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Glucose monomycolates based on single synthetic mycolic acids

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ABSTRACT

The preparation of 6-*O*-mycolylglucoses (GMMs) from single synthetic mycolic acids matching the overall structure of some of the major natural glucose monomycolates of *Mycobacterium tuberculosis* and other mycobacteria is reported.

1. Introduction

It has been known for nearly a century that mycolic acids form a characteristic component of the cells of mycobacteria and a number of related species (Barry et al., 1998; Minnikin, 1984; Asselineau et al., 2002; Verschoor et al., 2012). They are high molecular weight lipids consisting of around 70–90 carbons, with several chiral centres. These long chain β-hydroxy acids are present as complex mixtures of different homologues and different functional classes, either bound to the cell membrane, as penta-arabinose tetramycolates, or as non-membrane bound sugar esters, such as trehalose di- and monomycolates; free mycolic acids have been isolated from biofilms (Ojha et al., 2010). A mycobacterial antigen, glucose monomycolate (GMM, 6-*O*-mycoloyl-D-glucose) was isolated from different species of corynebacteria and mycobacteria (Brennan et al., 1970; Durand et al., 1979; Matsunaga et al., 1990, 1996), including *Mycobacterium tuberculosis*. MALDI-TOF analysis of GMM derived from *Mycobacterium smegmatis* shows a majority of peaks corresponding to α-mycolates (C_{74}, C_{76}, C_{77}, C_{78}, C_{79}, C_{80}) (Enomoto et al., 2005).

T-cell recognition is highly specific for the precise structure of natural GMM including the glucose, the linkage of the glucose to the mycolate, the *R,R*-stereochemistry of the hydroxyl acid part of the mycolate, and the length of the chains (Moody et al., 1997, 2000a,b, 2002). Antigen–protein complexes mediate T cells which respond in the human host by presenting GMM and other antigenic mycobacterial glycolipids by the CD1 family (Sieling et al., 1995; Moody et al., 1997, 2000a,b; Shamshiev et al., 1999, 2000, 2002). The particular importance of the related GroMM (glycerol monomycolate) in stimulating CD1-restricted T cells should also be noted (Layre et al., 2009). The mode of presentation of antigens to T cells and of their recognition has been reviewed (De Libero and Mori, 2014; Barral and Brenner, 2007). Cd1b tetramer staining of T cells has been used to prove that CD1b-glycolipid complexes bind the T cell receptor (Kasmar et al., 2011). GMM can induce a memory T cell response similar to a model protein antigen, and no B cell response; this has been applied in a new vaccination of cattle (Nguyen et al., 2009). Human T cells proliferate or produce interferon-in response to several types of mycobacterial lipid antigen presented by group 1CD1 proteins during latent or active tuberculosis infection, suggesting a function in host response to mycobacteria (Moody et al., 2000a,b; Ulrichs et al., 2003). Upon infection mycobacteria begin to produce GMM which provides a good indicator of local invasion. The GMM is produced more at 30 °C than 37 °C and is recognised by a GMM-specific, CD1-restricted T-cell line isolated from human skin. GMM-specific T-cells have also been shown to be able to approach the site of infection where $CD1c^+$ cells have accumulated; this indicates a role for CD1c molecules in eliciting responses to mycolate containing antigens (Morita et al., 2013).

Natural GMM and even some very simple synthetic analogues have been shown, among other mycolate esters, to be effective antigens in the serodiagnosis of TB and other infections such as by *Nocardia* and *Rhodococcus* (Yano et al., 1989). More recently, the GMM from, (+)-corynomycolic acid, which contains just two

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simple alkyl chains, has been shown to be a potent activator of both mouse and human Mincle (van der Peet et al., 2015).

Prandi in 2012 reported the preparation of glucose mycolates from natural mixtures of mycolic acids (Prandi, 2012). We have reported the synthesis of a range of unique mycolic acids corresponding in structure to those reported to be present in mycobacteria (Al Dulayymi et al., 2005, 2007; Koza and Baird, 2007; Koza et al., 2009a,b, 2013; Al Kremawi et al., 2010, 2014; Muzael et al., 2010). We have also reported the synthesis of the corresponding TDMs and TMMs from a range of these single mycolic acids (Al Dulayymi et al., 2014). We now report the synthesis of a number of examples of GMMs derived from single complete mycolic acids corresponding to the chain lengths and functionalities of major components of mycobacteria using the method described by Prandi (2012).

