Carbohydrate Research 347 (2012) 151–154

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

A convenient synthesis of glucose monomycolate

Jacques Prandi *

CNRS, IPBS (Institut de Pharmacologie et de Biologie Structurale), 205 route de Narbonne BP 64182, F-31077 Toulouse, France
Université de Toulouse, UPS, IPBS, F-31077 Toulouse, France

ARTICLE INFO

Article history:
Received 21 September 2011
Received in revised form 4 November 2011
Accepted 11 November 2011
Available online 19 November 2011

Keywords:
Mycobacteria
Tuberculosis
Glucose monomycolate

ABSTRACT

An efficient synthesis of glucose monomycolate, an important lipidic antigen from *Mycobacterium tuberculosis* is described.

Glucose monomycolate (GMM, 6-*O*-mycoloyl-D-glucose, Fig. 1) is a mycobacterial antigen isolated from various species of corynebacteria and mycobacteria,[1] including *Mycobacterium tuberculosis*, the etiologic agent of human tuberculosis. GMM and other antigenic mycobacterial glycolipids are presented to T cells by the CD1 family of proteins and the antigen–protein complexes mediate T cells response in the human host.[2,3] The CD1 proteins are a third type of antigen-presenting molecules, related to MHC-encoded class I and class II molecules and are also associated with the β2 microglobulin. In humans are found five non-polymorphic CD1 proteins, CD1a, CD1b, CD1c, and CD1d are expressed at the surface of antigen-presenting cells (APC), while CD1e is never found at the cell surface but is involved in the processing of antigens inside the APCs.[4]

Interest in these lipidic antigens has been boosted when it was demonstrated that an uncharacterized mixture of lipids from *M. tuberculosis*, could be used as a vaccine preparation and improved pulmonary pathology in a guinea pig model of infection by *M. tuberculosis*.[5] More recently, it had been established that GMM can induce a memory T cell response, just as protein antigens do, in an experimental vaccination of cattle.[6]

The structure of GMM is simple; it is a free glucose esterified on the 6-position by the mycolic acids (MAs). MAs are a family of α-branched-β-hydroxylated long-chain carboxylic acids (length up to 90 carbons), which are ubiquitous in the mycobacteria genus. However, depending on the species, the meromycolic chain carries variable functionalities in the proximal and distal positions, which are characteristic of the species.[7] The structure of mycolic acids from *M. tuberculosis* is shown in Figure 1.

Although GMM had been isolated and characterized from mycobacterial cultures[1] only tiny amounts can be obtained from natural sources after long and tedious purifications. Mentions of synthetic preparation of GMM are scattered in the literature[8,9] but neither protocols nor yields are reported. These synthesis involve acidic hydrolysis of trehalose dimycolate (TDM, 6,6′-*O*-dimycoloyl-α,α-trehalose) by 2 M trifluoroacetic acid for 2 h at 121 °C,[8] or coupling of a 3-*O*-TBDMS-protected C_{32} synthetic mycolic acid on a 1,2,3,4-tetra-*O*-TBDMS-glucose derivative,[9] a method derived from a reported synthesis of TDM,[10] which is an obvious model for the preparation of GMM. Two strategies had been described for the preparation of TDM. The first one uses the S_N2 displacement of bromide[11] or tosyl groups[12,13] on the 6 and 6′ positions of a protected trehalose derivative by mycolic acid salts. The second approach[10,14] involves the direct coupling on the 6,6′ hydroxyl groups of a suitably protected α,α-trehalose. In these cases, the 3-hydroxyl group of the mycolic acid must be protected and coupling reactions had been described under Mitsunobu conditions[14] or with DCC.[10] Both methods gave good yields of product and the latter one had been recently used for the preparation of TDM containing chiral synthetic mycolic acids.[15]

As we needed milligram quantities of GMM in the course of an ongoing program on lipidic mycobacterial antigens, we tried to prepare GMM according to the S_N2 method used by Toubiana for the synthesis of TDM[11] and mentioned by Moody[8] for the preparation of GMM. This method avoids the need to protect the hydroxyl group of the mycolic acids, which is mandatory in any DCC-type coupling.

