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Synthesis, Structural Elucidation, And Biochemical Analysis of Immunoactive Glucuronosyl Diacylglycerides of Mycobacteria and Corynebacteria

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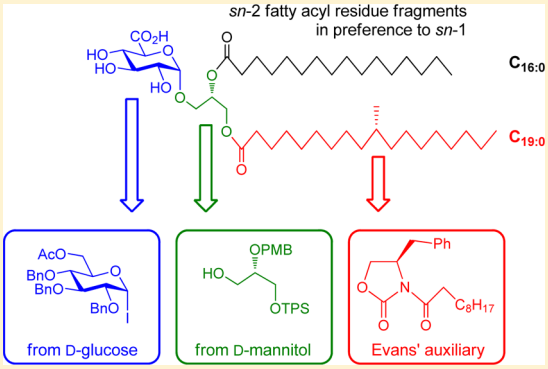
Supporting Information

ABSTRACT: Glucuronosyl diacylglycerides ($GlcAGroAc_2$) are functionally important glycolipids and membrane anchors for cell wall lipoglycans in the Corynebacteria. Here we describe the complete synthesis of distinct acyl-isoforms of $GlcAGroAc_2$ bearing both acylation patterns of (*R*)-tuberculostearic acid ($C_{19:0}$) and palmitic acid ($C_{16:0}$) and their mass spectral characterization. Collision-induced fragmentation mass spectrometry identified characteristic fragment ions that were used to develop "rules" allowing the assignment of the acylation pattern as $C_{19:0}$ (*sn*-1), $C_{16:0}$ (*sn*-2) in the natural product from *Mycobacterium smegmatis*, and the structural assignment of related $C_{18:1}$ (*sn*-1), $C_{16:0}$ (*sn*-2) $GlcAGroAc_2$ glycolipids from *M. smegmatis* and *Corynebacterium glutamicum*. A synthetic hydrophobic octyl glucuronoside was used to characterize the GDP-mannose-dependent mannosyltransferase MgtA from *C. glutamicum* that extends $GlcAGroAc_2$. This enzyme is an Mg^{2+}/Mn^{2+}-dependent metalloenzyme that undergoes dramatic activation upon reduction with dithiothreitol.



INTRODUCTION

The suborder Corynebacterineae includes a range of agriculturally, medically, and biotechnologically important bacteria. *Corynebacterium glutamicum* is used extensively in the biotechnology industry for the production of the amino acids L-glutamate and L-lysine and is now a focus for intensive metabolic engineering.[1] Many mycobacteria are responsible for human and animal diseases, especially *Mycobacterium tuberculosis*, the causative agent of tuberculosis. The fast-growing bacterium *Mycobacterium smegmatis* is generally considered to be nonpathogenic[2,3] and provides a readily handled fast-growing model for the basic biochemistry and genetics of *M. tuberculosis*.[4] All mycobacteria and corynebacteria contain an atypical cell wall in which the peptidoglycan layer is overlaid with layers of covalently linked arabinogalactan and long-chain mycolic acids. The mycolic acids effectively form the inner leaflet of an asymmetric bilayer with noncovalently associated lipids, glycolipids, and glycophospholipids forming the outer layer (Figure 1a).[5] These noncovalently associated lipids contribute to the bulk membrane lipids and can also act as membrane anchors for abundant cell wall lipoglycans that influence host immune interactions.[5]

The structure, biosynthesis, and function of several corynebacterium/mycobacterium glycolipids have been intensively studied, including the phosphatidylinositol mannosides (PIMs), lipomannan (LM), and lipoarabinomannan (LAM).[6−9] Recently, there has been increased interest in a range of glycosylglycerolipids that contain glucuronic acid linked to diacylglycerol (Figure 1b).[10,11] These immunoreactive α-glucuronosyl diacylglycerols were initially isolated from *M. smegmatis* MNC strain 13 by Brennan and colleagues[12] and recently shown to function as alternative membrane anchors for LM in *Corynebacterium glutamicum*.[13−15] Although depicted as nominal structures **1a** and **2a**, respectively, the precise arrangement of the ester-linked (*R*)-tuberculostearic acid **4** or oleic acid and palmitic acid on the glycerol backbone was not determined, and structures **1b** and **2b** are viable alternatives (Figure 2a,b). We have recently shown that structure **1a**, and only this natural arrangement, yielded a CD1d-presented antigen that could activate murine-derived NKT cells through V_α10-J_α50 T cell receptors,[16] highlighting the importance of acyl regiochemistry. Compound **1a** produced a Th2-biased response, characterized by the release of immunosuppressive cytokines, suggesting that mycobacterial-derived α-glucuronosyl diacylglycerols may have roles in modulating host−immune responses.[16] Besra and coworkers have also isolated the structurally related α-glucuronosyl

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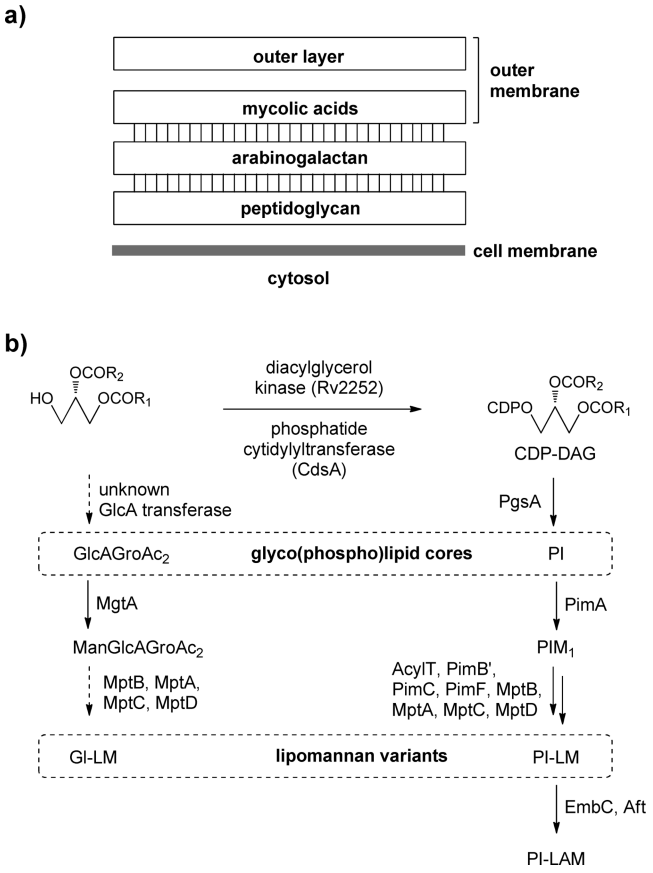

Figure 1. (a) Schematic of major structural elements of the mycobacterial and corynebacterial cell wall. (b) Outline of involvement of $GlcAGroAc_2$ in the biosynthesis of *C. glutamicum* Gl-LM and comparison with PI-LM/LAM biosynthesis in Corynebacterinae.

diacylglycerols (termed Gl-A) bearing oleic acid and palmitic acid from *C. glutamicum*. Gl-A was depicted as having the nominal structure **2b** (viz. the isomer of **2a**), although evidence for this acylation pattern was not provided.[17] In *C. glutamicum* it was reported that nominal **2b** undergoes mannosylation by the action of the glycosyltransferase MgtA (NCgl0452) to form nominal **3b** (termed Gl-X) (Figure 2a); however, the acylation pattern of this compound will be defined by its biosynthetic precursor Gl-A, and the structure **3a** represents a viable alternative.[17] Despite evidence that the *M. tuberculosis* ortholog (Rv0557) can complement a *C. glutamicum* deletion mutant of *MgtA*[17,18] and the existence of an *M. smegmatis MgtA* ortholog, there is no evidence that *M. smegmatis* can elongate its endogenous $GlcAGroAc_2$ species nor of the presence of any $GlcAGroAc_2$ species in *M. tuberculosis*. Indeed, knockout studies of *MgtA* in *M. tuberculosis* H37Rv failed to abolish production of any cell wall mannosylated glycans, although the rate of bacterial-induced cell death was increased.[19]

Methods for the synthesis of glucuronosyl diacylglycerides have not yet been reported. In this work, we have developed a synthetic approach to the putative *M. smegmatis* glycolipid structures **1a** and **1b** containing (*R*)-tuberculostearic acid at the *sn*-1 or *sn*-2 position. These authentic reference materials are used to establish rules governing the fragmentation of glucuronosyl diacylglycerides using negative-mode collision-induced fragmentation electrospray ionization mass spectrometry, allowing the unambiguous assignment of the natural material as possessing structure **1a**. We extend our analysis to the related oleic acid containing glycolipids from *M. smegmatis* and *C. glutamicum*, demonstrating that both possess structure **2a**. Next, we use these reference materials to guide a search for corresponding structures in *M. smegmatis* mc^2155 and *M. tuberculosis* H37Rv. Finally, we develop simplified structural analogues that can act as substrates for *C. glutamicum* MgtA, enabling its biochemical characterization.

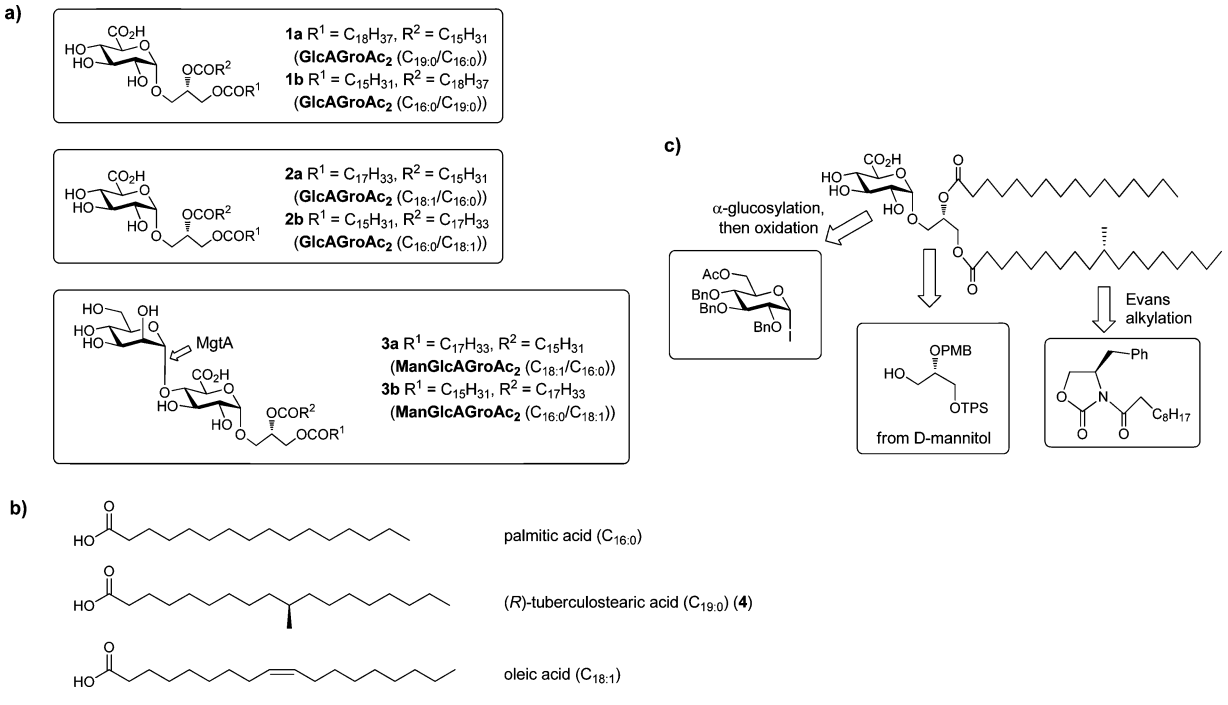

Figure 2. (a) Possible structures of glucuronosyl diacylglycerides isolated from *C. glutamicum* and *M. smegmatis*. (b) Structures of fatty acids. (c) Retrosynthesis for $GlcAGroAc_2$ (**1a**).