2. Experimental

2.1. General methods

Chemicals used were obtained from commercial suppliers (Sigma, Aldrich, and Alfa Aeser) or prepared from them by the methods described. Solvents which were required to be dry, *e.g.* ether, tetrahydrofuran were dried over sodium wire and benzophenone under nitrogen, while dichloro-methane and HMPA were dried over calcium hydride. All reagents and solvents used were of reagent grade unless otherwise stated. Silica gel (Merck 7736) and silica gel plates used for column chromatography and thin layer chromatography were obtained from Aldrich; separated components were detected using variously UV light, I_2 and phosphomolybdic acid solution in IMS followed by charring. Anhydrous magnesium sulfate was used to dry organic solutions. Infra-red (IR) spectra were carried out on a Perkin-Elmer 1600 F.T.I.R. spectrometer as liquid films or KBr disc (solid). Melting points were measured using a Gallenkamp melting point apparatus. NMR spectra were carried out on a Bruker Avance 400 or 500 spectrometer. Specific rotations were recorded in $CHCl_3$ on a POLAAR 2001 optical activity polarimeter. Mass spectra were recorded on a Bruker matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS) values are given plus sodium to an accuracy of 1 d.p. Accurate mass MALDI data were provided by Dr Paul Gates (Bristol University).

2.2. 6-O-[(R)-2-((R)-1-Hydroxy-18-((1S,2R)-2-((17S,18S)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl) tetracosanoyl]-α/β-D-glucopyranoside (4a)

(a) Dry cesium hydrogen carbonate (120 mg, 0.620 mmol) was added to a stirred solution of mycolic acid **2a** (70 mg, 0.050 mmol; prepared by the same general method as described in Al Dulayymi, et.al., 2007; Baols, 2014) in THF and DMF (1:1, 2.4 mL) at room temperature. The mixture was stirred at room temperature for 1 h; benzyl 2,3,4-tri-*O*-benzyl-6-*O*-tosyl-β-D-glucopyranoside **1** (44 mg, 0.060 mmol) was added. The mixture was stirred at 70 °C for 18 h. Sat. aq. $NaHCO_3$ (10 mL) was added and the product was extracted with dichloromethane (3 × 25 mL). The combined organic layers were evaporated. The product was purified by column chromatography eluting with petrol/ethyl acetate (4:1) to give a semi-solid, benzyl 2,3,4-tri-*O*-benzyl-6-*O*-[(*R*)-2-((*R*)-1-hydroxy-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoyl]-β-D-glucopyranoside **3a** (61 mg, 62%) $[\alpha]_D^{22} + 14$ (*c* 1.2, $CHCl_3$), [MALDI – Found $(M+Na)^+$: 1770.8, $C_{117}H_{198}NaO_9$ requires: 1770.5], which showed δ_H (400 MHz, $CDCl_3$): 7.40–7.28 (20H, m), 4.96 (1H, d, *J* 11.0 Hz), 4.95 (1H, d, *J* 11.0 Hz), 4.94 (1H, d, *J* 12.2 Hz), 4.9 (1H, d, *J* 12.2 Hz)), 4.8 (1H, d, *J* 11.0 Hz), 4.72 (1H, d, *J* 11.0 Hz), 4.63 (1H, d, *J* 12.2 Hz), 4.60 (1H, d, *J* 12.2 Hz), 4.54 (1H, br. d, *J* 12.1 Hz), 4.53 (1H, d, *J* 7.7 Hz), 4.23 (1H, dd, *J* 4.5, 12.1, Hz), 3.60 (2H, br. t, *J* 8.7 Hz), 3.53 (2H, br d, *J* 6.0 Hz), 3.49 (1H, br. d, *J* 9.0 Hz), 3.35 (3H, s), 3.0–2.95 (1H, m), 2.51–2.45 (2H, m), 1.77–1.60 (4H, m), 1.50–1.05 (139H, m), 0.89 (6H, t, *J* 6.8 Hz), 0.86 (3H, d, *J* 6.9 Hz), 0.7–0.64 (2H, m), 0.57 (1H, dt, *J* 4.0, 7.8 Hz), −0.32 (1H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.2, 138.4, 138.3, 137.7, 137.2, 128.5, 128.41, 128.4, 128.35, 128.1, 128.0, 127.97, 127.9, 127.8, 127.7, 102.3, 85.4, 84.5, 82.3, 77.8, 75.7, 75.1, 74.9, 72.9, 72.3, 71.1, 62.9, 57.7, 51.3, 35.6, 35.3, 32.4, 31.9, 30.5, 30.2, 30.0, 29.7, 29.65, 29.6, 29.4, 28.7, 27.6, 27.5, 26.2, 25.9, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max}/cm^{-1}: 3535, 2922, 2844, 1726, 1465, 1256, 1178.