Starting from 1,2,3,4,6-penta-*O*-trimethylsilyl-D-glucopyranose (**2**) (Fig. 2),[16] selective deprotection of the 6-*O*-silyl group with potassium carbonate was attempted but no 1,2,3,4-tetra-*O*-

* Tel.: +33 5 61 17 54 85; fax: +33 5 61 17 59 00.
E-mail address: jacques.prandi@ipbs.fr

0008-6215/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2011.11.007

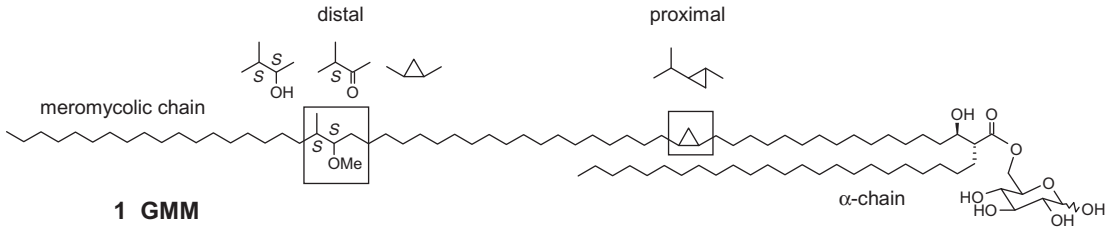

Figure 1.

trimethylsilyl-glucose derivative (**3**) could be isolated because of the very fast removal of the anomeric trimethyl silyl group under the reaction conditions. We then tried to use the more resistant *tert*-butyldimethylsilyl ethers instead of the trimethylsilyl ethers and prepared the per-*tert*-butyldimethylsilylated D-glucose derivative **4**, using an excess of *tert*-butyldimethylsilyl trifluoromethane sulfonate and pyridine in dichloromethane.[17] Selective deprotection of the 6-*O*-silyl group of **4** could be achieved, but isolated yields of 1,2,3,4-tetra-*O*-TBDMS-glucose **5** were poor (<20%); once again, considerable concomitant deprotection of the anomeric silyl group was observed during the reaction. Despite this disappointing yield, alcohol **5** was tosylated to give **6** in good yield (81%) and submitted to the S_N2 displacement by MAs. After four days, the reaction of **6** with the cesium salts of MAs in HMPA at 80 °C[12] afforded **7**, which was isolated in 37% yield. The formation of polar unidentified products was also observed during the reaction. Final deprotection of **7** to GMM was also troublesome. Acidic hydrolysis (HCl in CH_2Cl_2–MeOH) or fluoride deprotection (NBu_4F, THF–$CHCl_3$) could not be brought to completion. Even if some minor amounts of GMM could be recovered, this procedure was far from satisfactory.

We looked for an alternative to the silyl group in this synthesis and chose to start from benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside **8** (Fig. 2) as the anomeric benzyl group was expected to be much more stable than any anomeric silyl group. Glycoside. **8** can be easily obtained in three steps and a >60% yield from D-glucose.[18]

Tosylation of **8** proceeded smoothly and gave the expected tosylate **9** in good yield (82%). Tosylate **9** was then cleanly displaced by the cesium salt of MAs in a THF–DMF mixture following a procedure used for the preparation of mycoloyl-arabinan fragments.[19] After two and a half days of reaction at 70 °C, a rewarding 58% yield (based on a mean molecular weight of 1240 g mol^{-1} for the engaged MAs) of protected GMM **10** was obtained. Final deprotection of the benzyl groups of **10** was done by catalytic hydrogenation with carbon-supported palladium hydroxide as catalyst (H_2, $Pd(OH)_2$–C, petroleum ether–ethyl acetate–methanol mixture).