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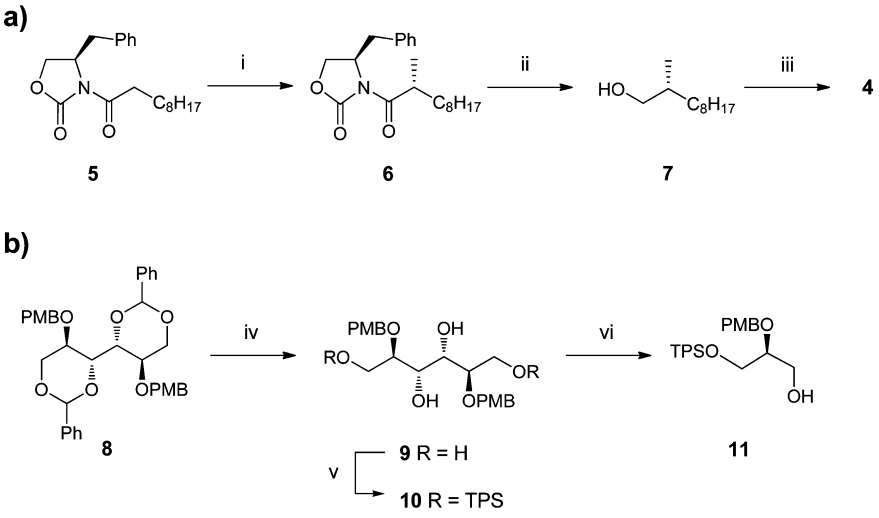
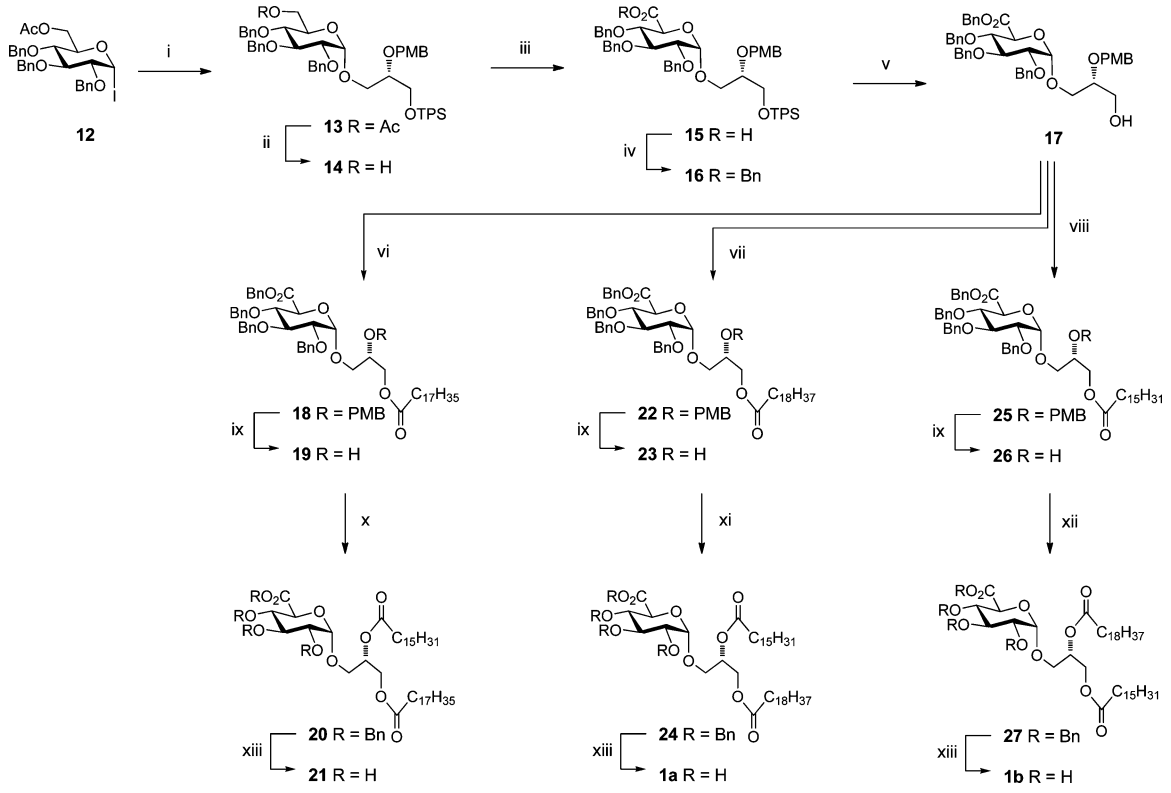
Scheme 1. (a) Synthesis of (*R*)-Tuberculostearic Acid and (b) Synthesis of Homochiral Glycerol Fragment 11[a]



[a]Reagents and conditions for part a: (i) NaHDMS, THF, MeI, 86%; (ii) $NaBH_4$, THF/H_2O, 93%; (iii) refs 22 and 23. Reagents and conditions for part b: (iv) aq HCl, hexane/MeOH, 39%; (v) TPSCl, imidazole, DMF (94%); (vi) (1) $NaIO_4$, THF/H_2O, (2) $NaBH_4$, $EtOH/H_2O$, 91%, two steps.

Scheme 2. Synthesis of $GlcAGroAc_2$ Isomers[a]



[a]Reagents/conditions (i) **11**, Bu_4NI, TTBP, CH_2Cl_2, 6 d, 63%; (ii) NaOMe, MeOH, CH_2Cl_2, 75%; (iii) cat. TEMPO, $PhI(OAc)_2$, CH_2Cl_2/H_2O, 87%; (iv) BnOH, HBTU, DIPEA, cat. DMAP, CH_2Cl_2, 84%; (v) HF·pyr, THF, 94%; (vi) stearic acid, COMU, DIPEA, cat. DMAP, DMF, 50° C, 24 h, 72%; (vii) (*R*)-tuberculostearic acid, COMU, DIPEA, cat. DMAP, DMF, 50 °C, 24 h, 63%; (viii) two portions of palmitic acid, COMU, DIPEA, cat. DMAP, DMF, 50 °C, 24 h, 87%; (ix) CAN, $MeCN/H_2O$; (x) palmitic acid, COMU, DIPEA, DMAP, DMF, rt, 30 h, 60% over two steps; (xi) palmitic acid, COMU, DIPEA, DMAP, DMF, rt, 2 d, 67% over 2 steps; (xii) two portions of (*R*)-tuberculostearic acid, COMU, DIPEA, DMAP, DMF, rt, 2 d, 75% over two steps; (xiii) H_2, $Pd(OH)_2$, MeOH/THF/AcOH; 60% for **21**, 85% for **1a**, 90% for **1b**.

RESULTS AND DISCUSSION

Total Synthesis of $GlcAGroAc_2$ ($C_{19:0}/C_{16:0}$) and $GlcAGroAc_2$ ($C_{16:0}/C_{19:0}$).[20] Our strategy toward the synthesis of compound **1a** and isomer **1b** is summarized in Figure 2c. Glycosylations using glucuronosyl donors are known to be more difficult than using glucosyl donors owing to the strongly electron-withdrawing nature of the carboxyl of C6. For this reason, we elected to use a glucosyl donor that could be oxidized at the 6-position of the sugar after forming the challenging 1,2-*cis* α-glycosidic linkage. Several syntheses of enantiopure (*R*)-

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tuberculostearic acid (**4**) have been reported that utilize resolution approaches,[21] chiral pool starting materials,[22−25] and catalytic enantioselective reactions.[26] The approach used here employs an Evans' chiral auxiliary to set the 10-(*R*)-methyl stereocenter[27] and then merges with the approaches of Seeberger[22] and Painter[23] to conclude the synthesis (Scheme 1a). Diastereoselective methylation of *N*-decanoyloxazolidinone (**5**)[28] via the chelated *Z*-enolate was achieved by deprotonation with NaHDMS followed by addition of iodomethane and yielded **6** as a single stereoisomer as determined by ^{1}H NMR spectroscopy, in 86% yield.[28] Reductive cleavage of the chiral auxiliary of **6** proceeded smoothly using $NaBH_4$ in THF affording the alcohol **7** in 93% yield with recovery of 76% of the chiral auxiliary. Also obtained was 3% of the *N*-acylphenylalaninol resulting from hydride attack at the endocyclic carbonyl, which was easily removed by chromatography. The optical rotation of **7** ($[\alpha]_D$ = +10.4) was in excellent agreement with that reported previously ($[\alpha]_D$ = +11.0).[22] The synthesis of (*R*)-tuberculostearic acid **4** was concluded following the route outlined by Seeberger[22] involving tosylation of **7**, Cu(I)-catalyzed sp^3-sp^3 cross-coupling with a THP-protected C8 fragment, and deprotection and finally oxidation according to the method of Painter.[23]

Given the need to install different acyl groups at the *sn*-1 and *sn*-2 positions of the glyceride following glycosylation, we elected to prepare an orthogonally protected glycerol fragment **11** bearing a *p*-methoxybenzyl group at *sn*-2 and a *tert*-butyldiphenylsilyl group at the *sn*-1 position. The dibenzylidene diether **8** was prepared in two steps from D-mannitol[29,30] and was debenzylidenated using dilute HCl in a mixture of hexane and MeOH providing the tetraol **9** in a moderate 39% yield (Scheme 1b). Regioselective silylation of the primary alcohols using TPSCl (TPS: *tert*-butyldiphenylsilyl) and imidazole in DMF afforded disilyl ether **10** in 94% yield. The vicinal diol was oxidatively cleaved by exposure to $NaIO_4$ in THF/H_2O, and the crude glyceraldehyde reduced with $NaBH_4$ in $EtOH/H_2O$ to afford the selectively protected *sn*-glycerol **11** in 91% yield over two steps.

Unlike 1,2-*trans* glycosidic linkages, which can be readily constructed by virtue of neighboring group participation, stereoselective formation of 1,2-*cis*-glycosidic linkages can be problematic. We chose to explore the glycosyl iodide method, owing to the outstanding α-stereoselectivities observed in glycosylations of a range of primary alcohols.[31−33] Attempted glycosylation of **11** with freshly prepared **12**[34] under promotion by Ph_3PO in CH_2Cl_2, according to Kobashi's protocol,[35] resulted in the formation of significant amounts of a 1,3-disilylated glycerol, arising from intermolecular silyl group transfer between molecules of the acceptor. Speculating that silyl transfer was catalyzed by the release of HI in the glycosylation reaction, the hindered base tris-*tert*-butylpyrimidine (TTBP)[36] was added, but resulted in a poorer yield. Changing from Ph_3PO to a more powerful promoter, Bu_4NI, in the presence of TTBP, allowed isolation of the α-glucoside **13** in 63% yield with no evidence of the formation of the β-anomer (Scheme 2).

With the glucoside in hand, our attention turned to oxidation of the sugar to the glucuronoside. Deprotection of glycoside **13** with NaOMe in $MeOH/CH_2Cl_2$ afforded the primary alcohol **14** in 91% yield (Scheme 2). Oxidation of **14** using TEMPO (2,2,6,6-tetramethylpiperidin-1-oxyl) and BAIB (bis(acetoxy)-iodobenzene)[37] in a biphasic solvent system consisting of CH_2Cl_2/H_2O gave the D-glucuronic acid **15** in 87% yield. Treatment of **15** with benzyl alcohol and HBTU in the presence of *N*-methylmorpholine (NMM) and DMAP in CH_2Cl_2 afforded the D-glucuronate **16** in 84% yield.

The closing stages of the synthesis required the selective installation of the acyl chains. We chose to first install the primary acyl group as 1,2-acyl migration favors the primary ester, and as the conditions for subsequent PMB removal were perceived to be milder than that required for TPS deprotection. Cleavage of the silyl ether of **16** using HF.pyridine in THF gave the alcohol **17** in 94% yield (Scheme 2). Our initial exploratory efforts targeted the $C_{18:0}/C_{16:0}$ isomer **21** as a model. However, initial attempts to esterify the primary alcohol of **17** with stearic acid and DIC or HBTU resulted in sluggish reactions with variable yields. Therefore we investigated the third generation uronium-type reagent COMU, based on Oxyma (ethyl 2-cyano-2-(hydroxyimino)acetate).[38−40] Superior reactivity has been ascribed to COMU over classical peptide coupling reagents owing to the exclusive formation of *O*-acyl Oxyma intermediates compared to the less reactive predominantly *N*-acyl species present using HOBt-based reagents such as HBTU.[38] Alcohol **17** was treated with stearic acid, COMU, DIPEA, and catalytic DMAP in DMF at 50 °C for 24 h. After purification with flash chromatography the monoacylated species **18** was isolated in 72% yield. The PMB group of **18** was cleaved under oxidative conditions using CAN in $MeCN/H_2O$. Attempts to purify the secondary alcohol **19** by silica gel chromatography led to acyl group migration; in practice, best results were obtained when the crude secondary alcohol was directly acylated with palmitic acid using COMU, DIPEA, and DMAP, affording the diacylglyceride **20** in a modest 60% yield over two steps. The benzyl ethers and benzyl ester of **20** were cleaved simultaneously using $H_2/Pd(OH)_2$, delivering **21** in 60% yield.

The synthesis of the two targets **1a** and **1b** was achieved from **17** using a similar approach (Scheme 2). Thus, esterification of the primary hydroxyl with (*R*)-tuberculostearic acid **4** (**17** → **22**), oxidative removal of the PMB group and esterification of the secondary hydroxyl with palmitic acid (**22** → **23** → **24**) and finally deprotection afforded $GlcAGroAc_2$ ($C_{19:0}/C_{16:0}$) **1a**. In the case of **1b**, some minor procedural improvements were implemented. Addition of COMU in two portions, omission of DIPEA, and use of excess DMAP gave improved yields. Thus, the alcohol **17** was treated with palmitic acid, COMU and DMAP in DMF for 24 h before addition of a second portion of palmitic acid, COMU, and DMAP. The reaction was stirred for a further 24 h and the resultant monoglyceride **25** was isolated in 87% yield. Removal of the PMB group followed by esterification of the liberated alcohol **26** with **4** under the modified COMU/DMAP reaction conditions gave the diglyceride **27** in 75% yield over two steps. Finally, hydrogenolysis of **27** ($H_2/Pd(OH)_2$) afforded $GlcAGroAc_2$ ($C_{16:0}/C_{19:0}$) **1b** in 90% yield.