(b) Palladium hydroxide 10% on charcoal (6 mg) was added to a stirred solution of the above product (45 mg, 0.02 mmol) in dry CH_2Cl_2/CH_3OH (2 mL, 1:1) at room temperature then the suspension was stirred for 18 h under hydrogen. The reaction was diluted with dichloromethane (20 mL), and filtered through a bed of celite. The filtrate was evaporated and the product was purified by column chromatography eluting with chloroform/ methanol (10:1) to give a semi-solid, the title compound **4a** (32 mg, 94%) as a mixture of two isomers (α,β) in ratio 3:2, $[\alpha]_D^{22} + 35$ (*c* 1.2, $CHCl_3$), [MALDI – Found $(M+Na)^+$: 1410.3035, $C_{89}H_{174}NaO_9$ requires: 1410.3050], which showed δ_H (400 MHz, $CDCl_3$): 5.13 (0.6H, d, *J* 3.6 Hz, H – 1α), 4.48 (0.4H, d, *J* 7.8 Hz, H – 1β), 4.42 (1H, br. d, *J* 12 Hz, H – 6α,β), 4.27 (1H, dd, *J* 5.6, 12.0 Hz, H – 6α,β), 3.96 (0.6H, ddd, *J* 2.2, 5.6, 9.8 Hz, H – 5α), 3.68–3.59 (1.6H, m, H – 4α + CH–OH mycolic acid), 3.47 (0.4H, ddd, *J* 2.2, 5.6, 9.0 Hz, H – 5β), 3.43–3.26 (5H, including s for the methoxy group at 3.30), 3.19 (0.4H, br. t, *J* 7.8 Hz, H – 2β), 2.96–2.92 (1H, m), 2.41–2.34 (1H, m), 1.68–1.0 (148H, m), 0.83 (6H, t, *J* 6.8 Hz), 0.80 (3H, d, *J* 6.9 Hz), 0.64–0.58 (2H, br. m), 0.53–0.48 (1H, dt, *J* 3.8, 7.8 Hz), −0.42 (1H, br., q, *J* 5.0 Hz); δ_C (101 MHz, $CDCl_3$) for α and β isomers: 175.1, 96.5, 92.2, 85.5, 73.6, 73.5, 72.5, 72.1, 70.5, 70.3, 69.2, 63.5, 63.4, 57.6, 52.7, 52.5, 35.2, 35.0, 32.3, 31.8, 30.4, 30.1, 29.83, 29.78, 29.6, 29.57, 29.5, 29.4, 29.2, 28.6, 27.4, 27.3, 26.0, 25.3, 22.6, 15.6, 14.7, 14.0, 10.8; ν_{max}/cm^{-1}: 3456, 2919, 2850, 1728, 1148, 1106, 992, 721, 427.

2.3. 6-O-[(R)-2-((R)-1-Hydroxy-18-((1S,2R)-2-((17S,18S)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl) hexacosanoyl]-α/β-D-glucopyranoside (4b)

(a) Using the same method as above cesium hydrogen carbonate (100 mg, 0.510 mmol) and mycolic acid **2b** (61 mg, 0.04 mmol; prepared by the same general method as described in Al Dulayymi et.al., 2007b) in THF and DMF (1:1, 2.4 mL) with glucopyranoside **1** (37 mg, 0.05 mmol) gave a semi-solid, benzyl 2,3,4-tri-*O*-benzyl-6-*O*-[(R)-2-((R)-1-hydroxy-18-((1S,2R)-2-((17S,18S)-17-methoxy-18-methylhexatriacontyl) cyclopropyl)-octadecyl)hexacosanoyl]-β-D-glucopyranoside **3b** (52 mg, 63%), $[\alpha]_D^{22} + 13$ (*c* 1.1, $CHCl_3$), [MALDI – Found $(M+Na)^+$: 1798.9, $C_{119}H_{202}NaO_9$ requires: 1798.5], which showed δ_H (400 MHz, $CDCl_3$): 7.40–7.28 (20H, m), 4.96 (1H, d, *J* 11.0 Hz), 4.95 (1H, d, *J* 11.0 Hz), 4.93 (1H, d, *J* 12.2 Hz), 4.9 (1H, d, *J* 12.2 Hz), 4.8 (1H, d, *J* 11.0 Hz), 4.72 (1H, d, *J* 11.0 Hz), 4.63 (1H, d, *J* 12.0 Hz), 4.60 (1H, d, *J* 12.0 Hz), 4.54 (1H, br d, *J* 11.5 Hz), 4.53 (1H, d, *J* 7.7 Hz), 4.23 (1H, dd, *J* 4.5, 11.5, Hz), 3.67 (2H, br, t, *J* 8.7 Hz), 3.53 (2H, br d, *J* 6.0 Hz), 3.5 (1H, br d, *J* 9.0 Hz), 3.35 (3H, s), 3.0–2.95 (1H, m), 2.51–2.45 (2H, m), 1.77–1.60 (4H, m), 1.50–1.05 (143H, m), 0.89 (6H, t, *J* 6.6 Hz), 0.86 (3H, d, *J* 6.9 Hz), 0.7–0.60 (2H, m), 0.57 (1H, dt, *J* 4.0, 7.8 Hz), −0.32