Synthesis of 2,3-Di-*O*-palmitoyl-α,α-trehalose *via* a Novel Tri-protected Trehalose Intermediate

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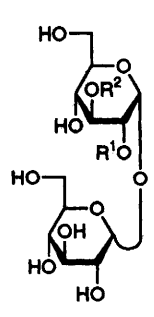
2′,3′;4,6;4′,6′-Tri-*O*-cyclohexylidene-α,α-trehalose **4** is prepared from α,α-trehalose, regioselectively acylated at C-2 with palmitate; after further acylation with palmitate at C-3, deacetalisation gives 2,3-di-*O*-palmitoyl-α,α-trehalose **2**.

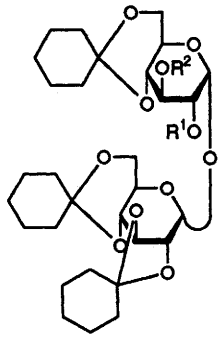
Pathogenic mycobacteria produce a range of complex cell surface glycopeptidolipid, diacylglycosylphenolphthiocerol and lipooligosaccharide lipid antigens.[1] Recently, relatively simple 2,3-di-*O*-acyl-α,α-trehalose mycobacterial antigens have been characterised,[2–4] as exemplified by the main component **1** from *Mycobacterium tuberculosis*.[2] The chemical synthesis of these lipids offers the possibility of economically producing large amounts of antigens for use in rapid serodiagnosis,[5] particularly in developing countries where the combination of tuberculosis and AIDS is an increasing problem. A synthesis of 2,3-di-*O*-acyl-α,α-trehaloses has been reported recently,[6] involving selective acylation of the 2,3-*O*-dibutylstannylene derivative of 4,6;4′,6′-di-*O*-benzylidene-α,α-trehalose, protection of the monoesters by cyclising silylation with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, further acylation and deprotection. In this communication, we report the efficient synthesis of 2,3-di-*O*-palmitoyl-α,α-trehalose **2** *via* the regioselective acylation of a novel asymmetrically protected trehalose.

Following a previous glucose protection strategy,[7] anhydrous trehalose, in dry dimethylformamide, was heated under reflux and reduced pressure (water pump) for 4 h with 8 molar equivalents of 1,1-dimethoxycyclohexane in the presence of toluene-4-sulfonic acid. Reaction of the crude product with acetic anhydride in pyridine gave a mixture of acetates which were resolved by flash silica gel chromatography to give the main tri-protected product [**3**, 42%, TLC, R_f 0.63 in ethyl acetate–light petroleum (b.p. 40–60 °C) (1 : 1)], accompanied by tetra-protected (3%, R_f 0.86), di-protected (39%, R_f 0.38) and mono-protected (6%, R_f 0.25) derivatives. The structure of the key product **3**, $[\alpha]_D^{20}$ + 108 (*c* 1, $CHCl_3$), m.p. 90–94 °C, was confirmed by the combination of electron impact mass spectrometry [found M^+, *m/z* 666; $C_{35}H_{50}O_{13}$ requires 666.76] and NMR spectroscopy (^1H, 200 MHz; ^{13}C, 50.3 MHz; $CDCl_3$). The NMR spectra showed distinctive proton signals at δ_H 5.27 (1 H, $J_{1,2}$ 3.8 Hz, 1-H) and 5.32 (1 H, $J_{1',2'}$ 3.0 Hz, 1′-H) and carbon signals at δ_C 94.09 (1 C, C-1) and 93.39 (1 C, C-1′) and only two CH_3CO_2– signals (δ_H 2.03 and 2.09), as required for the asymmetric substitution pattern. Deacylation of **3** with 3% NaOMe in chloroform–methanol (1 : 1) and flash chromatography gave **4** (62%, m.p. 138–140 °C), $[\alpha]_D^{22}$ + 112 (*c* 1, $CHCl_3$).

