# Synthesis and Structure of 2,3-Di-*O*-acyl-α,α-trehalose Lipid Antigens from *Mycobacterium fortuitum*

**Paul A. Wallace,[a] David E. Minnikin*[a] and Malin Ridell[b]**

[a] *Department of Chemistry, University of Newcastle, Newcastle upon Tyne, UK NE1 7RU*
[b] *Department of Medical Microbiology, University of Gothenburg, S-413 46, Gothenburg, Sweden*

The absolute structure of a 2,3-di-*O*-acyl-α,α-trehalose lipid antigen from *Mycobacterium fortuitum* has been established as **2** by synthesis of the natural lipid and its regioisomer **3**.

*Mycobacterium fortuitum* is an opportunistic pathogen which is becoming increasingly important because of its effect on immunocompromised AIDS patients.[1] Located within the cell envelope of *M. fortuitum* are three acylated α,α-trehalose lipid antigens, identified by Hamid *et al.*,[2] Gautier *et al.*[3] and Sempere *et al.*[4] The most non-polar lipid is a 2,3,6-tri-*O*-acyl-α,α-trehalose, the next a 2,3,4-tri-*O*-acyl-α,α-trehalose and the most polar lipid a 2,3-di-*O*-acyl-α,α-trehalose. All three glycolipids proved to be strongly antigenic and cross-reacted with sera raised against *Mycobacterium tuberculosis*, which also contains a series of 2,3-di-*O*-acyl-α,α-trehaloses,[5–7] having structures related to **1**.[8]

Preparative reverse-phase high-performance thin-layer chromatography (Merck $F_{254}$ RP-18 HPTLC plates)[8] separated the 2,3-di-*O*-acyl-α,α-trehaloses from *M. fortuitum*[2] into five glycolipids (**A**–**E**) with $R_f$ 0.87, 0.86, 0.85, 0.83 and 0.80 (chloroform–methanol–water, 6 : 15 : 0.1, twice), respectively. The major fatty acyl component present in these glycolipids is (*E*)-2-methyloctadec-2-enoic acid, the other acyl components being $C_{16}$–$C_{18}$ straight-chain fatty acids.[2]

The natural product **2** and its regioisomer **3** were synthesised from α,α-trehalose. Formation of 2′,3′,4,6;4′,6′-tri-*O*-cyclohexylidene-α,α-trehalose **4**,[10] followed by completely regioselective acylation at C-2, provided **5** and **6**. This reaction used one molar equivalent of either palmitic acid, to give compound **5**, or (*E*)-2-methyloctadec-2-enoic acid,[2] to give compound **6**, using dicyclohexylcarbodiimide (DCC) (1.2 mol. equiv.) and 4-dimethylaminopyridine (DMAP) (1.1 mol. equiv.) in dry dichloromethane as solvent and activated 4 Å molecular sieves.[9] Compound **5** has been previously reported.[10] Compound **6** (86%) showed $[\alpha]_D^{21}$ +71.2 (*c* 2, $CHCl_3$), found $M^+$, *m/z* 861, $C_{49}H_{80}O_{12}$ requires 861.16; $\delta_H$ (200 MHz) (see Table 1 for ring protons) 0.84 (t, 3 H, *J* 6.2 Hz, $MeCH_2$), 1.23 (s, 24 H, $-[CH_2]_n-$), 1.78 (s, 3H, $-CH{=}CMe-$), 2.20 (t, 2 H, *J* 7.5 Hz, $-CH_2CH{=}CMe-$), 6.82 (t 1 H, *J* 7.4 Hz $-C{=}CMe-$); $\delta_C$ (50.3 MHz) 12.38 ($-CH{=}CMe-$), 14.16 ($MeCH_2$), 36.07 ($-CH_2CH{=}CMe-$), 128.67 ($-CH{=}CMe-$), 146.01 ($-CH{=}CMe-$), 170.21 (–CO–, C-2).

(*E*)-2-Methyloctadec-2-enoic acid (1.1 mol. equiv.) was converted to the acid chloride, by reaction with oxalyl chloride, and this allowed the reaction with **5** (1.0 mol. equiv.) and 4-pyrollidinopyridine (1.2 mol. equiv.) in dry dichloromethane at room temp. Purification by flash column silica gel chromatography gave **7** (62%), $[\alpha]_D^{20}$ +54.2 (*c* 4, $CHCl_3$), found $M^+$, *m/z* 1099, $C_{65}H_{110}O_{13}$ requires 1099.58; $\delta_H$ (200 MHz) (see Table 1 for ring protons) 0.85 (t, 6 H, *J* $MeCH_2$), 1.23 (s, 48 H, $-[CH_2]_n-$), 1.79 (s, 3 H, $-CH{=}CMe-$), 2.16 (t, 2 H, *J* 7.4 Hz, $-CH_2CH{=}CMe-$), 2.32 (m, 2 H, $-CH_2CO-$), 6.73 (t, 1 H, *J* 7.3 Hz, $-CH{=}CMe-$); $\delta_C$ (50.3 MHz) 12.46 ($-CH{=}CMe-$) 14.15 ($MeCH_2$), 33.75 ($-CH_2CO-$), 36.25 ($-CH_2CH{=}CMe-$), 127.27 ($-CH{=}CMe-$), 145.15 ($-CH{=}CMe-$), 169.70 (–CO–, C-3), 172.42 (–CO–, C-2).