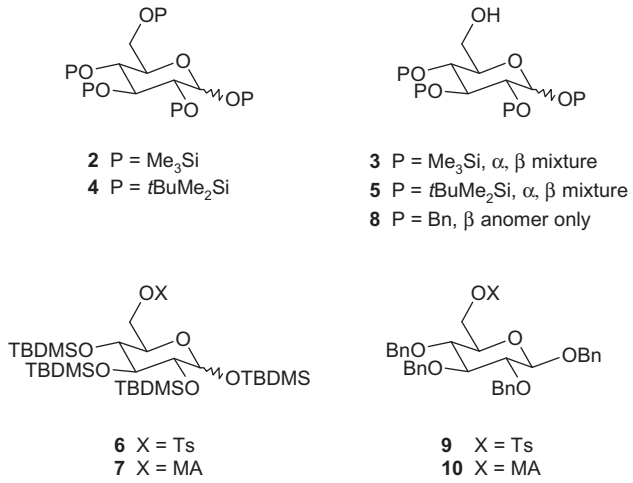

Figure 2.

The reaction was complete in 24 h and gave a high yield of GMM after chromatographic purification (91%).

To establish the identity of synthetic GMM **1** with the product isolated from natural sources, we first checked that no reductive opening of the cyclopropane rings had occurred during the hydrogenolysis of the benzyl ethers. Although the catalytic conditions we used are known to leave cyclopropane rings intact[20] we quantified by ^{1}H NMR spectroscopy (400 MHz) the signals for the *cis*-cyclopropane ring (found around −0.4 ppm) using the signal corresponding to the C*H*OMe group of the meromycolic chain as an internal standard, this signal is found isolated around 2.9 ppm.[21] An identical ratio was found (3.87 for GMM **1** and 3.80 for Benzyl GMM **10**, see Supplementary data) showing that no detectable cyclopropane cleavage occurred during the hydrogenolysis reaction.

The MALDI-TOF spectrum for synthetic GMM (Fig. 3) was then recorded and gave a complex pattern, as expected from the heterogeneity of the mycolic acid mixture. Observed peak values for synthetic GMM were compared to the reference masses recorded for GMM in the Colorado State University data base[22] and with the known composition of the starting mycolic acid mixture.[23]

Glucosyl esters of α-mycolic acids with 76, 78, 80, and 82 carbon atoms gave peaks at m/z = 1294.3, 1322.3, 1350.3, and 1378.4 and corresponding peaks were found at m/z = 1146.1, 1174.1, 1202.2, and 1230.2 in the mycolic acid methyl esters (MAMes) spectrum.[23] The major species, at m/z = 1466.5 corresponds to a glucose esterified with a methoxy-mycolic acid with a total number of 87 carbon atoms, this peak nicely correlates to a peak at m/z = 1318.3 found in the MAMes spectrum.[23] Peaks for GMM substituted by other members of this methoxy-mycolic acids family were found at m/z = 1410.4 (C_{83}), 1438.4 (C_{85}), 1494.4 (C_{89}) and 1522.5 (C_{91}), in full agreement with observed peaks at m/z = 1262.2, 1290.3, 1346.3 and 1374.3 in the MAMes spectrum. Finally, derivatives of keto-mycolic acids, the third family of mycolic acids produced by *M. tuberculosis* are visible at m/z = 1380.4, 1408.4, and 1436.4 (80, 82, and 84 carbon atoms, respectively, corresponding peaks for MAMes at m/z = 1232.2, 1260.2, and 1288.3). The observed data for synthetic GMM are in full agreement with expected values and clearly showed that the heterogeneity of synthetic GMM is fully representative of the natural mixture of mycolic acids, which is produced by *M. tuberculosis* H37Rv strain.

In conclusion, GMM from *M. tuberculosis* has been prepared from D-glucose by a six-step sequence (overall yield of 26%) and an efficient incorporation of the engaged mycolic acids in the final product. GMM from any other mycobacterial species might also be prepared using this sequence. This procedure makes this important mycobacterial antigen readily available by synthesis and opens the way for further biological studies.