Structural Elucidation of Natural $GlcAGroAc_2$ Compounds from *C. glutamicum* and *M. smegmatis*. With authentic synthetic targets **1a** and **1b** in hand, the collision-induced dissociation (CID) mass spectra of the $[M - H]^-$ of both compounds were compared with that of the natural product. ESI CID-MS/MS of the deprotonated isomers **1a** and **1b** (*m*/*z* 785) revealed the formation of fragment ions with identical *m*/*z* values but with clear differences in relative intensity (Figure 3a,b). For example, fragmentation of the $[M - H]^-$ of both isomers via loss of two different ketenes derived from the palmitic acyl and tuberculostearic acyl chains gives fragment ions at *m*/*z* 547 and 505, respectively. For **1a**, the ion at *m*/*z* 547 is formed in greater abundance than the ion at *m*/*z* 505 (ratio of 3:1), indicating that loss of the ketene from the palmitate at the

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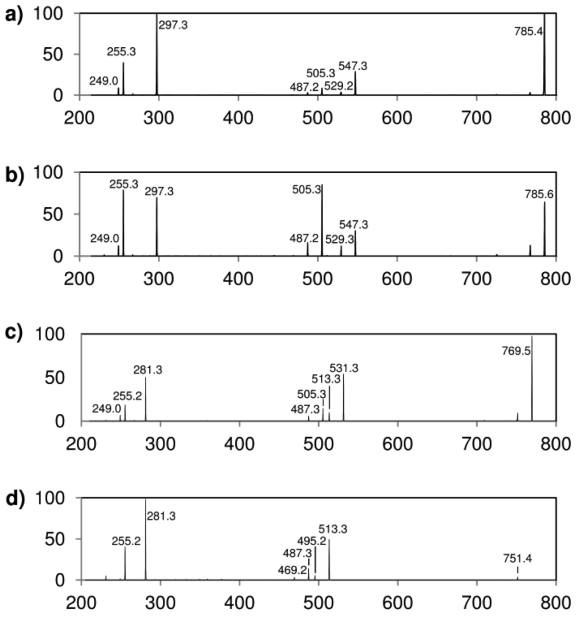

Figure 3. Multistage mass spectrometry (MS^n) experiments involving collision-induced dissociation of negative ions formed via ESI. (a) MS^2 spectrum of deprotonated **1a**; (b) MS^2 spectrum of deprotonated **1b**; (c) MS^2 spectrum for the ion at m/z = 769.8 from *C. glutamicum*, assigned as **2a**; (d) MS^3 spectrum of collision-induced dissociation of m/z 751.4, derived from the ion m/z = 931.6, assigned as **3a**. All experiments were performed on a Finnigan hybrid LTQ-FT-MS.

sn-2 position is the preferred fragmentation pathway. In contrast, the CID MS/MS spectrum of **1b** has a reciprocal intensity ratio with the peaks at m/z 547 and 505 in a 1:3 ratio, consistent with preferential loss of the ketene derived from the *sn*-2 tuberculostearate. This data confirms that cleavage of the fatty acyl residue at the *sn*-2 position yields a more intense product ion than for cleavage at the *sn*-1 position.

The structure of **1** isolated from *M. smegmatis* MNC strain 13 was originally assigned as **1a** ($C_{19:0}/C_{16:0}$). However, no definitive evidence was provided to verify this structure and this assignment appears to have been arbitrary.[12] In the original report of the FAB-MS/MS spectrum of deprotonated native **1**, distinct fragment ions at m/z 547 and 505 are observed, corresponding to loss of ketenes via cleavage of the palmitic acyl and tuberculostearic acyl groups respectively.[12] The former ion (m/z 547) is more abundant than the latter (m/z 505) (approx 3:1), identical to the fragment ratios observed with synthetic **1a** and clearly different from the ratios observed with **1b**. In fact, the MS^2 spectrum of **1a** (Figure 3a) appears identical to the native glycolipid from *M. smegmatis* in all respects including the relative intensity of the peaks at m/z 255 and 297 and m/z 487 and 539.[12] This therefore provides strong evidence that the natural glycolipid from *M. smegmatis* MNC strain 13 is **1a**, confirming the original assignment of Brennan and co-workers.[12] As well, structure **1a** is consistent with related $C_{19:0}/C_{16:0}$ phosphoglyceride-containing mycobacterial natural products, suggesting a common biogenesis of the diacylglyceride fragment.[23,26,41−43]

In the case of the deprotonated glucuronosyl diacylglycerides, the characteristic peaks observed at m/z 547 and 505 correspond to the secondary and primary alcohols **28** and **29**, respectively, and are generated through loss of ketenes derived from the acyl residues (Figure 4a). Similarly, cleavage of palmityl and tuberculostearyl groups can also occur through neutral loss as the carboxylic acids by elimination, to give alkene fragments **30** and **31** observed at m/z 529 and 487, respectively (Figure 4a). These ions are formed in a 1:1 ratio and unlike the phospholipids these peaks are not useful for assignment of fatty acid distribution. The carboxylate anions of the fatty acids at m/z 297 and 255 are also less suitable for assigning the fatty acid positions as they exhibit only minor intensity differences. Thus, the fragments ions generated from loss of the ketenes from the

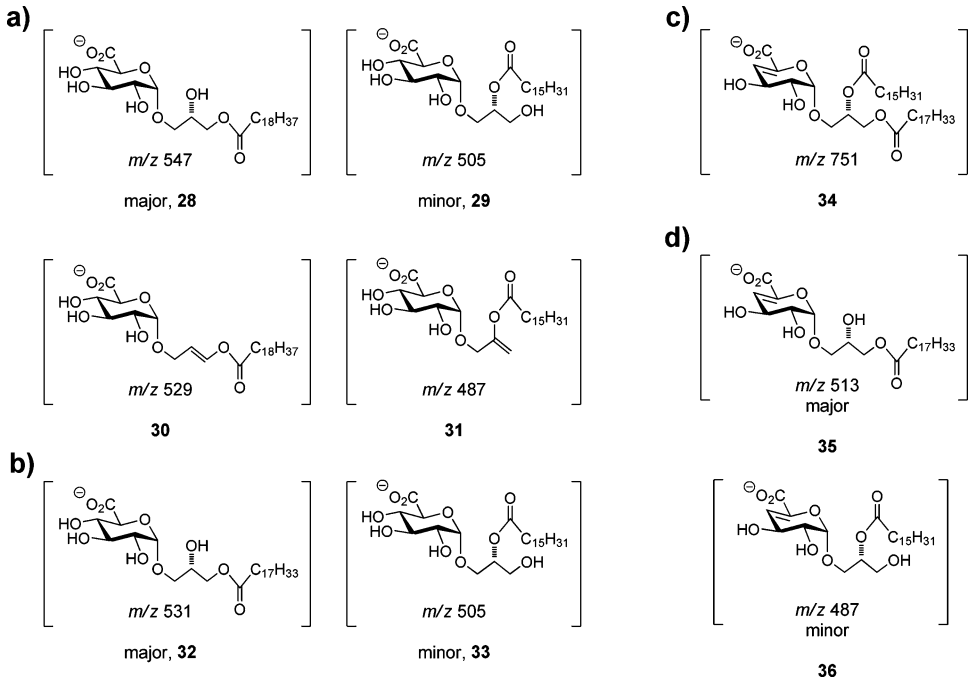

Figure 4. Proposed fragment ions formed in the CID spectra of deprotonated $GlcAGroAc_2$ and $ManGlcAGroAc_2$ shown in Figure 5. Fragment ions of $[M - H]^-$ from: (a) **1a**, (b) **2a**, (c) **3a**, (d) **34**.

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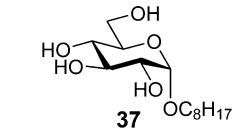
acyl residues are the most diagnostic for assignment of the acylation pattern of glucuronosyl diacylglycerides.

With the establishment of "rules" for the assignment of the *sn*-1 and *sn*-2 acyl chains based on the CID spectra of deprotonated **1a** and **1b**, we next applied these findings to the structures of other $GlcAGroAc_2$ compounds. Brennan and co-workers also reported FAB-MS/MS spectra for the fragmentation of $[M - H]^-$ of natural **2** isolated from *M. smegmatis* MNC strain 13, which revealed ion fragments at *m*/*z* 531 (assigned as **32**) and *m*/*z* 505 (assigned as **33**), corresponding to cleavage of palmitic and oleic acids, respectively (Figure 3b).[12] The former ion (*m*/*z* 531) is approximately 3-fold more abundant than the latter (*m*/*z* 505), which supports the oleyl group being attached at the *sn*-1 position and the palmityl group at *sn*-2, thus confirming the structure **2a** as the structure of the *M. smegmatis* natural product. Besra and co-workers did not report fragmentation spectra for **2** isolated from *C. glutamicum*.[17] We therefore studied a crude lipid extract from *C. glutamicum* by CID ESI-MS/MS containing **2**. Fragmentation of the parent ion (*m*/*z* 769) yielded ion fragments at *m*/*z* 531 and 505, corresponding to cleavage of palmitic and oleic acids, respectively (Figure 3c). The fact that the former ion (*m*/*z* 531) is approximately 3-fold more abundant than the latter (*m*/*z* 505) supports structure **2a**, which is the reverse acylation pattern of that originally assigned by Besra and co-workers[17] but identical to the material from *M. smegmatis*. We also studied **3**, which is present within the same *C. glutamicum* lipid extract. Fragmentation of the parent ion (*m*/*z* 931.6) produces one major ion (*m*/*z* 751.4) assigned as **34**, presumably derived from elimination of the mannopyranosyloxy group (Figure 4c). Isolation and subsequent fragmentation of this ion resulted in the MS^3 spectrum shown in Figure 3d, which displays the characteristic ion fragment ions at *m*/*z* 513 (assigned as **35**) and *m*/*z* 487 (assigned as **36**), corresponding to loss of palmitic and oleic acids, respectively (Figure 4d). The 3:1 ratio of **35**/**36** is consistent with the presence of oleic acid at the *sn*-1 position and structure **3a**, which is the same acylation pattern as established for its biosynthetic precursor **2a**, and is the reverse of that assigned by Besra and co-workers.[17]

We next screened total lipid extracts of log-phase *M. smegmatis* mc^2155 and *M. tuberculosis* H37Rv for the presence of **1** or **2** using Finnigan LTQ and Agilent LCMS triple-quadrupole and QToF instruments. Using the authentic reference materials **1a** and **1b**, a limit of detection was established defining the sensitivity limit of the analysis. Based on these results, we conclude that within these strains grown under the specified conditions, **1** and **2** are not present at levels of more than 100 ng per mg of dried lipid extract.

Synthesis of Hydrophobic Glycosides for Study of $ManGlcAGroAc_2$ biosynthesis. While the GDP-mannose-dependent mannosyltransferase MgtA responsible for the biosynthesis of $ManGlcAGroAc_2$ from $GlcAGroAc_2$ has been cloned,[17] relatively little is known about the biochemical properties of this enzyme. Preliminary experiments revealed that the synthetic glycolipids **1a**, **1b**, or **21** do not act as substrates for MgtA from a *C. glutamicum* cell free extract, possibly because they do not have the correct acylation pattern for *C. glutamicum*, or more probably owing to the poor physicochemical properties of these glycolipids which results in poor solubility in the cell free system, even with inclusion of Triton X-100. This disappointing result provided an incentive to investigate simplified substrate analogues **37**, **38**, **39** and **40**. We elected to prepare these compounds as octyl glycosides as such hydrophobic derivatives are readily purified by reverse-phase chromatography, and allow simple solvent partitioning of products from radiolabeled sugar donors in enzymic assays. As well, the detergent-like behavior of octyl glycosides ensures excellent solubility and distribution within aqueous and micellar phases within the cell free systems to be used for biochemical assays.



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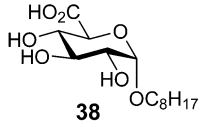
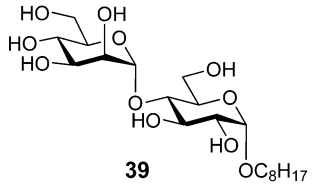
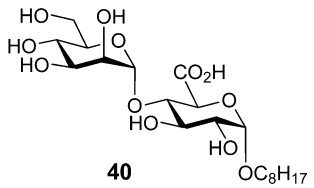


38



39



40

A key requirement for our approach was the ability to generate large amounts of octyl α-D-glucopyranoside **37**. Adasch and co-workers reported that treatment of D-glucose with 10 equiv of octanol in dioxane containing 7% H_2SO_4 gave **37** in 26% yield, with reduced equivalents of octanol leading to oligomerization of D-glucose.[44] However, their procedure involves a complex product isolation procedure requiring distillation of excess octanol and chromatography prior to recrystallization. In this work, we investigated the use of DMF as a cosolvent. After considerable optimization, we found that use of 12 equiv of octanol in DMF, AcCl as a source of HCl, and a reaction temperature of 60 °C for 6 h provided the cleanest conversion to octyl D-glucopyranoside as a mixture of anomers (Scheme 3a). As a general rule, the Krafft points of alkyl α-D-glucosides are higher than the corresponding β-anomers, allowing separation of the pure α-anomers by crystallization at temperatures between their Krafft points.[44] The mixture of anomers were readily separated by careful recrystallization above the Krafft point of the β-anomer, affording **37** in 30% yield. The chemoselective oxidation of **37** was achieved using TEMPO in aqueous $Ca(OCl)_2$ and KBr[45] affording **38** in 90% yield.