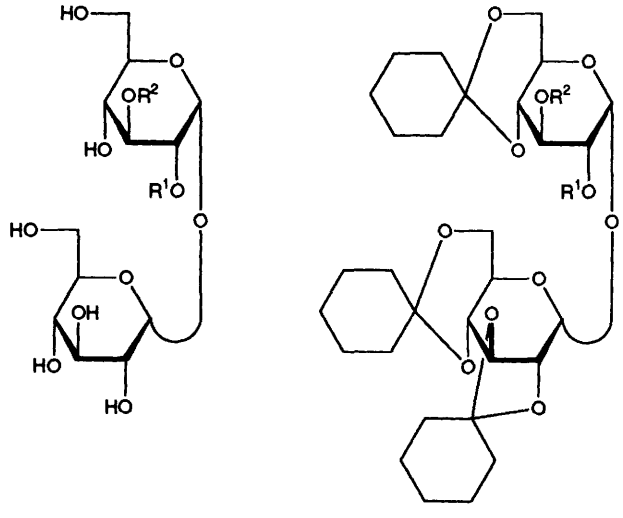
Palmitoyl chloride (1.0 mol. equiv.) reacted with **6** in the presence of imidazole (1.2 mol. equiv.) in dry THF at 70 °C for 3 h. Purification by flash silica gel column chromatography gave **8** (39%), $[\alpha]_D^{20}$ +30.4 (*c* 1, $CHCl_3$), found $M^+$, *m/z* 1099, $C_{65}H_{110}O_{13}$ requires 1099.58; $\delta_H$ (200 MHz) (see Table 1 for ring protons) 0.84 (t, 6 H, *J* 6.6 Hz, $MeCH_2$), 1.23 (s, 48 H, $-[CH_2]_n-$), 1.81 (s, 3 H, $-CH{=}CMe-$), 2.18 (t, 2 H, *J* 7.4 Hz, $-CH_2CH{=}CMe-$), 2.35 (m, 2 H, $-CH_2CO-$), 6.83 (t, 1 H, *J* 7.5 Hz, $-CH{=}CMe-$).

The cyclohexylidene protecting groups of **7** and **8** were removed by treatment with acetic acid and water (3 : 1) at 85 °C for 16 h. Purification using preparative TLC (chloro-

**Table 1** 200 MHz NMR chemical shifts of carbohydrate ring protons and the alkene fatty acid proton

| | Proton shift (δ) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | H-1<br>H-1′ | H-2<br>H-2′ | H-3<br>H-3′ | H-4<br>H-4′ | H-5<br>H-5′ | H-6a<br>H-7a′ | H-6e<br>H-6e′ | –C*H*=CMe–<br>— |
| **2** | 5.14 | 4.88 | 5.58 | [← | 3.44–4.36 | | →] | 6.72 |
| | 5.24 | [← | | 3.44–4.36 | | | →] | — |
| **3** | 5.09 | 4.88 | 5.49 | [← | 3.40–4.31 | | →] | 6.78 |
| | 5.33 | [← | | 3.40–4.31 | | | →] | — |
| **4** | 5.12 | [← | | 3.42–3.82 | | | →] | — |
| | 5.38 | [← | | 3.42–3.82 | | | →] | — |
| **5** | 5.26 | 4.75 | [← | 3.89–4.09 | | | →] | — |
| | 5.34 | [← | | 3.39–4.09 | | | →] | — |
| **6** | 5.08 | 4.61 | [← | 3.22–3.94 | | | →] | 6.82 |
| | 5.08 | [← | | 3.22–3.94 | | | →] | — |
| **7** | 5.26 | 5.01 | 5.45 | [← | 3.45–4.12 | | →] | 6.73 |
| | 5.34 | [← | | 3.45–4.12 | | | →] | — |
| **8** | 5.22 | 4.98 | 5.42 | [← | 3.47–4.15 | | →] | 6.83 |
| | 5.33 | [← | | 3.47–4.15 | | | →] | — |
| **C**[a] | 5.18 | 4.84 | 5.55 | [← | 3.39–4.41 | | →] | 6.71 |
| | 5.28 | [← | | 3.39–4.41 | | | →] | — |

[a] Diacyl trehalose fraction **C** from *M. fortuitum*.