1. Experimental

1.1. General methods

All reactions were run in anhydrous solvents under a dry argon atmosphere using commercial reagents used as received. THF was

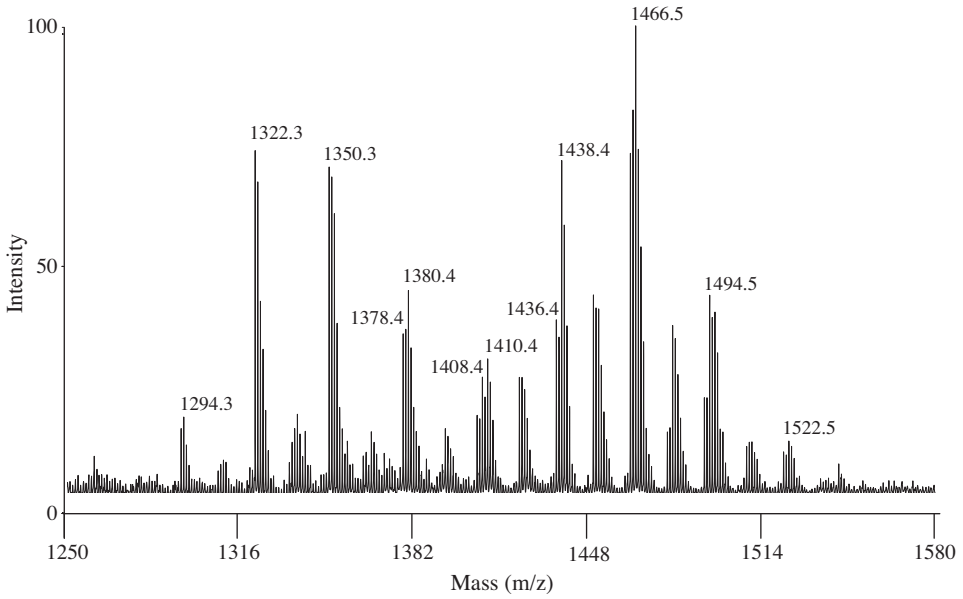

Figure 3. MALDI-TOF mass spectrum in positive ion mode of synthetic GMM.

dried by distillation from sodium benzophenone, dichloromethane from phosphorus pentoxide, and DMF was distilled from BaO at reduced pressure and kept on activated 4 Å molecular sieves. Mycolic acids were obtained from *M. tuberculosis* H37Rv strain, by saponification of purified arabinogalactan complex. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV400 and DPX300 spectrometers working at 400 MHz (100.6 MHz for ^{13}C) and 300 MHz (75.5 MHz for ^{13}C) and chemical shifts (δ) are expressed in part per million (ppm) from tetramethylsilane. MALDI-TOF spectra were obtained on a 4700 Proteomics Analyser from Applied Biosystems, using dihydroxybenzoic acid as the matrix and a Nd:YAG laser pulse at 355 nm. Chromatographic purifications (flash chromatography) were done using Silica 60 (35–70 nm) from SDS (Peypin France). Elemental analysis were obtained from the analytical service of the Laboratoire de Chimie de Coordination (Toulouse, France).

1.2. Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-tosyl-β-D-glucopyranoside (9)

Glycoside **8** (294 mg, 0.54 mmol) was dissolved in dichloromethane (3 mL) and the solution was cooled to 0 °C. Pyridine (0.7 mL), DMAP (30 mg, 0.24 mmol) and tosyl chloride (425 mg, 2.2 mmol) were sequentially added at 0 °C. The mixture was warmed to room temperature and was stirred overnight. The mixture was diluted with EtOAc and hydrolyzed with 3 M HCl (3 mL). The organic phase was decanted and washed twice with saturated $NaHCO_3$ and brine. Chromatography of the residue (95:5→80:20 petroleum ether–EtOAc) gave the title compound as a white solid (319 mg, 82%). $[\alpha]_D^{25}$ −3.3 (*c* 1.46, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 2.39 (s, 3H, C*H*$_3$-tosyl), 3.37–3.51 (m, 3H, H-2, H-4, H-5), 3.60 (m, 1H, H-3), 4.14 (dd, $^{2}J_{H,H}$ = 10.5 Hz, $^{3}J_{H,H}$ = 4.5 Hz, 1H, H-6), 4.26 (dd, $^{2}J_{H,H}$ = 10.5 Hz, $^{3}J_{H,H}$ = 1.5 Hz, 1H, H-6), 7.15–7.20 and 7.76–7.80 (2 m, 2 × 2H, H-Ar tosyl). ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ 21.6 (*C*H$_3$-tosyl), 68.7 (C-6), 71.0, 74.9, 75.0, 75.7 (*C*H$_2$-Ph), 72.7 (C-5), 76.9 (C-4), 82.0 (C-2), 84.4 (C-3), 102.1 (C-1), 127.7, 127.7, 127.8, 127.9, 128.0, 128.0, 128.1, 128.1, 128.3, 128.4, 128.4, 128.5, 129.8, 137.1, 137.6, 138.2, 138.3, 144.8 (C-Ar). Anal. Calcd for $C_{41}H_{42}O_8S$ (694.8 g mol^{-1}): C, 70.87; H, 6.09. Found: C, 71.14, H, 6.19.

