **12** to the alcohol **13** was followed by tosylation to give **14**. Nucleophlic substitution with cyanide on **14** gave the nitrile **15**, which was hydrolysed to the acid **16**. Acid catalysed esterification converted the acid **16** to the desired ester **17** (Scheme 1).

The phosphonium salt prepared from methyl 4-bromobutyrate, reacted with the aldehyde **10**, in the presence of sodium bis-trimethylsilyl amide to give the acid **18** with only the *Z* diasteromer formed as indicated by NMR (Scheme 2). It seemed likely that the excess base used to ensure generation of the yield had hydrolysed the ester to the acid. A similar reaction using 4-carboxybutyl triphenylphosphonium bromide afforded the acid **22** again with only the *Z* diasteroisomer indicated by NMR (Scheme 2). Selective reduction of the vinylic acids acid **18** and **22** gave the saturated acids **20** and **24**, respectively. The unsaturated (**18**, **22**) and saturated (**20**, **24**) cyclopropane acids were converted into the corresponding unsaturated (**19**, **23**) and saturated (**21**, **25**) esters, with DOWEX® 50WX2-100 H^{+} resin in methanol for 4 h (Scheme 2).

The (1*R*,2*S*) long-chain ω-19 cyclopropane acids (**18**, **20**, **22**, **24**) and esters (**11**, **12**, **17**, **19**, **21**, **23**, **25**) and the racemic C24 methyl ester analogue (Hartmann et al., 1994) were assayed for inhibition of mycolic acid biosynthesis, using a cell-wall preparation from *M. smegmatis* (Wheeler et al., 1993a,b) (Table 1). The assay essentially measures elongation of endogenous fatty acid precursors associated with the cell wall extracts, it being unlikely that enzymes for de novo mycolate synthesis are present in high concentrations in these preparations. Inhibition by these long-chain compounds probably reflects binding to the essential Δ^{5}-desaturase, which appears to initiate mycolate synthesis; this was dramatically shown for the cyclopropene analogue **3** (Wheeler et al., 1993a). Stimulation may be a result of direct incorporation of the long-chain cyclopropane compounds, but extensive studies would be necessary to establish this.

Preliminary studies had indicated that racemic *cis*-5, 6-methanomethyloctadecanoate (**RacC24**) was a marginal inhibitor of mycolic acid biosynthesis in *M. smegmatis*. (Hartmann, 1993; Hartmann et al., 1994). In the present study, **RacC24** actually stimulated mycolate synthesis but the (1*R*,2*S*) enantiomer **17** showed small inhibition. Considering the saturated analogues first, the 23-carbon chain length analogue **12** was also a weak inhibitor. However, the 25- and 26-carbon analogues **21** and **25** showed significant stimulation of mycolate biosynthesis. The saturated

acid analogues **20** and **24** appeared to stimulate biosynthesis although the 25-carbon acid **20** showed a larger stimulation than the 26-carbon analogue **24**. This was also the case with the olefinic acids **18** and **22**. In the case of the vinyl esters, these were also found to be stimulating, with the 25-carbon analogue **19** showing less stimulation than the 23-carbon analogue **11** and the 26-carbon analogue **23** which showed similar stimulation values. The C24 acid **16** was not tested for mycolic acid inhibition.

Considering the four saturated cyclopropane esters (**12**, **17**, **21**, **25**), it appears that the C23 and C24 compounds (**12**, **17**) are able to interact with the active site of possibly a key Δ^5-desaturase and cause mild inhibition of mycolate synthesis. The extra carbon atom in the C25 ester **21** seems to disallow such interactions, giving mild stimulation; the addition of a further carbon in the C26 ester **25** reinforces this trend. The increased inhibition of the C24 (1*R*,2*S*) form **17** over the racemic form (**RacC24**) suggests that this enantiomer has enhanced affinity for an enzymic active site.

The presence of an additional double bond does not abolish stimulation of mycolate synthesis (Table 1). The increased rigidity conferred by the *trans* unsaturation in the C23 ester **11** negates the inhibitory effect seen in the saturated analogue **12**. Some interesting trends were discernable for the C25 and C26 compounds. The unsaturated methyl esters (**19**, **23**) were notably more stimulatory than the corresponding saturated esters (**21**, **25**). However, the behaviour of the unsaturated (**18**, **22**) and saturated acids (**20**, **24**) was not so well distinguished. This possibly reflects the differential effect of any lipases needed to hydrolyse the esters, the unsaturated esters (**19**, **23**) apparently being better substrates than the saturated analogues (**21**, **25**). In view of the relatively high stimulation of mycolate synthesis by some of the unsaturated analogues, such as compounds **11**, **18** and **23**, it would be of interest to know if their constituent double bonds are preserved through into intact mycolates. It is interesting that the most potent stimulators **11**, **18**, **20** and **23** (Table 1) have either odd chain lengths (**11**, **18**, **20**) or a double bond (**23**), suggesting that these substrates may not bind well to a desaturase active site but are capable of being elongated. Concerning different activities of cyclopropane acids and the corresponding esters, the only point of interest was that C25 acids (**18**, **20**) were stronger mycolate stimulators than their esters (**19**, **21**) but the reverse was found for the C26 acids (**22**, **24**) and esters (**23**, **25**). It may be surmised that the C26 esters are more readily hydrolysed by lipases due to their even numbered chain lengths being more biocompatible.

The diverse effects on mycolic acid synthesis shown by these long-chain ω-19 cyclopropane acids and esters indicate that specific interactions are taking place with key biosynthetic enzymes. These enzymes seem to be catholic in their tastes, perhaps demonstrating that recognition is governed, largely by the ω-19 *cis*-cyclopropane part of the molecule. These results support the hypothesis (Takayama and Qureshi, 1984; Wheeler et al., 1993a,b) that long-chain acids with ω-19 *cis* unsaturations have importance in the biosynthesis of mycolic acids.

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