**1**; $R^1$ = $CO[CH_2]_{16}Me$, $R^2$ = $CO[CH(Me)CH_2]_2[CH_2]_{16}Me$
**2**; $R^1$ = $CO[CH_2]_{14}Me$, $R^2$ = $CO[CHC(Me)][CH_2]_{14}Me$
**3**; $R^1$ = $CO[CHC(Me)][CH_2]_{14}Me$, $R^2$ = $CO[CH_2]_{14}Me$
**4**; $R^1$ = $R^2$ = H
**5**; $R^1$ = $CO[CH_2]_{14}Me$, $R^2$ = H
**6**; $R^1$ = $CO[CHC(Me)][CH_2]_{14}Me$, $R^2$ = H
**7**; $R^1$ = $CO[CH_2]_{14}Me$ $R^2$ = $CO[CHC(Me)][CH_2]_{14}Me$
**8**; $R^1$ = $CO[CHC(Me)][CH_2]_{14}Me$ $R^2$ = $CO[CH_2]_{14}Me$

form–methanol–water, 100:14:0.8) gave the natural product **2** (29%, $R_f$ 0.13), $[\alpha]_D^{19}$ +20.4 (*c* 0.5, $CHCl_3$), found $M^+$, *m/z* 859, $C_{47}H_{86}O_{13}$ requires 859.19; $\delta_H$ (200 MHz) (see Table 1 for ring protons) 0.85 (t, 6 H, *J* 6.7 Hz, *Me*$CH_2$), 1.23 (s, 48 H, –$[CH_2]_n$–), 1.79 (s, 3 H, –CH=C*Me*–), 2.10 (2 H, t, *J* 7.5 Hz, –$CH_2$CH=CMe), 2.30 (m, 2 H, –$CH_2$CO–), 6.72 (s, 3 H, *J* 7.5 Hz, –C*H*=CMe–), and its regioisomer **3** (44%, $R_f$ 0.13), $[\alpha]_D^{19}$ +18.2 (*c* 0.5, $CHCl_3$), found $M^+$, *m/z* 859, $C_{47}H_{86}O_{13}$ requires 859.19; $\delta_H$ (200 MHz) (see Table 1 for ring protons) 0.86 (t, 6 H, *J* 6.7 Hz, *Me*$CH_2$), 1.23 (s, 48 H, –$[CH_2]_n$–), 1.82 (s, 3 H, –CH=C*Me*–), 2.06 (2 H, t, *J* 7.4 Hz, –$CH_2$CH=CMe–), 2.31 (m, 2 H, –$CH_2$CO–), 6.78 (s, 3 H, *J* 7.5, –C*H*=CMe–). The NMR data demonstrate that compound **2** is homogeneous with no evidence of acyl migration; the modest yield of **2** may be attributable to incomplete hydrolysis, as evidence by some less polar components.

NMR studies of these two synthetic glycolipids showed similar results to those for model compounds based on 4,6-*O*-benzylidene-α-D-glucopyranose. These models were synthesised, having the same fatty acyl moieties as compounds **2** and **3**. In these model compounds, the monosaccharide corresponding to compound **2** showed the unsaturated fatty acyl vinyl $^1H$ NMR signal at δ 6.73. In the model corresponding to compound **3**, the same proton was observed at δ 6.80. This provided conclusive evidence of our assignments. For the synthetic glycolipid **2**, the acyl double bond proton was observed at δ 6.72 and in **3** at δ 6.78. In the natural glycolipid, this proton resonated at δ 6.71, with other proton resonances at δ 5.18 (H-1), 5.28 (H-1′), 4.84 (H-2) and 5.55 (H-3).

Reverse-phase HPTLC of **2** and **3** in chloroform–methanol–water (6:15:0.1, twice) showed both compounds having $R_f$ 0.85 which corresponds to fraction **C** of the glycolipids from *M. fortuitum*. This natural glycolipid also showed $[\alpha]_D^{16}$ +20.8 (*c* 0.5, $CHCl_3$) and $\delta_H$ (200 MHz) (see Table 1 for ring protons), 0.85 (t, 6 H, *J* 6.6 Hz, *Me*$CH_2$), 1.23 (s, 48 H, –$[CH_2]_n$–), 1.79 (s, 3 H, –CH=C*Me*–) 6.71 (t, 1 H, *J* 7.4 Hz, –C*H*=CMe–). Compound **2** showed $[\alpha]_D^{19}$ +20.4 (*c* 0.5, $CHCl_3$).

The absolute structure **2** of a major component of the *M. fortuitum* 2,3-di-*O*-acyl-α,α-trehalose lipid antigens has been established. The present paper introduces methods for the selective acylation of the key 'triprotected' trehalose intermediate **4**, opening up routes for the synthesis of the important diacyl trehalose antigens **1** from *M. tuberculosis*.[8] Syntheses of 2,3-di-*O*-palmitoyl-α,α-trehalose have been reported previously.[10,11]

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