Treatment of **37** with benzaldehyde dimethyl acetal and catalytic *p*-toluenesulfonic acid afforded the benzylidene acetal **41** in 83% yield (Scheme 3b). The benzylidene acetal **41** was acetylated by treatment with acetic anhydride and pyridine to afford the acetylated benzylidene acetal **42** in 91% yield. Regioselective reduction of the acetylated benzylidene acetal **42** with trifluoroacetic acid and triethylsilane in dichloromethane[46] afforded the alcohol **43** in 83% yield. TMSOTf activation of a mixture of the trichloroacetimidate **44**[47] and alcohol **43** with 0.06 equiv of TMSOTf at −60 °C for 2 h and then at room temperature for another 2 h afforded the disaccharide **45** directly in 90% yield. Debenzylation of the disaccharide **45** was achieved by treatment with H_2 and catalytic $Pd(OH)_2$, affording the primary alcohol **46** in 84% yield. This primary alcohol was treated with NaOMe in MeOH to afford **39** in 72% yield. Alternatively, oxidation of the primary alcohol **46** with TEMPO and BAIB in CH_2Cl_2/water, afforded the carboxylic acid **47** in 90% yield. In this case the choice of an organic soluble co-oxidant was influenced by the lipophilic nature of the alcohol, which prevents its efficient partitioning into the aqueous phase, as is required for biphasic TEMPO oxidations using $Ca(OCl)_2$. Finally, the carboxylic acid **47** was treated with

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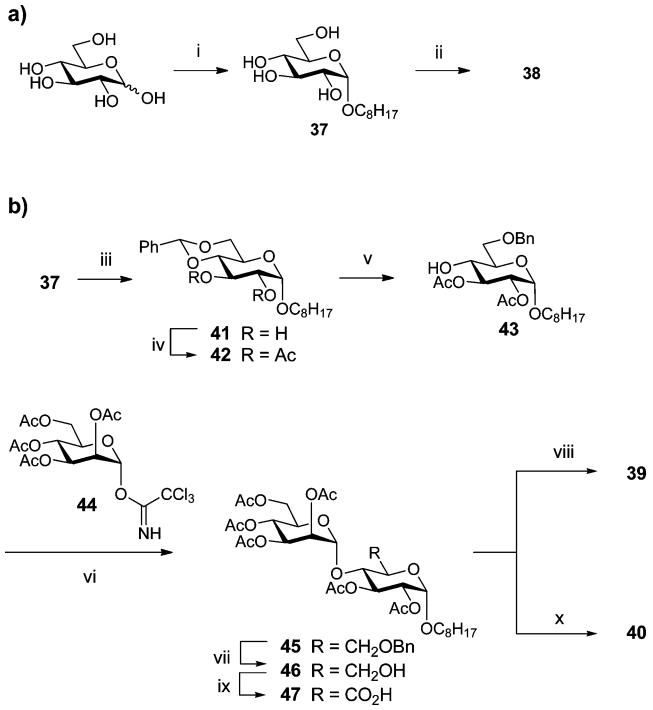
Scheme 3. (a) Synthesis of Octyl Monosaccharides 37 and 38 and (b) Synthesis of Octyl Disaccharides 39 and 40[a]



[a]Reagents and conditions for part a: (i) AcCl, octanol, DMF, 60 °C, 30%; (ii) TEMPO, KBr, $Ca(OCl)_2$, CH_2Cl_2, H_2O, 0 °C, 90%. Reagents and conditions for part b: (iii) $PhCH(OMe)_2$, *p*-toluenesulfonic acid, $CHCl_3$, 83%; (iv) Ac_2O, pyridine, 91%; (v) Et_3SiH, TFA, CH_2Cl_2, 83%; (vi) TMSOTf, CH_2Cl_2, 85%; (vii) H_2, $Pd(OH)_2$, EtOAc/EtOH, 84%; (viii) NaOMe, MeOH, 72%; (ix) TEMPO, BAIB, H_2O, CH_2Cl_2, 90%; (x) NaOMe, MeOH, 65%.

NaOMe in MeOH to cleave all the acetate esters, affording **40** in 65% yield.

Evaluation of Hydrophobic Glycosides in a *C. glutamicum* Cell-Free System: Characterization of MgtA. The synthetic analogues **37**–**40** were examined for their ability to act as substrates for *C. glutamicum* mannosyltransferases in a cell-free system obtained by nitrogen cavitation of log phase cells. The in vitro biosynthesis of PIMs and $ManGlcAGroAc_2$ can be reconstituted in this system following addition of GDP-[2-^{3}H]mannose as a mannosyl donor and the lipophilic products isolated by biphasic partitioning of the suspension into 1-butanol and separation of radiolabeled products by HPTLC. In the absence of an exogenous acceptor, the major products labeled are $ManGlcAGroAc_2$ (Gl-A) **3a**, polyprenylmannose phosphate (PPM), and acylphosphatidyl-*myo*-inositol dimannoside ($AcPIM_2$),[48] which are derived largely from existing endogenous pools of direct intermediates rather than de novo synthesis from PI (Figure 5a). Upon addition of synthetic glucuronoside **38**, a new lipid is synthesized. The formation of this product requires the 6-position to be oxidized as octyl glucoside **37** did not yield a new product. The new product formed from **38** coeluted with synthetic **40** and was sensitive to digestion by jack bean α-mannosidase but was resistant to snail β-mannosidase demonstrating the presence of an α-linked mannosyl group (Figure S1, Supporting Information). Negative-ion LC–MS analysis of the total lipid extract from the in vitro assay revealed a new species (m/z = 467.1), with a fragmentation pattern that matches synthetically prepared **40**

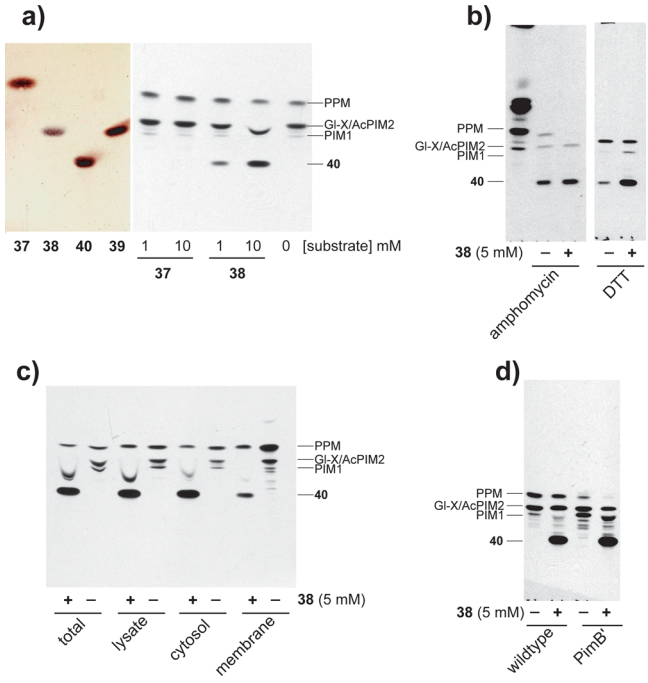


Figure 5. Biochemical characterization of *C. glutamicum* MgtA. (a) Left: Orcinol-stained HPTLC of synthetic **37**–**40**. Right: Fluorogram of HPTLC of octyl α-D-glucoside **37** (1, 10 mM) and octyl α-D-glucuronoside **38** (1, 10 mM) incubated with GDP-[^{3}H]mannose in centrifuged lysate of midlog *C. glutamicum*. (b) Left: *C. glutamicum* cytosolic fraction was incubated with amphomycin, prior to addition of GDP-[^{3}H]mannose. Right: Cytosolic fraction was oxidized using 50 mM $CuCl_2$, prior to addition of 10 mM DTT. (c) Midlog culture of *C. glutamicum* was lysed by sonication (total), and unbroken cells were removed by centrifugation (lysate). The lysate was fractioned to afford cytosolic and membrane fractions. Fractions were incubated in the presence of 5 mM octyl α-D-glucuronoside **38** and GDP-[^{3}H]mannose for 2 h, and the lipid was extracted and separated on HPTLC. (d) Wild-type and Δ*PimB′ C. glutamicum*-derived cytosolic fractions were incubated with **38** (5 mM).

(Figure S2, Supporting Information). We conclude that the product formed in the in vitro assay is **40**. The synthesis of **40** was not inhibited by amphomycin, a selective inhibitor of polyprenolphosphomannose synthesis, indicating that the mannosyltransferase involved in the conversion **38** → **40**, is most likely the GDP-mannose dependent mannosyltransferase, MgtA (Figure 5b).

The synthetic substrate **38** facilitated the further characterization of MgtA. Incubation of **38** with *C. glutamicum* total lysate, a cleared lysate, and fractionated cytosol and membrane/cell wall fractions revealed that the majority of MgtA activity was localized to the cytosol, which is consistent with MgtA being a peripheral membrane protein that under the lysis conditions can be released into the cytosol (Figure 5c). Interestingly, in the presence of 10 mM DTT, the enzyme activity was dramatically enhanced (Figure 5b). This is an unusual observation for a glycosyltransferase and suggests that the activity of the enzyme can be regulated by the redox status of the bacterium. A screen for metal ion dependence revealed that the activity of MgtA was stimulated by Mg^{2+} and Mn^{2+} in preference to various other metal ions (Ca^{2+}, Cu^{2+}, Fe^{3+}, and Zn^{2+}) (Figure S1, Supporting Information).

Incubation of **38** with *C. glutamicum* Δ*PimB′* resulted in similar levels of mannosylation, providing evidence that the

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enzymatic activity monitored by the use of **38** arises from MgtA (Figure 5d). Incubation of **38** with a cell-free extract derived from *M. smegmatis* mc^{2}155 provided no evidence for the formation of **40**, suggesting that under conditions of exponential growth this strain does not express MgtA (Figure S1, Supporting Information).

CONCLUSIONS

Glucuronosyl diacylglycerols of varying lipid composition have been isolated from *C. glutamicum* and *M. smegmatis*. However, the precise arrangement of the acyl chain on the glycerol backbone has not been defined. To assist in resolving their structure, we chemically synthesized two candidate structures **1a** and **1b** to use as reference materials. Using collision-induced dissociation and multistage mass spectrometry, we were able to establish that these synthetic materials yielded characteristic fragmentation spectra. The fragmentation profiles established for glucuronosyl diacylglycerides is consistent with related observations using electrospray ionization multistage mass spectrometry for glycosyldiglycerides,[49] glycosylphosphatidic acids,[50] phosphatidylinositol phosphates,[51] phosphatidylethanolamines,[26,52,53] and phosphatidylinositol mannosides.[23,41,42] That the fragmentation patterns of the glucuronosyl diacylglycerides should match those for these other classes of molecules is not immediately obvious. Previous mechanistic studies of phospholipids has shown that charge-driven fragmentation determines the production of diagnostic fragment ions.[52] In the case of neutral glycosylglycerides, analysis is typically performed on positive ions, which fragment through charge-remote mechanisms.[49] In this case, fragmentation of the negative ions also occurs through charge-remote mechanisms, yielding larger peaks derived from loss of the acyl group on the secondary (*sn*-2) position relative to the primary (*sn*-1) position. This work has allowed (re)assignment of structures **1a**, **2a**, and **3a** for the glucuronosyl diacylglycerides from *M. smegmatis* and *C. glutamicum* and notably demonstrates the identity of the GlcAGroAc$_2$ ($C_{18:0}/C_{16:0}$) structure **2a** from these two organisms.

We next investigated whether compounds **1a**, **2a**, and **3a** could be detected in the common laboratory strains *M. smegmatis* mc^{2}155 and *M. tuberculosis* H37Rv. Using Q-trap and LCMS-QToF and LCMS-triple quadrupole mass spectrometers, we were unable to find any evidence for these compounds within the limit of detection. Compounds **1a** and **1b** were originally isolated from *M. smegmatis* MNC strain 13, and the absence of this compound from *M. smegmatis* mc^{2}155 provides a note of caution for future studies of the biosynthesis of these compounds within this laboratory strain. *M. smegmatis* strain mc^{2}155 ultimately derives from the heterogeneous American type culture collection (ATCC) 607[54] and is a mutant selected on the basis of an efficient plasmid transformation phenotype,[2] and its relationship to MNC strain 13 is unclear. The absence of any detectable GlcAGroAc$_2$ species from *M. tuberculosis* H37Rv to within the limit of detection is consistent with the absence of a clear phenotype observed upon deletion of Rv2188c (*PimB′*) in this organism, and there are no literature reports on this compound in any strains of *M. tuberculosis*.[18]

MgtA (Rv0557 in *M. tuberculosis*; MSMEG1113 in *M. smegmatis*) was originally annotated as PimB, a GDP-mannose-dependent α-mannosyltransferase that catalyzes the conversion of (Ac)PIM$_1$ to (Ac)PIM$_2$ in mycobacteria.[55] However, deletion of the corresponding ortholog (NCgl0452) from *C. glutamicum* revealed that MgtA catalyzes the transfer of mannose to GlcAGroAc$_2$ to form ManGlcAGroAc$_2$.[17] The genes responsible for the synthesis of PIM$_2$ were subsequently identified as Rv2188c, MSMEG4253, and NCgl2106 in *M. tuberculosis*, *M. smegmatis*, and *C. glutamicum*, respectively, and annotated *PimB′*.[13,56] Complementation studies with Rv2188c and Rv0557 in a Δ*MgtA*/Δ*PimB′ C. glutamicum* background have confirmed these roles for the *M. tuberculosis* orthologs.[18] However, in vitro studies using *C. glutamicum* Δ*MgtA* and Δ*PimB′* mutants showed that cell-free extracts could each catalyze the synthesis of both AcPIM$_2$ and ManGlcAGroAc$_2$, revealing a relaxed specificity for the *C. glutamicum* PimB′ and MgtA transferases within a single mutant background.[18] In addition, in vitro studies of *M. tuberculosis* MgtA expressed within a *C. glutamicum* Δ*MgtA*/Δ*PimB′* double knockout revealed that this enzyme was equally able to mannosylate AcPIM$_1$ or GlcAGroAc$_2$, whereas PimB′ expressed within the same double mutant exhibited a preference for mannosylation of AcPIM$_1$.[18]

In order to study the GlcAGroAc$_2$ mannosylating mannosyltransferase MgtA, we synthesized the artificial substrate octyl α-D-glucuronoside **38**. Our data, including studies with Δ*PimB′ C. glutamicum*, demonstrate that this compound is exclusively a substrate for MgtA. This substrate was used to undertake the initial biochemical characterization of MgtA and confirms that this enzyme is a GDP-mannose-dependent mannosyltransferase. Further, using **38** we show that MgtA is a metalloenzyme with a preference for Mg^{2+}/Mn^{2+}, and also demonstrate that MgtA undergoes a dramatic enhancement of activity under reducing conditions, an atypical feature for a glycosyltransferase. Finally, using the artificial substrate **38**, we demonstrate the absence of MgtA activity within *M. smegmatis* mc^{2}155.