1.3. 6-*O*-Mycoloyl-D-glucopyranose (GMM) (1)

Mycolic acids from *M. tuberculosis* H37Rv (39 mg, 0.03 mmol) were dissolved in a mixture of THF and DMF (THF–DMF 5:1, 2.4 mL). To this solution were added the tosylate **9** (34.2 mg, 0.054 mmol) and dry cesium hydrogen carbonate (64 mg, 0.33 mmol). The mixture was brought to 70 °C and left at this temperature for 2.5 days. TLC analysis (petroleum ether–EtOAc, 7:1) of the reaction mixture showed the starting tosylate **9** at R_f = 0.2, MAs as a single spot at R_f = 0.29 and **10** as (at least) three very close spots with R_f ranging from 0.44 to 0.48. The reaction mixture was then cooled to room temperature, diluted with EtOAc, and the organic phase was washed twice with water. Chromatography of the residue (7:1 petroleum ether–EtOAc) and pooling the highly-moving spots (R_f from 0.44 to 0.48) gave benzyl 2,3,4-tri-*O*-benzyl-6-*O*-mycoloyl-β-D-glucopyranoside (**10**) as a white amorphous solid (31.8 mg, 58%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ −0.41, 0.47 and 0.57 (3 m, *cis*-cyclopropane, myco.), 0.78 (d, $^{3}J_{H,H}$ = 7.0 Hz, CH–C*H*$_3$ myco.), 0.81 (t, $^{3}J_{H,H}$ = 7.0 Hz, CH$_2$–C*H*$_3$ myco.), 1.11–1.34 (m, CH$_2$ myco.), 2.30–2.45 (m, C*H*–CO myco.), 2.88 (m, C*H*-OMe, myco.), 3.26 (s, OC*H*$_3$ myco.), 3.39–3.49 (m, H-2, H-4, H-5), 3.59 (m, H-3, C*H*–OH myco.), 4.14 (dd, $^{2}J_{H,H}$ = 11.5 Hz, $^{3}J_{H,H}$ = 4.5 Hz, H-6), 4.45 (d, $^{3}J_{H,H}$ = 7.5 Hz, H-1), 4.46 (br d, $^{2}J_{H,H}$ = 11.5 Hz, H-6), 4.50–4.89 (8d, C*H*$_2$-Ph). ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 °C): δ 62.8 (C-6), 71.1, 74.9, 75.1, 75.7 (*C*H$_2$-Ph), 72.7 (C-4), 77.8 (C-2), 82.0 (C-5), 84.5 (C-3), 85.4, 102.3 (C-1), 175.2 (CO myco.).