Treatment of **4** with palmitic acid (1 molar equiv.), dicyclohexylcarbodiimide (DCC) (1.2 molar equiv.) and 4-dimethylaminopyridine (DMAP) (1.1 molar equiv.) in dry dichloromethane,[8] in the presence of activated 4 Å molecular sieves, gave essentially a single component on TLC; purification by flash chromatography yielded **5** (75%, syrup) (found M^+ *m/z* 821; $C_{46}H_{49}O_6$ requires 821.1); $[\alpha]_D^{20}$ + 80.4 (*c* 1, $CHCl_3$). The regioselective esterification at C-2 was confirmed by NMR spectroscopy with the expected signal for 2-H at δ_H 4.75 (1 H, dd, $J_{1,2}$ 3.7, $J_{2,3}$ 9.6 Hz) and the absence of an



1; R^1 = $CO[CH_2]_{16}Me$, R^2 = $CO[CH(Me)CH_2]_2[CH_2]_{18}Me$
2; R^1 = R^2 = $CO[CH_2]_{14}Me$



3; R^1 = R^2 = Ac
4; R^1 = R^2 = H
5; R^1 = $CO[CH_2]_{14}Me$, R^2 = H
6; R^1 = R^2 = $CO[CH_2]_{14}Me$

expected triplet for 3-H at about δ_H 5.35. The ^{13}C spectrum showed only one set of signals for the unit $-CO_2CH-$ (δ_C 71.39, C-2; 173.44, carbonyl).

Further esterification of **5** with palmitic acid (2.0 molar equiv.), DCC (2.4 molar equiv.) and DMAP (2.2 molar equiv.) gave, after flash chromatography, **6** (68%, syrup), $[\alpha]_D^{21}$ + 51.6 (*c* 2, $CHCl_3$); δ_H 4.94 (1 H, dd, $J_{1,2}$ 4.0, $J_{2,3}$ 9.9 Hz, 2-H), 5.09 (1 H, d, $J_{1,2}$ 3.0 Hz, 1'-H), 5.26 (1 H, dd, $J_{1,2}$ 3.7 Hz, 1-H), 5.46 (1 H, t, $J_{2,3}$ 9.9 Hz, 3-H); δ_C 170.49 (1 C, carbonyl of ester at C-2), 169.53 (1 C, carbonyl of ester at C-3).

The cyclohexylidene protection groups of **6** were removed by treatment with 10% aqueous HCl–tetrahydrofuran (2 : 1) at 20 °C for 16 h. Flash chromatography, with chloroform–methanol (100% $CHCl_3$ to 3 : 1 to 2 : 1 to 1 : 1 gradient), of the product gave 2,3-di-*O*-palmitoyl-α,α-trehalose **2** (31%, m.p. 132–134 °C), $[\alpha]_D^{22}$ + 43.4 (*c* 1, $CHCl_3$); δ_H 44.84 (1 H, dd, $J_{1,2}$ 3.3, $J_{2,3}$ 9.1 Hz, 2-H), 5.08 (1 H, d, $J_{1,2}$ 2.2 Hz, 1'-H), 5.23 (1 H, d, $J_{1,2}$ 3.3 Hz, 1-H), 5.37 (1 H, t, $J_{2,3}$ 9.0 Hz, 3-H); δ_C 171.5 (1 C, carbonyl of ester at C-3), 178.8 (1 C, carbonyl of ester at C-2). The natural lipid antigen isolated from *M. tuberculosis*[2] has δ_H 4.83 (2-H), 5.05 (1'-H), 5.25 (1-H), 5.40 (3-H); δ_C 173.8 (carbonyl of ester at C-3), 177.8 (carbonyl of ester at C-2). The synthetic glycolipid is also cochromatographed on TLC with natural diacyl trehalose glycolipid antigens from *M. tuberculosis*[2] and *Mycobacterium fortuitum*.[4]

Efficient syntheses of both diacylated (**2**,**6**) and 2-acylated (**5**) trehalose derivatives are described above. The regioselective acylation is in accord with the previous synthesis of **2**[6] but it is achieved without the necessity of activation as a 2,3-*O*-dibutylstannylene derivative and subsequent protection of the 2',3' positions with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane. The present synthesis involves the use of inexpensive reagents and the synthesis of **2** can be cut to four steps (trehalose → **4** → **5** → **6** → **2**) if the acetylation–deacylation step is omitted. This would require that the initial purification is of compound **4**, which is quite satisfactory since the true identity of **4** has been established by preparation and characterisation of its diacetate **3**. The synthesis of the native glycolipid antigens **1** from *M. tuberculosis*[2] will require the prior preparation of the dimethyl branched fatty acid and sequential esterification. The way is now open for the efficient production of a range of 2,3-di-*O*-acyl-α,α-trehaloses and thereby the determination of the precise epitopes of these important glycolipid antigens.

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