This work describes the first comprehensive study of the chemical synthesis and structural analysis of glucuronosyl diacylglycerides, as well as the processing mannosyltransferase MgtA. Synthetic approaches to these glycolipids are of importance as isolating these glycolipids in sufficient quantities and purity from their natural sources is impractical. Understanding the structure−activity relationships of these glycolipids and their impact on the immunopathology of mycobacteria represent important topics for future study.

EXPERIMENTAL SECTION

General Experimental Methods. Pyridine was distilled over KOH before use. Dichloromethane and THF were dried over alumina according to the method of Pangborn et al.[57] Reactions were monitored using thin-layer chromatography (TLC), performed with silica gel 60 F_{254}. Detection was effected by charring in a mixture of 5% sulfuric acid in methanol, vanillin stain (6% vanillin, 1% H_2SO_4 in EtOH), 10% phosphomolybdic acid in EtOH, and/or visualizing with UV light. Flash chromatography was performed according to the method of Still et al.[58] using silica gel 60. Melting points were obtained using a hot-stage microscope (corrected). $[\alpha]_D$ values are given in deg 10^{-1} cm^2 g^{-1}.

(4*R*)-3-((2′*R*)-2′-Methyldecanoyl)-4-benzyl-2-oxazolidinone (6). NaHMDS (60 mL, 60 mmol, 1.0 M in THF) was added dropwise to a stirred solution of (4*R*)-3-decanoyl-4-benzyl-2-oxazolidinone (**5**)[28] (7.50 g, 22.6 mmol) in dry THF (80 mL) at −78 °C under N_2 and the resultant mixture stirred for 1 h. MeI (7.0 mL, 112 mmol) was added via syringe, and the mixture was stirred for 2 h at −78 °C at which time TLC indicated consumption of starting material and formation of a less polar product. The reaction was quenched with satd aq NH_4Cl (40 mL), and the volatile components were removed by rotary evaporation. The residue was extracted with CH_2Cl_2 (3 × 50 mL), and the combined organic phase was washed with 1 M sodium sulfite (50 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography (10:90 EtOAc/petroleum spirits) to give **6** (6.73 g, 86%) as a colorless oil: $[\alpha]_D$ −64.4 (*c* 1.0 in $CHCl_3$) (lit.[28]

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$[\alpha]_D$ −73.5 (*c* 1.0, CH_2Cl_2)); ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (3 H, t, *J* = 7.0 Hz, CH_2CH_3), 1.22 (3 H, d, *J* = 7.0 Hz, $CHCH_3$), 1.26−1.35 (12 H, m, $(CH_2)_6CH_2$), 1.40−1.42 (1 H, m, $NCOCHCH_2$), 1.71−1.75 (1 H, m, $NCOCHCH_2$), 2.77 (1 H, m, dd, *J* = 9.5, 13.5 Hz, CH_2Ph), 3.27 (1 H, dd, *J* = 3.0, 13.5 Hz, CH_2Ph), 3.68−3.74 (1 H, m, CHCO), 4.15−4.22 (2 H, m, CH_2O), 4.66−4.70 (1 H, m, CHN), 7.21−7.35 (5 H, m, Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2 (1 C, CH_2CH_3), 17.5 (1 C, $CHCH_3$), 22.8, 27.4, 29.4, 29.6, 29.8, 32.0, 33.6, 37.8 (8 C, $CH(CH_3)(CH_2)_7CH_3$), 38.1, 55.5, 66.1 (3 C, $OCH_2CH(CH_2Ph)NH$), 127.4, 129.0, 129.6, 135.5 (6 C, Ph), 153.2, 177.5 (2 C, OCONHCOCH); HRMS (ESI^+) calcd for $C_{21}H_{31}NNaO_3$ $[M + Na]^+$ *m*/*z* 368.2196, found 368.2196.

(*R*)-2-Methyldecan-1-ol (7). A freshly prepared solution of $NaBH_4$ (947 mg, 29.6 mmol) in water (3.5 mL) was added to a stirred mixture of **6** (1.28 g, 3.70 mmol) in THF (11 mL) at 0 °C. The mixture was stirred overnight at rt and then neutralized with 1 M HCl with cooling (ice/water). The aqueous phase was extracted with EtOAc (3 × 20 mL), and the combined organic phase was washed with water (30 mL) and brine (30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography (15:85 EtOAc/petroleum spirits) to give **7** (600 mg, 94%) as a colorless oil: $[\alpha]_D$ +10.4 (*c* 3.2 in $CHCl_3$) (lit.[22] $[\alpha]_D$ +11.0 (*c* 2.3 in $CHCl_3$)); ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (3 H, t, *J* = 6.8 Hz, CH_2CH_3), 0.91 (3 H, d, *J* = 6.7 Hz, $CHCH_3$), 1.07−1.64 (15 H, m, $CH(CH_2)_7CH_3$), 3.41 (1 H, dd, *J* = 6.6, 10.5 Hz, $HOCH_2CH$), 3.50 (1 H, dd, *J* = 5.7, 10.5 Hz, $HOCH_2CH$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2 (1 C, CH_2CH_3), 16.7 (1 C, $CHCH_3$), 22.8, 27.1, 29.5, 29.8, 30.1, 32.0, 33.3 (7 C, $CH(CH_2)_7CH_3$), 35.9 (1 C, $CHCH_3$), 68.6 ($HOCH_2$). Next to elute was (2*R*)-*N*-((1*R*)-2-hydroxyethyl-1-benzyl)-2-methyldecanamide (36 mg, 3%) as a colorless solid, ^1H NMR (400 MHz, $CDCl_3$) δ 0.87 (3 H, t, *J* = 6.8 Hz, CH_2CH_3), 1.03 (3 H, d, *J* = 6.9 Hz, $CHCH_3$), 1.22−1.59 (14 H, m, $(CH_2)_7CH_3$), 2.07−2.16 (1 H, m, $CH(CH_3)CH_2$), 2.83 (1 H, dd, *J* = 7.7, 13.8 Hz, CH_2Ph), 2.92 (1 H, dd, *J* = 7.1, 13.8 Hz, CH_2Ph), 3.14 (1 H, br s, OH), 3.60 (1 H, dd, *J* = 5.1, 11.0 Hz, $HOCH_2$), 3.68 (1 H, dd, *J* = 3.5, 11.0 Hz, $HOCH_2$), 4.17 (1 H, m, CONHCH), 5.76 (1 H, d, *J* = 7.2 Hz, CONH), 7.21−7.31 (5 H, m, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 18.0 (2 C, 2 × CH_3), 22.8, 27.5, 29.4, 29.6, 29.8, 33.0, 34.4, 37.1 (8 C, $(CH_2)_7CH_3$, CH_2Ph), 41.8 (1 C, COCH), 52.9 (1 C, CONHCH), 64.7 (1 C, $HOCH_2$), 126.8, 128.8, 129.3, 137.8 (6 C, Ph), 177.6 (1 C, NHCOCH); HRMS (ESI^+) calcd for $C_{20}H_{33}NO_2H$ $[M + H]^+$ *m*/*z* 320.2584, found 320.2584. Last to elute was (4*R*)-4-benzyl-2-oxazolidinone (490 mg, 75% recovered).

2,5-Di-*O*-(4-methoxybenzyl)-D-mannitol (9). HCl (12 M, 0.5 mL) was added to **8**[29,30] (2.78 g, 4.64 mmol) in a mixture of MeOH (100 mL) and hexane (65 mL) at rt with vigorous stirring. The mixture was stirred for 5 h, water (10 mL) was added, and the resulting mixture was stirred for an additional 1 h. The resultant two layers were separated, and the bottom layer was treated with satd aq Na_2CO_3 (4 mL). Additional Na_2CO_3 powder was added to adjust the pH to between 8 and 9. The mixture was filtered and the filtrate concentrated to give a residue, which was taken up in boiling EtOAc (200 mL). The hot mixture was filtered, the filtrate concentrated, and the residue recrystallized (EtOAc/hexane) to give **9** (0.74 g, 38%) as colorless needles: mp 99−101 °C (lit.[59] mp 98.5−100 °C); $[\alpha]_D$ −1.0 (*c* 1.0 in MeOH) (lit.[60] $[\alpha]_D$ −9.0 (*c* 1.9 in MeOH)); ^1H NMR (500 MHz, $CDCl_3$) δ 2.00 (4 H, br s, 4 × OH), 3.58 (2 H, ddd, *J* = 5.0, 6.4, 9.1 Hz, H2,5), 3.75−3.84 (4 H, m, H1(a,b),6(a,b)), 3.80 (6 H, 2 × OCH_3), 3.93 (2 H, d, *J* = 6.4 Hz, H3,4), 4.52, 4.59 (4 H, 2d, *J* = 11.3 Hz, 2 × CH_2Ph), 6.87−7.26 (8 H, AA′XX′, Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.4 (2 C, OCH_3), 61.4, 70.0, 72.4, 79.6 (8 C, C1,2,3,4,5,6 and 2 × CH_2Ph), 114.1, 129.8, 130.0, 159.6 (12 C, Ar).

1,6-Di-*O*-(*tert*-butyldiphenylsilyl)-2,5-di-*O*-(4-methoxybenzyl)-D-mannitol (10). TBDPSCl (200 μL, 0.78 mmol) was added to a stirred mixture of **9** (150 mg, 0.36 mmol) and imidazole (97.0 mg, 1.42 mmol) in DMF (1 mL) at 0 °C under N_2. The mixture was warmed to rt and stirred for 4 h. The mixture was diluted with EtOAc (20 mL), washed with water (3 × 10 mL) and brine (10 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography (15:85 then 20:80 EtOAc/petroleum spirits) to give **10** (300 mg, 94%) as a pale yellow oil: $[\alpha]_D$ −12.5 (*c* 1.0 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.05 (18 H, s, 2 × *tert*-butyl), 3.10 (2 H, d, *J* = 5.5 Hz, 2 × OH), 3.69 (2 H, ddd, *J* = 4.5, 5.0, 5.5 Hz, H2,5), 3.78 (6 H, s, 2 × OCH_3), 3.83 (2 H, dd, *J* = 5.0, 11.0 Hz), 3.75 (2 H, dd, *J* = 5.0, 11.0 Hz, H1b,6b), 3.90 (2 H, dd, *J* = 4.5, 11.0 Hz, H1a,6a), 4.00 (2 H, t, *J* = 5.5 Hz, H3,4), 4.47, 4.61 (4 H, 2 × d, *J* = 11.0 Hz, 2 × CH_2Ar), 6.80−7.69 (28 H, m, Ar,Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.3, 27.0 (8 C, $C(CH_3)_3$), 55.4 (2 C, 2 × OCH_3), 64.3, 70.0, 73.0, 80.5 (8 C, C1,2,3,4,5,6 and 2 × CH_2Ph), 113.9, 127.9, 129.6, 129.9, 130.6, 133.3, 133.4, 135.8, 135.8, 159.3 (36 C, Ar,Ph); HRMS (ESI^+) calcd for $C_{54}H_{66}NaO_8Si_2$ $[M + Na]^+$ *m*/*z* 921.4188, found 921.4192.

1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(4-methoxybenzyl)-*sn*-glycerol (11). $NaIO_4$ (1.96 g, 9.18 mmol) was added to a stirred solution of **10** (1.50 g, 1.67 mmol) in a mixture of THF (25 mL) and water (5 mL) at rt. The mixture was stirred for 3 h and then diluted with EtOAc (100 mL). The organic layer was separated, washed with water (2 × 40 mL) and brine (2 × 30 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give the crude aldehyde. The residue was dissolved in a mixture of ethanol (27 mL) and water (3 mL), cooled to 0 °C, and treated with $NaBH_4$ (758 mg, 20.0 mmol). The mixture was stirred for 2 h and then quenched with the careful addition of AcOH to adjust to pH 8. The mixture was extracted with EtOAc (3 × 30 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (20:80 EtOAc/petroleum spirits) to give **11** (1.36 g, 91% over two steps) as a pale yellow oil: $[\alpha]_D$ −27.5 (c 1.0 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.06 (9 H, s, *tert*-butyl), 2.03 (1 H, dd, *J* = 5.0, 6.0 Hz, OH), 3.58−3.62 (1 H, m, H2), 3.67 (1 H, dd, *J* = 6.0, 11.5 Hz, H3a), 3.77−3.80 (1 H, obscured m, H3b), 3.72 (1 H, dd, *J* = 6.0 Hz, 10.5 Hz, H1a), 3.78 (1 H, dd, *J* = 5.0, 10.5 Hz, H1b), 3.80 (3 H, s, OCH_3), 4.44, 4.56 (2 H, 2d, *J* = 11.5 Hz, CH_2Ar), 6.84−7.68 (14 H, m, Ar,Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.3, 27.0 (4 C, $C(CH_3)_3$), 55.4 (1 C, OCH_3), 63.0, 63.7, 72.0 (3 C, C1,2,3), 79.4 (1 C, CH_2Ar), 114.0, 127.9, 129.5, 129.9, 130.6, 133.3, 133.4, 135.7, 135.8, 159.4 (18 C, Ar,Ph); HRMS (ESI^+) calcd for $C_{27}H_{34}O_4SiNH_4$ $[M + NH_4]^+$ *m*/*z* 468.2565, found 468.2565.