Glycoside **10** (31.8 mg, 0.019 mmol) was dissolved in a mixture of petroleum ether, EtOAc and CH_3OH (2 mL of a 5:4:1 v/v/v mixture) and treated under a hydrogen atmosphere with a catalytic amount of 20% palladium hydroxide on charcoal. The mixture was stirred for one day at room temperature and centrifuged (10 min, 1000*g*). The clear supernatant was removed and the black solid residue was taken up in $CHCl_3$–CH_3OH, shaken and centrifuged as above two more times. After pooling the organic phase and evaporation of the solvents, the residue was filtered on silica (CH_2Cl_2–CH_3OH 6:1) to give GMM **1** (α,β mixture) as a white amorphous solid. (24.4 mg, 91%). ^{1}H NMR (400 MHz, 4:1 $CDCl_3$–CD_3OD, 25 °C): δ −0.41 (m, $^{3}J_{H,H}$ = 5.0 Hz, $^{3}J_{H,H}$ = 4.0 Hz, *cis*-cyclopropane, myco.), 0.48 (ddd, $^{3}J_{H,H}$ = 9.0 Hz, $^{3}J_{H,H}$ = 7.5 Hz, $^{3}J_{H,H}$ = 4.0 Hz,

cis-cyclopropane, myco.), 0.57 (m, *cis*-cyclopropane, myco.), 0.78 and 0.82 (2d, $^{3}J_{H,H}$ = 7.5 Hz, CH–C*H*$_3$ myco.), 0.80 (br t, $^{3}J_{H,H}$ = 7.5 Hz, CH$_2$–C*H*$_3$ myco.), 1.15–1.34 (m, C*H*$_2$ myco.), 2.35 (m, C*H*–COO and C*H*–CO myco.), 2.92 (m, C*H*–COCH$_3$ myco.), 3.12 (dd, $^{3}J_{H,H}$ = 9.0 Hz, $^{3}J_{H,H}$ = 8.0 Hz, H-2β), 3.22–3.37 (m, H-2α, H-3α,β, H-4β), 3.27 (s, –C–O–C*H*$_3$ myco.), 3.43 (ddd, $^{3}J_{H,H}$ = 9.5 Hz, $^{3}J_{H,H}$ = 6.0 Hz, $^{3}J_{H,H}$ = 2.5 Hz, H-5β), 3.58 (m, C*H*–OH myco.), 3.60 (dd, $^{3}J_{H,H}$ = 9.5 Hz, $^{3}J_{H,H}$ = 9.5 Hz, H-4α), 3.91 (ddd, $^{3}J_{H,H}$ = 9.5 Hz, $^{3}J_{H,H}$ = 6.5 Hz, $^{3}J_{H,H}$ = 2.5 Hz, H-5α), 4.19 (2 dd, H-6α,β), 4.38 (2 dd, H-6α,β), 4.42 (d, $^{3}J_{H,H}$ = 8.0 Hz, H-1β), 5.07 (d, $^{3}J_{H,H}$ = 3.5 Hz, H-1α). ^{13}C NMR (100.6 MHz, 4:1 $CDCl_3$–CD_3OD, 25 °C): δ 10.6, 13.7, 14.5, 15.5, 22.4, 22.4, 15.2, 25.2, 25.8, 27.2, 27.2, 28.5, 28.9, 29.1, 29.1, 29.2, 29.2, 29.3, 29.4, 29.4, 29.6, 29.7, 30.0, 30.3, 31.7, 31.7, 32.2, 34.7, 35.1, 52.5, 52.6, 57.4, 63.4 (C-6), 69.1, 70.2, 70.3, 72.0, 72.3, 73.4, 73.6, 85.5, 92.2 (C-1α), 96.5 (C-1β), 175.1 (CO myco.). MALDI-TOF (positive mode) see Figure 3.

Acknowledgements

We thank Sophie Tannière and Baptiste Cottin for technical assistance, and Luis-Fernando Garcia-Alles for a generous gift of mycolic acids from *M. tuberculosis* H37Rv strain.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.11.007.