1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(4-methoxybenzyl)-*sn*-glyceryl 2,3,4-Tri-*O*-benzyl-6-*O*-acetyl-α-D-glucopyranoside (13). TMSI (1.22 mL, 8.99 mmol) was added to a solution of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-glucopyranose[61] (4.00 g, 7.49 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C under N_2. The resultant mixture was stirred for 1 h at 0 °C, at which point TLC indicated consumption of all starting material. The mixture was azeotroped with dry toluene (3 × 10 mL) and then placed under high vacuum for 1 h. Crude **12** was dissolved in dry CH_2Cl_2 (50 mL) and cannulated into a stirred mixture of **11** (1.34 g, 2.99 mmol), TBAI (3.86 g, 10.5 mmol), 2,4,6-tri-*tert*-butylpyrimidine (2.05 g, 8.24 mmol), and freshly activated 4 Å molecular sieves in dry CH_2Cl_2 (50 mL). The reaction was stirred for 6 d at rt under N_2 at which point TLC indicated consumption of the glycerol. The mixture was diluted with EtOAc, quenched with 1 M $Na_2S_2O_3$, and stirred for 1 h. The mixture was then filtered through a layer of Celite and the organic phase washed with water (50 mL), brine (50 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was diluted with Et_2O and the excess TBAI filtered. The filtrate was concentrated and the residue purified by flash chromatography (20:80 then 25:75 then 30:70 EtOAc/petroleum spirits) to give **13** (1.73 g, 63%) as a pale yellow oil: $[\alpha]_D$ +29.6 (*c* 1.24 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.10 (9 H, s, *tert*-butyl), 2.02 (3 H, s, $COCH_3$), 3.54 (1 H, dd, $J_{3,4}$ = 9.0, $J_{4,5}$ = 9.9 Hz, H4), 3.57−3.60 (1 H, m, H2′), 3.55 (1 H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.6 Hz, H2), 3.77−3.81 (3 H, m, H1′(a,b),3′a), 3.79 (3 H, s, OCH_3), 3.89 (1 H, ddd, $J_{4,5}$ = 10.1, $J_{5,6}$ = 2.1, $J_{5,6}$ = 4.1 Hz, H5), 3.93 (1 H, dd, *J* = 3.5, 10.5 Hz, H3′b), 4.04 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H3), 4.20 (1 H, dd, $J_{5,6}$ = 2.1, $J_{6,6}$ = 12.0 Hz, H6a), 4.30 (1 H, dd, $J_{5,6}$ = 4.2, $J_{6,6}$ = 12.0 Hz, H6), 4.60, 4.63 (2 H, 2 × d, *J* = 11.5 Hz, CH_2Ph), 4.60, 4.92 (2 H, 2 × d, *J* = 11.0 Hz, CH_2Ph), 4.70, 4.74 (2 H, 2 × d, *J* = 12.0 Hz, CH_2Ph), 4.86, 5.05 (2 H, 2 × d, *J* = 10.8 Hz, CH_2Ph), 4.92 (1 H, d, $J_{1,2}$ = 3.2 Hz, H1), 6.80, 7.26 (4 H, 2 × d, *J* = 8.7 Hz), 7.29−7.72 (25 H, m, Ar,Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.4 (1 C, $C(CH_3)_3$), 21.0 (1 C, $COCH_3$), 27.0 (3 C, $C(CH_3)_3$), 55.4 (1 C, OCH_3), 63.2, 63.7 (2 C, C6,1′), 68.8, 68.9 (2 C, C2,5), 72.0, 72.9, 75.2, 75.8 (4 C, 4 × CH_2Ph), 77.4, 78.4 (2 C, C2′,3′), 80.2 (1 C, C4), 82.0 (1 C, C3), 97.5 (1 C, C1), 113.8, 127.8−128.6, 129.3, 129.8, 130.9, 133.4, 133.5, 135.7, 135.8, 138.1, 138.4, 138.8, 159.2

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(30 C, Ar,Ph), 170.7 (1 C, C═O); HRMS (ESI$^+$) calcd for $C_{56}H_{64}SiNaO_{10}$ [M + Na]$^+$ m/z 947.4161, found 947.4157.

1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(4-methoxybenzyl)-*sn*-glyceryl 2,3,4-Tri-*O*-benzyl-α-D-glucopyranoside (14). NaOMe in MeOH (0.05 M, 1 mL) was added to a stirred solution of **13** (174 mg, 0.188 mmol) in CH_2Cl_2 (3 mL). The mixture was stirred overnight and then neutralized with Amberlite 120R (H$^+$ form) resin. The mixture was filtered and the filtrate concentrated to give a residue, which was purified by flash chromatography (30:70 EtOAc/petroleum spirits) affording **14** (151 mg, 91%) as a colorless oil: $[\alpha]_D$ +27.3 (*c* 0.99 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.13 (9 H, s, *tert*-butyl), 1.77 (1 H, br s, OH), 3.59 (1 H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.7 Hz, H2), 3.61−3.66 (2 H, m, H4,2′), 3.73−3.85 (6 H, m, H1′(a,b),3′a,5,6(a,b)), 3.81 (3 H, s, OCH_3), 3.96 (1 H, dd, *J* = 3.2, 10.5 Hz, H3′b), 4.09 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H3), 4.65 (2 H, s, CH_2Ph), 4.73, 4.78 (2 H, 2 × d, *J* = 11.5 Hz, CH_2Ph), 4.73, 4.97 (2 H, 2 × d, *J* = 11.0 Hz, CH_2Ph), 4.91, 5.07 (2 H, 2 × d, *J* = 11.0 Hz, CH_2Ph), 4.93 (1 H, d, $J_{1,2}$ = 3.5 Hz, H1), 6.86, 7.29 (4 H, 2 × d, *J* = 8.5 Hz), 7.32−7.75 (25 H, m, Ar,Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.3, 27.0 (4 C, C(CH_3)$_3$), 55.3 (1 C, OCH_3), 61.8, 63.6, 68.6, 71.0, 72.0, 72.9, 75.1, 75.7, 77.5, 78.4, 80.3, 81.9 (12 C, C2,3,4,5,6,1′,2′,3′ and 4 × CH_2Ph), 97.5 (1 C, C1), 113.8, 127.6, 127.8, 127.8, 127.8, 127.9, 128.0, 128.1, 128.5, 128.5, 129.3, 129.8, 130.9, 133.4, 133.5, 135.7, 135.7, 138.4, 138.4, 138.9, 159.1 (36 C, Ar,Ph); HRMS (ESI$^+$) calcd for $C_{54}H_{62}NaO_9Si$ [M + Na]$^+$ m/z 905.4055, found 905.4064.

1-*O*-(*tert*-Butyldiphenylsilyl-2-*O*-(4-methoxybenzyl)-*sn*-glyceryl 2,3,4-Tri-*O*-benzyl-α-D-glucopyranosiduronic Acid (15). Compound **14** (180 mg, 0.204 mmol), TEMPO (19.0 mg, 0.122 mmol), and BAIB (394 mg, 1.22 mmol) were stirred in a mixture of CH_2Cl_2/H_2O (2:1, 6 mL) at rt for 3 h and then quenched with 0.25 M $Na_2S_2O_3$. The aqueous phase was extracted with EtOAc (3 × 5 mL). The organic layer was washed with water, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography (30:70 then 40:60 EtOAc/petroleum spirits with 2% AcOH) to give **15** (160 mg, 87%) as a pale yellow oil: $[\alpha]_D$ +15.3 (*c* 1.09 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.05 (9 H, s, *tert*-butyl), 3.58 (1 H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.6 Hz, H2), 3.62 (1 H, dd, *J* = 6.0, 10.5 Hz, H3′a), 3.75 (3 H, s, OCH_3), 3.60−3.77 (4 H, m, H4,1′(a,b),2′), 3.90 (1 H, dd, *J* = 2.5, 10.5 Hz, H3′b), 4.02 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H3), 4.30 (1 H, d, $J_{4,5}$ = 10.0 Hz, H5), 4.55 (2 H, s, CH_2Ph), 4.64, 4.71 (2 H, 2 × d, *J* = 12.0 Hz, CH_2Ph), 4.66, 4.84 (2 H, 2 × d, *J* = 10.6 Hz, CH_2Ph), 4.82, 4.98 (2 H, 2 × d, *J* = 10.9 Hz, CH_2Ph), 4.91 (1 H, d, *J* = 3.5 Hz, H1), 6.79, 7.21 (4 H, 2 × d, *J* = 8.5 Hz), 7.23−7.68 (25 H, m, Ar,Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.3, 27.0 (4 C, C(CH_3)$_3$), 55.3 (1 C, OCH_3), 63.4, 69.3, 69.9, 71.9, 73.0, 75.3, 75.9, 78.3, 79.3, 79.5, 81.3 (11 C, C2,3,4,5,1′,2′,3′ and 4 × CH_2Ph), 98.0 (1 C, C1), 113.8, 127.8, 127.8, 127.8, 127.9, 128.0, 128.0, 128.0, 128.2, 128.48, 128.50, 128.53, 129.3, 129.8, 130.7, 133.37, 133.43, 135.69, 135.73, 137.7, 138.1, 138.6, 159.1 (36 C, Ar,Ph), 173.7 (1 C, C6); HRMS (ESI$^+$) calcd for $C_{54}H_{60}NaO_{10}Si$ [M + Na]$^+$ m/z 919.3848, found 919.3851.

Benzyl (1-*O*-*tert*-Butyldiphenylsilyl-2-*O*-(4-methoxybenzyl)-*sn*-glyceryl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosid)uronate (16). Benzyl alcohol (27 μL, 0.264 mmol) was added to a stirred mixture of **15** (158 mg, 0.176 mmol), HBTU (133 mg, 0.352 mmol), DIPEA (77 μL, 0.44 mmol), and DMAP (8.6 mg, 0.07 mmol) in CH_2Cl_2 (4 mL). The mixture was stirred at rt for 2 h, quenched with water, and diluted with EtOAc and the organic phase separated. The organic layer was concentrated under reduced pressure to give a residue, which was purified by flash chromatography (10:90 to 15:85 EtOAc/petroleum spirits) to give **16** (146 mg, 84%) as a colorless oil: $[\alpha]_D$ +9.47 (*c* 0.97 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.05 (9 H, s, *tert*-butyl), 3.59 (1 H, m, H3′a), 3.61 (1 H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.5 Hz, H2), 3.71−3.79 (4 H, H4,1′(a,b),2′), 3.76 (3 H, s, OCH_3), 3.94 (1 H, dd, *J* = 2.0, 10.8 Hz, H3′b), 4.01 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H3), 4.33 (1 H, d, $J_{4,5}$ = 10.0 Hz, H5), 4.46, 4.76 (2 H, 2 × d, *J* = 10.8 Hz, CH_2Ph), 4.53, 4.57 (2 H, 2 × d, *J* = 11.5 Hz, CH_2Ph), 4.65, 4.71 (2 H, 2 × d, *J* = 12.1 Hz, CH_2Ph), 4.81, 4.96 (2 H, 2 × d, *J* = 10.9 Hz, CH_2Ph), 4.93 (1 H, d, *J* = 3.5 Hz, H1), 5.13, 5.16 (2 H, 2 × d, *J* = 12.2 Hz, CH_2Ph), 6.78, 7.20 (4 H, 2 × d, *J* = 8.5 Hz), 7.12−7.15 (2 H, m), 7.25−7.68 (28 H, Ar,Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.3, 27.0 (4 C, C(CH_3)$_3$), 55.4 (1 C, OCH_3), 63.5, 67.6, 69.3, 70.8, 72.0, 73.0, 75.2, 75.9, 78.3, 79.6, 79.9, 81.3 (12 C, C2,3,4,5,1′,2′,3′ and 5 × CH_2Ar), 98.2 (1 C, C1), 127.7, 127.75, 127.83, 127.85, 127.88, 127.90, 128.1, 128.4, 128.50, 128.53, 128.57, 128.65, 129.3, 129.8, 130.9, 133.4, 133.5, 135.2, 137.7, 135.8, 138.1, 138.3, 138.8, 159.1 (42 C, Ar,Ph), 169.9 (1 C, C6); HRMS (ESI$^+$) calcd for $C_{61}H_{66}NaO_{10}Si$ [M + Na]$^+$ m/z 1009.4318, found 1009.4318.

Benzyl (2-*O*-(4-Methoxybenzyl)-*sn*-glyceryl 2,3,4-Tri-*O*-benzyl-α-D-glucopyranosid)uronate (17). HF·pyridine (70%, 290 μL) was added to a mixture of **16** (140 mg, 0.142 mmol) in dry THF (4.5 mL). The mixture was stirred under N_2 overnight, diluted with EtOAc (30 mL), and poured into satd aq $NaHCO_3$. The organic layer was separated and then washed with satd aq $NaHCO_3$ (5 × 10 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography (40:60 to 45:55 EtOAc/petroleum spirits) to give **17** (100 mg, 94%) as a colorless oil: $[\alpha]_D$ +28.9 (*c* 1.13 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.22 (1 H, br s, OH), 3.58 (1 H, dd, *J* = 5.4, 10.5 Hz, H3′a), 3.66 (1 H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.6 Hz, H2), 3.67 (1 H, dd, *J* = 5.5, 11.4 Hz, H1′a), 3.72 (1 H, m, H2′), 3.76−3.80 (2 H, m, H4,1′b), 3.80 (3 H, s, OCH_3), 3.86 (1 H, dd, *J* = 4.7, 10.5 Hz, H3′b), 4.01 (1 H, dd, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H3), 4.33 (1 H, d, $J_{4,5}$ = 10.0 Hz, H5), 4.49, 4.77 (2 H, 2 × d, *J* = 10.8 Hz, CH_2Ph), 4.59, 4.80 (2 H, 2 × d, *J* = 11.4 Hz, CH_2Ph), 4.66, 4.68 (2 H, 2 × d, *J* = 11.0 Hz, CH_2Ph), 4.81 (1 H, d, *J* = 3.2 Hz, H1), 4.83, 4.96 (2 H, 2 × d, *J* = 10.9 Hz, CH_2Ph), 5.17, 5.20 (2 H, 2 × d, *J* = 12.3 Hz, CH_2Ph), 6.85−7.34 (24 H, m, Ar,Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.4 (1 C, OCH_3), 62.7, 67.4, 68.9, 70.8, 71.9, 73.6, 75.2, 75.9, 77.1, 79.6, 79.7, 81.3 (12 C, C2,3,4,5,1′,2′,3′ and 5 × CH_2Ar), 98.2 (1 C, C1), 114.0, 127.8, 127.9, 128.06, 128.09, 128.11, 128.4, 128.5, 128.55, 128.57, 128.62, 128.7, 129.6, 130.4, 135.2, 138.0, 138.1, 138.6, 159.4 (30 C, Ar,Ph), 169.6 (1 C, C6); HRMS (ESI$^+$) calcd for $C_{45}H_{48}NaO_{10}$ [M + Na]$^+$ m/z 771.3140, found 771.3147.

Benzyl (1-*O*-Stearyl-2-*O*-(4-methoxybenzyl)-*sn*-glyceryl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosid)uronate (18). DIPEA (34 μL, 0.197 mmol) was added to a mixture of **17** (37 mg, 0.049 mmol), stearic acid (28 mg, 0.099 mmol), and COMU (42 mg, 0.099 mmol) in DMF (1.5 mL). The mixture was stirred under N_2 for 20 min at which point the solution turned from pale yellow to dark orange. DMAP (2.4 mg, 0.02 mmol) was added, and the reaction was heated to 50 °C and stirred overnight. The mixture was diluted with EtOAc, washed with satd aq $NaHCO_3$, water, and brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (10:60:30 EtOAc/toluene/petroleum spirits) to give **18** (36 mg, 72%) as a colorless oil: $[\alpha]_D$ +22.2 (*c* 1.2 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.91 (1 H, t, *J* = 7.0 Hz, CH_2CH_3), 1.28−1.62 (30 H, m, OCOCH_2(CH_2)$_{14}$-C$H_2$$CH_3$), 2.30 (2 H, t, *J* = 7.7 Hz, OCOC$H_2$$CH_2$), 3.54 (1 H, m, H3′a), 3.61 (1 H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.6 Hz, H2), 3.77 (1 H, dd, $J_{3,4}$ = 9.1, $J_{4,5}$ = 9.9 Hz, H4), 3.78 (3 H, s, OCH_3), 3.80−3.84 (3 H, m, H2′,3′b), 4.00 (1 H, dd, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H3), 4.16 (1 H, dd, *J* = 5.0, 11.8 Hz, H1′a), 4.31 (1 H, d, $J_{4,5}$ = 9.9 Hz, H5), 4.32 (1 H, dd, *J* = 4.1, 11.8 Hz, H1′b), 4.47, 4.76 (2 H, 2 × d, *J* = 10.8 Hz, CH_2Ph), 4.61 (2 H, s, CH_2Ph), 4.65, 4.76 (2 H, 2 × d, *J* = 12.0 Hz, CH_2Ph), 4.82, 4.96 (2 H, 2 × d, *J* = 10.9 Hz, CH_2Ph), 4.86 (1 H, d, $J_{1,2}$ = 3.5 Hz, H1), 5.15, 5.18 (2 H, 2 × d, *J* = 12.3 Hz, CH_2Ph), 6.82−7.34 (24 H, m, Ar,Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3 (1 C, CH_3), 22.8, 25.0, 29.3, 29.46, 29.50, 29.6, 29.79, 29.80, 29.82, 29.84, 32.1, 34.3 (16 C, OCO(CH_2)$_{16}$$CH_3$), 55.4 (1 C, OCH_3), 63.2 (1 C, C1′), 67.4 (1 C, C3′), 70.8 (1 C, C5), 68.4, 71.8, 73.3, 75.0, 75.2, 75.9 (6 C, C2′ and 5 × CH_2Ar), 79.6, 79.8 (2 C, C2,4), 81.3 (1 C, C3), 98.1 (1 C, C1), 113.9, 127.76, 127.77, 127.9, 128.0, 128.1, 128.4, 128.5, 128.55, 128.56, 128.58, 128.7, 129.5, 130.3, 135.2, 138.1, 138.2, 138.7, 159.4 (30 C, Ar,Ph), 169.7 (1 C, C6), 173.7 (1 C, $OCOCH_2$); HRMS (ESI$^+$) calcd for $C_{63}H_{82}NaO_{11}$ [M + Na]$^+$ m/z 1037.5759, found 1037.5758.

Benzyl (1-*O*-Stearyl-2-*O*-palmityl-*sn*-glyceryl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosid)uronate (20). Cerium ammonium nitrate (60.0 mg, 109 μmol) was added to a stirred solution of **18** (35.0 mg, 34.5 μmol) in MeCN/H_2O (9:1, 3 mL) at rt. The mixture was stirred at rt for 2 h, diluted with EtOAc (10 mL) and H_2O (5 mL), and stirred for 10 min. The organic layer was separated and the aqueous layer extracted with EtOAc (2 × 5 mL). The combined organic extract was washed with water (2 × 5 mL) and brine (5 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The crude alcohol **19** was placed on high vacuum for 2 h and then used directly in the next step. DIPEA (25 μL,

140 μmol) was added to a stirred mixture of crude **19** (36 mg), palmitic acid (36 mg, 142 μmol), and COMU (61 mg, 142 μmol) in DMF (2 mL). The mixture was stirred for 10 min under N_2, and then DMAP (1.7 mg, 14 μmol) was added. The reaction was stirred for 30 h at rt and then diluted with EtOAc, washed sequentially with 1 M HCl (5 mL), 0.5 M K_2CO_3 (3 × 5 mL), water (5 mL), and brine (10 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (10:90 then 15:85 EtOAc/petroleum spirits) to give **20** (24 mg, 60% over two steps) as a colorless oil: $[\alpha]_D$ +19.1 (*c* 0.5 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91, 0.91 (6 H, 2 × t, *J* = 7.1 Hz, 2 × CH_3), 1.26−1.61 (56 H, m, stearyl and palmityl CH_2), 2.25, 2.31 (4 H, m, 2 × $OCOCH_2CH_2$), 3.56 (1 H, dd, *J* = 5.1, 10.7 Hz, H3′a), 3.59 (1 H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.6 Hz, H2), 3.75 (1 H, dd, $J_{3,4}$ = 9.0, $J_{4,5}$ = 9.9 Hz, H4), 3.82 (1 H, dd, *J* = 5.2, 10.7 Hz, H3′b), 3.97 (1 H, dd, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H3), 4.22 (1 H, dd, *J* = 6.0, 11.9 Hz, H1′a), 4.28 (1 H, d, $J_{4,5}$ = 9.9 Hz, H5), 4.44 (1 H, dd, *J* = 3.8, 11.8 Hz, H1′b), 4.45, 4.75 (2 H, 2 × d, *J* = 10.8 Hz, CH_2Ph), 4.63, 4.75 (2 H, 2 × d, *J* = 11.7 Hz, CH_2Ph), 4.76 (1 H, d, $J_{1,2}$ = 3.5 Hz, H1), 4.80, 4.92 (2 H, 2 × d, *J* = 10.8 Hz, CH_2Ph), 5.17 (2 H, s, CH_2Ph), 5.25 (1 H, m, H2′), 7.13−7.35 (20 H, m, Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3 (2 C, 2 × CH_3), 22.8, 25.0, 29.25, 29.29, 29.5, 29.7, 29.8, 29.9, 32.1, 34.2, 34.3 (30 C, stearyl and palmityl CH_2), 62.5 (1 C, C1′), 66.9 (1 C, C3′), 69.7 (1 C, C2′), 70.9 (1 C, C5), 67.4, 73.5, 75.2, 76.0 (4 C, 4 × CH_2Ph), 79.7 (2 C, C2,4), 81.2 (1 C, C3), 98.2 (1 C, C1), 127.8, 127.9, 128.0, 128.1, 128.4, 128.5, 128.6, 128.66, 128.71, 135.2, 138.0, 138.2, 138.7 (24 C, Ar), 169.6 (1 C, C6), 173.2, 173.5 (2 C, 2 × $OCOCH_2$); HRMS (ESI^+) calcd for $C_{71}H_{104}NaO_{11}$ $[M + Na]^+$ *m*/*z* 1155.7471, found 1155.7472.

2-*O*-Palmityl-1-*O*-stearyl-*sn*-glyceryl-α-D-glucopyranosiduronic Acid (21). Pd(OH)$_2$−C (20%, 37 mg) was added to a degassed solution of **20** (22 mg, 19.4 μmol) in MeOH/THF (3:2, 2.5 mL) containing AcOH (50 μL). The mixture was stirred under H_2 atmosphere for 2 h. The mixture was filtered through a layer of Celite, rinsed with MeOH/THF (3:2), and then concentrated under reduced pressure. Flash chromatography ($CHCl_3$ with 1% AcOH then 7:2:0.1:0.1 $CHCl_3$/MeOH/H_2O/AcOH) gave **21** (9 mg, 60%) as a colorless powder: $[\alpha]_D$ +29.3 (*c* 0.25 in $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.85, 0.85 (6 H, 2 × t, *J* = 7.2 Hz, 2 × CH_3), 1.23−1.50 (56 H, m, stearyl and palmityl CH_2), 2.24−2.30 (4 H, m, 2 × $OCOCH_2$), 3.21−3.23 (1 H, m, H2), 3.36−3.40 (2 H, m, H3,4), 3.55 (1 H, dd, *J* = 5.0, 10.9 Hz, H3′a), 3.69 (1 H, dd, *J* = 5.6, 10.9 Hz, H3′b), 3.78 (1 H, d, $J_{4,5}$ = 9.8 Hz, H5), 4.16 (1 H, dd, *J* = 6.9, 12.0 Hz, H1′a), 4.32 (1 H, dd, *J* = 2.6, 12.0 Hz, H1′b), 4.69 (1 H, d, $J_{1,2}$ = 3.5 Hz, H1), 4.80, 4.91 (2 H, 2 × br s, 2 × OH), 5.11 (1 H, m, H2′); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.9 (2 × CH_3), 22.1, 24.4, 24.4, 28.4, 28.70, 28.74, 28.9, 29.1, 32.3, 33.4, 33.5 (30 C, stearyl and palmityl CH_2), 62.2 (1 C, C1′), 65.5 (1 C, C3′), 69.6, 71.4, 71.7, 71.8, 72.6 (5 C, C2,3,4,5,2′), 99.4 (1 C, C1), 171.0 (1 C, C6), 172.3, 172.5 (2 C, 2 × $OCOCH_2$); HRMS (ESI^+) *m*/*z* 795.5596 ($C_{43}H_{80}NaO_{11}$ $[M + Na]^+$ requires 795.5593).

Benzyl (1-*O*-((*R*)-10-Tuberculostearyl)-2-*O*-(4-methoxybenzyl)-*sn*-glyceryl 2,3,4-Tri-*O*-benzyl-α-D-glucopyranosid)uronate (22). DIPEA (111 μL, 0.742 mmol) was added to a stirred mixture of **17** (95 mg, 0.127 mmol), (*R*)-tuberculostearic acid (**4**) (61 mg, 0.204 mmol), and COMU (174 mg, 0.406 mmol) in DMF (4 mL). The mixture was stirred under N_2 for 20 min, at which point the solution turned from pale yellow to dark orange. DMAP (3.1 mg, 0.025 mmol) was added, and the reaction was heated to 50 °C and stirred overnight. The mixture was diluted with EtOAc, washed with satd aq $NaHCO_3$, water, and brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (10:60:30 EtOAc/toluene/petroleum spirits) to give **22** (83 mg, 63%) as a colorless oil: $[\alpha]_D$ +20.6 (*c* 1.3 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (3 H, d, *J* = 6.6 Hz, $CHCH_3$), 0.90 (3 H, t, *J* = 7.1 Hz, CH_2CH_3), 1.08−1.57 (30 H, m, tuberculostearyl CH + CH_2), 2.26 (3 H, t, *J* = 7.7 Hz, $OCOCH_2$), 3.50 (1 H, dd, *J* = 7.5, 12.0 Hz, H3′a), 3.58 (1 H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.6 Hz, H2), 3.74 (1 H, dd, $J_{3,4}$ = 9.1, $J_{4,5}$ = 10.0 Hz, H4), 3.74 (3 H, s, OCH_3), 3.76−3.81 (2 H, m, H2′,3′b), 3.97 (1 H, dd, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H3), 4.13 (1 H, dd, *J* = 5.0, 11.8 Hz, H1′a), 4.27 (1 H, d, $J_{4,5}$ = 9.8 Hz, H5), 4.29 (1 H, dd, *J* = 4.1, 11.7 Hz, H1′b), 4.43, 4.73 (2 H, 2 × d, *J* = 10.8 Hz, CH_2Ph), 4.58 (2 H, s, CH_2Ph), 4.62, 4.72 (2 H, 2 × d, *J* = 12.0 Hz, CH_2Ph), 4.78, 4.93 (2 H, 2 × d, *J* = 10.9 Hz, CH_2Ph), 4.82 (1 H, d, $J_{1,2}$ = 3.5 Hz, H1), 5.12, 5.15 (2 H, 2 × d, *J* = 12.2 Hz, CH_2Ph), 6.79−7.30 (24 H, m, Ar,Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2 (1 C, CH_2CH_3), 19.8 (1 C, $CHCH_3$), 22.8, 25.0, 27.2, 29.3, 29.47, 29.49, 29.7, 29.8, 30.1, 30.2, 32.0, 32.9, 34.3, 37.2 (16 C, $(CH_2)_8CH(CH_3)(CH_2)_7CH_3$), 55.4 (1 C, OCH_3), 63.2 (1 C, C1′), 68.3 (1 C, C3′), 70.8 (1 C, C5), 67.4, 71.8, 73.3, 75.0, 75.1, 75.9 (6 C, C2′ and 5 × CH_2Ar), 79.6 (1 C, C2), 79.72 (1 C, C4), 81.2 (1 C, C3), 98.1 (1 C, C1), 113.9, 127.8, 127.9, 128.0, 128.1, 128.39, 128.43, 128.5, 128.55, 128.57, 128.7, 129.5, 130.3, 136.2, 138.0, 138.2, 138.6, 159.3 (30 C, Ar,Ph), 169.6 (1 C, C6), 173.7 (1 C, $OCOCH_2$); HRMS (ESI^+) calcd for $C_{64}H_{84}NaO_{11}$ $[M + Na]^+$ *m*/*z* 1051.5906, found 1051.5909.