References

1. Brennan, P. J.; Lehane, D. P.; Thomas, D. W. *Eur. J. Biochem.* **1970**, *13*, 117–123.
2. Lawton, A. P.; Kronenberg, M. *Immunol. Cell Biol.* **2004**, *82*, 295–306.
3. Zajonc, D. M.; Kronenberg, M. *Curr. Opin. Struct. Biol.* **2007**, *17*, 521–529.
4. de la Salle, H.; Marriotti, S.; Angenieux, C.; Gilleron, M.; Garcia-Allès, L.-F.; Malm, D.; Berg, T.; Paoletti, S.; Maître, B.; Mourey, L.; Salamero, J.; Cazenave, J. P.; Hanau, D.; Mori, L.; Puzo, G.; De Libero, G. *Science* **2005**, *310*, 1321–1324.
5. Dascher, C. C.; Hiromatsu, K.; Xiong, X.; Morehouse, C.; Watts, G.; Liu, G.; McMurray, D. N.; LeClair, K. P.; Porcelli, S. A.; Brenner, M. B. *Int. Immunol.* **2003**, *15*, 915–925.
6. Nguyen, T. K. A.; Koets, A. P.; Santema, W. J.; van Eden, W.; Rutten, V. P. M. G.; Van Rhijn, I. *Vaccine* **2009**, *27*, 4818–4825.
7. Marrakchi, H.; Bardou, F.; Lanéelle, M.-A.; Daffé, M. In *The Mycobacterial Cell Envelope*; Daffé, M., Reyrat, J.-M., Eds.; ASM Press: Washington, DC, 2008; pp 41–62.
8. Moody, D. B.; Reinhold, B. B.; Guy, M. R.; Beckman, E. M.; Frederique, D. E.; Furlong, S. T.; Ye, S.; Reinhold, V. N.; Sieling, P. A.; Modlin, R. L.; Besra, G. S.; Porcelli, S. A. *Science* **1997**, *278*, 283–286.
9. Moody, D. B.; Guy, M. R.; Grant, E.; Cheng, T.-Y.; Brenner, M. B.; Besra, G. S.; Porcelli, S. A. *J. Exp. Med.* **2000**, *192*, 965–976.
10. Datta, A. K.; Takayama, K.; Nashed, M. A.; Anderson, L. *Carbohydr. Res.* **1991**, *218*, 95–109.
11. Toubiana, R.; Das, B. C.; Defaye, J.; Mompon, B.; Toubiana, M.-J. *Carbohydr. Res.* **1975**, *44*, 308–312.
12. Liav, A.; Goren, M. B. *Carbohydr. Res.* **1986**, *155*, 229–235.
13. Azuma, I.; Sakurai, T.; Ishida, H.; Kitajima, T.; Yamazaki, M. *Carbohydr. Res.* **1991**, *212*, 47–53.
14. Jenkins, I. D.; Goren, M. B. *Chem. Phys. Lipids* **1986**, *41*, 225–235.
15. Al Dulayymi, J. a. R.; Baird, M. S.; Maza-Iglesias, M.; Vander Bekken, S.; Grooten, J. *Tetrahedron Lett.* **2009**, *50*, 3702–3705.
16. Birkofer, L.; Ritter, A.; Bentz, F. *Chem. Ber.* **1964**, *97*, 2196–2201.
17. Corey, E. J.; Cho, H.; Ruecker, C.; Hua, D. H. *Tetrahedron Lett.* **1981**, *22*, 3455–3458.
18. Lu, W.; Navidpour, L.; Taylor, S. D. *Carbohydr. Res.* **2005**, *340*, 1213–1271.
19. Ishiwata, A.; Akao, H.; Ito, Y.; Sunagawa, M.; Kusunose, N.; Kashiwazaki, Y. *Bioorg. Med. Chem.* **2006**, *14*, 3049–3061.
20. Nicolaou, K. C.; Sasmal, P. K.; Rassias, G.; Reddy, M. V.; Altmann, K.-H.; Wartmann, M.; O'Brate, A.; Giannakakou, P. *Angew. Chem., Int. Ed.* **2003**, *42*, 3515–3520.
21. Uenishi, Y.; Fujita, Y.; Kusunose, N.; Yano, I.; Sunagawa, M. *J. Microbiol. Method* **2008**, *72*, 149–156.
22. http://mrl.colostate.edu/mtb_db/pages/mld.aspx.
23. Laval, F.; Lanéelle, M.-A.; Déon, C.; Monsarrat, B.; Daffé, M. *Anal. Chem.* **2001**, *73*, 4537–4544.