Benzyl (1-*O*-((*R*)-10-Tuberculostearyl)-2-*O*-palmityl-*sn*-glyceryl 2,3,4-Tri-*O*-benzyl-α-D-glucopyranosid)uronate (24). Cerium ammonium nitrate (129 mg, 0.236 mmol) was added to a stirred solution of **22** (81 mg, 0.079 μmol) in MeCN/H_2O (11:1, 6 mL) at rt. The mixture was stirred at rt for 2 h, diluted with EtOAc (10 mL) and H_2O (5 mL), and stirred for 10 min. The organic layer was separated and the aqueous layer extracted with EtOAc (2 × 5 mL). The combined organic extract was washed with water (2 × 5 mL) and brine (5 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was placed on high vacuum for 2 h and then used directly in the next step. DIPEA (27 μL, 0.157 μmol) and palmitic acid (40.4 mg, 0.157 μmol) were added to a stirred mixture of the crude alcohol **23** (75 mg) and COMU (67.4 mg, 0.157 μmol) in DMF (2 mL). The mixture was stirred for 10 min under N_2, and then DMAP (0.2 mg, 16 μmol) was added. The reaction was stirred overnight, a second portion (as above) of DIPEA, palmitic acid, COMU, and DMAP was added, and the mixture was stirred for a further 24 h. The mixture was diluted with EtOAc (20 mL), washed sequentially with 1 M HCl (10 mL), 0.5 M K_2CO_3 (3 × 10 mL), water (10 mL), and brine (10 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (5:65:30 then 10:60:30 EtOAc/toluene/petroleum spirits) to give **24** (60.4 mg, 67% over two steps) as a colorless oil: $[\alpha]_D$ +19.1 (*c* 1.16 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (3 H, d, *J* = 6.5 Hz, $CHCH_3$), 0.90, 0.90 (6 H, 2 × t, *J* = 7.0 Hz, 2 × CH_2CH_3), 1.08−1.60 (55, m, palmityl and tuberculostearyl CH + CH_2), 2.25−2.31 (4 H, m, 2 × $OCOCH_2$), 3.56 (1 H, dd, *J* = 5.1, 10.7 Hz, H3′a), 3.59 (1 H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.6 Hz, H2), 3.75 (1 H, dd, $J_{3,4}$ = 9.1, $J_{4,5}$ = 9.9 Hz, H4), 3.81 (1 H, dd, *J* = 5.2, 10.7 Hz, H3′b), 3.96 (1 H, dd, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H3), 4.22 (1 H, dd, *J* = 6.0, 11.9 Hz, H1′a), 4.27 (1 H, d, $J_{4,5}$ = 9.9 Hz, H5), 4.43 (1 H, dd, *J* = 3.7, 11.9 Hz, H1′b), 4.45, 4.75 (2 H, 2 × d, *J* = 10.8 Hz, CH_2Ph), 4.62, 4.77 (2 H, 2 × d, *J* = 12.0 Hz, CH_2Ph), 4.76 (1 H, d, $J_{1,2}$ = 3.5 Hz, H1), 4.80, 4.94 (2 H, 2 × d, *J* = 10.8 Hz, CH_2Ph), 5.16 (2 H, s, CH_2Ph), 5.24 (1 H, m, H2′), 7.12−7.14 (2 H, m), 7.25−7.35 (18 H, m, Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3 (2 × CH_2CH_3), 19.8 (1 C, $CHCH_3$), 22.8, 25.0, 27.2, 29.2, 29.3, 29.46, 29.49, 29.52, 29.67, 29.71, 29.8, 29.9, 30.16, 30.20, 32.1, 32.9, 34.2, 34.3, 37.3 (30 C, palmityl and tuberculostearyl CH + CH_2), 62.5 (1 C, C1′), 66.9 (1 C, C3′), 69.8 (1 C, C2′), 70.9 (1 C, C5), 67.4, 73.5, 75.2, 76.0 (4 C, 4 × CH_2Ph), 79.7 (2 C, C2,4), 81.2 (1 C, C3), 98.2 (1 C, C1), 127.8, 127.9, 128.0, 128.1, 128.4, 128.5, 128.57, 128.64, 128.7, 135.1, 138.0, 138.2, 138.6 (24 C, Ph), 169.6 (1 C, C6), 173.1, 173.5 (2 C, 2 × $OCOCH_2$); HRMS (ESI^+) calcd for $C_{72}H_{106}NaO_{11}$ $[M + Na]^+$ *m*/*z* 1169.7627, found 1169.7628.

1-*O*-((*R*)-10-Tuberculostearyl)-2-*O*-palmityl-*sn*-glyceryl α-D-glucopyranosiduronic Acid (1a). Pd(OH)$_2$−C (20%, 20 mg) was added to a degassed solution of **24** (25 mg, 21.8 μmol) in MeOH/THF (3:2, 2.5 mL) containing AcOH (50 μL). The mixture was stirred under H_2 atmosphere for 2 h. The mixture was filtered through a layer of Celite, rinsed with MeOH/THF (3:2), and then concentrated under reduced pressure. The residue was dissolved in minimal AcOH/$CHCl_3$ and purified by silica gel chromatography (99:1 $CHCl_3$/AcOH then 7:2:0.1:0.1 $CHCl_3$/MeOH/H_2O/AcOH) to give **1a** (14.5 mg, 85%) as a colorless glass: $[\alpha]_D$ +39.2 (*c* 0.25 in DMSO); ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.81 (3 H, d, *J* = 6.5 Hz, $CHCH_3$), 0.85, 0.85 (6 H, 2 × t, *J* = 7.0 Hz, 2 × CH_2CH_3), 1.03−1.23 (51 H, m), 1.50 (4 H, m, palmityl and tuberculostearyl CH + CH_2), 2.24−2.31 (4 H, m, 2 × $OCOCH_2$), 3.22 (1 H, dd, $J_{1,2}$ = 3.7, $J_{2,3}$ = 9.4 Hz, H2), 3.55 (1 H, dd, *J* = 5.3, 10.9 Hz, H3′a), 3.69 (1 H, dd, *J* = 5.2, 10.7 Hz, H3′b), 3.77 (1 H, d, $J_{4,5}$ = 9.9 Hz, H5), 4.16 (1 H, dd, *J* = 6.8, 12.0 Hz, H1′a), 4.32 (1 H, dd, *J* = 2.7, 12.0 Hz, H1′b), 4.69 (1 H, d, $J_{1,2}$ = 3.3 Hz, H1), 5.11 (1 H, m, H2′); ^{13}C

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NMR (125 MHz; DMSO-d_6) δ 13.9 (1 C, 2 × CH_2CH_3), 19.6 (1 C, $CHCH_3$), 22.1, 24.4, 24.4, 26.40, 26.44, 28.43, 28.45, 28.69, 28.71, 28.8, 29.0, 29.0, 29.1, 29.36, 29.37, 31.3, 32.1, 33.4, 33.5, 36.42, 36.44 (30 C, palmityl and tuberculostearyl CH + CH_2), 62.2 (1 C, C1′), 65.5 (1 C, C3′), 69.6 (1 C, C2′), 71.4, 71.8, 72.6 (4 C, C2,3,4,5), 99.4 (1 C, C1), 171.1 (1 C, C6), 172.2, 172.5 (2 C, 2 × CO_2CH_2); HRMS (ESI$^+$) calcd for $C_{44}H_{82}NaO_{11}$ [M + Na]$^+$ m/z 809.5749, found 809.5739.

Benzyl (1-*O*-Palmityl-2-*O*-(4-methoxybenzyl)-*sn*-glyceryl 2,3,4-Tri-*O*-benzyl-α-D-glucopyranosid)uronate (25). Palmitic acid (45.3 mg, 0.177 mmol), COMU (75.8 mg, 0.177 mmol), and DMAP (21.6 mg, 0.177 mmol) was added to a stirred solution of **17** (87.6 mg, 0.117 mmol) in DMF (2 mL). The mixture was stirred under N_2 for 24 h, a second portion of palmitic acid, COMU, and DMAP (as above) was added, and the mixture was stirred for a further 24 h. The mixture was diluted with EtOAc, washed with satd aq $NaHCO_3$, water, and brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (10:60:30 EtOAc/toluene/petroleum spirits) to give **25** (100 mg, 87%) as a colorless oil: $[\alpha]_D$ +22.8 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 (3 H, t, *J* = 7.0 Hz, CH_3), 1.27−1.33 (24 H, m), 1.57−1.61 (2 H, m, $(CH_2)_{13}$), 2.28 (2 H, t, *J* = 7.5 Hz, $OCOCH_2$), 3.53 (1 H, t, *J* = 7.5, 12.0 Hz, H3′a), 3.61 (1 H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.6 Hz, H2), 3.76 (1 H, dd, $J_{3,4}$ = 9.1, $J_{4,5}$ = 10.0 Hz, H4), 3.78 (3 H, s, OCH_3), 3.79−3.83 (2 H, m, H2′,3′b), 4.00 (1 H, dd, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H3), 4.16 (1 H, dd, *J* = 5.0, 11.8 Hz, H1′a), 4.30 (1 H, d, $J_{4,5}$ = 9.7 Hz, H5), 4.32 (1 H, dd, *J* = 4.1, 11.7 Hz, H1′b), 4.46, 4.75 (2 H, 2 × d, *J* = 10.8 Hz, CH_2Ph), 4.60 (2 H, s, CH_2Ph), 4.65, 4.75 (2 H, 2 × d, *J* = 12.0 Hz, CH_2Ph), 4.81, 4.96 (2 H, 2 × d, *J* = 10.9 Hz, CH_2Ph), 4.85 (1 H, d, $J_{1,2}$ = 3.5 Hz, H1), 5.15, 5.18 (2 H, 2 × d, *J* = 12.2 Hz, CH_2Ph), 6.82−6.84 (2 H, m), 7.13−7.15 (2 H, m), 7.24−7.33 (20 H, m, Ar,Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2 (1 C, CH_3), 22.8, 25.0, 29.3, 29.4, 29.5, 29.6, 29.77, 29.79, 29.80, 29.83, 32.1, 34.3 (14 C, $(CH_2)_{14}$), 55.4 (1 C, OCH_3), 63.2 (1 C, C1′), 68.4 (1 C, C3′), 70.8 (1 C, C5), 67.4, 71.8, 73.3, 75.0, 75.1, 75.9 (6 C, C2′ and 5 × CH_2Ar), 79.6 (1 C, C2), 79.7 (1 C, C4), 81.3 (1 C, C3), 98.1 (1 C, C1), 113.9, 127.75, 127.76, 127.9, 128.0, 128.1, 128.4, 128.49, 128.54, 128.55, 128.57, 128.7, 129.5, 130.3, 135.2, 138.1, 138.2, 138.7, 159.4 (30 C, Ar,Ph), 169.6 (1 C, C6), 173.7 (1 C, CO_2CH_2); HRMS (ESI$^+$) calcd for $C_{61}H_{78}O_{11}$ [M + Na]$^+$ m/z 1009.5436, found 1009.5441.

Benzyl (1-*O*-Palmityl-2-*O*-(*R*)-tuberculostearyl-*sn*-glyceryl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosid)uronate (27). Cerium ammonium nitrate (127 mg, 0.231 mmol) was added to a stirred solution of **25** (76 mg, 77 μmol) in MeCN/H_2O (11:1, 6 mL) at rt. The mixture was stirred at rt for 2 h, diluted with EtOAc (20 mL) and H_2O (5 mL), and stirred for 10 min. The organic layer was separated, washed with water (2 × 5 mL) and brine (5 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was placed on high vacuum for 2 h and then used directly in the next step. (*R*)-Tuberculostearic acid (**4**) (23 mg, 77 μmol in 0.5 mL DMF), COMU (36 mg, 85 μmol), and DMAP (14 mg, 116 μmol) were added to a stirred solution of the crude alcohol **26** in DMF (1 mL). The mixture was stirred under N_2 for 24 h, a second equal portion of (*R*)-tuberculostearic acid, COMU, and DMAP (as above) was added, and the mixture was stirred for a further 24 h. The mixture was diluted with EtOAc, washed with satd aq $NaHCO_3$, water, and brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (5:60:35 EtOAc/toluene/petroleum spirits) to give **27** (67 mg, 76%) as a colorless oil: $[\alpha]_D$ +19.5 (*c* 1.35 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (3 H, d, *J* = 6.5 Hz, $CHCH_3$), 0.90, 0.90 (6 H, 2 × t, *J* = 7.0 Hz, 2 × CH_2CH_3), 1.07−1.60 (55 H, 3 × m, palmityl and tuberculostearyl CH + CH_2), 2.25−2.29 (4 H, m, 2 × $OCOCH_2CH_2$), 3.56 (1 H, dd, *J* = 5.1, 10.8 Hz, H3′a), 3.59 (1 H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.6 Hz, H2), 3.75 (1 H, dd, $J_{3,4}$ = 9.1, $J_{4,5}$ = 9.8 Hz, H4), 3.81 (1 H, dd, *J* = 5.2, 10.8 Hz, H3′b), 3.96 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H3), 4.22 (1 H, dd, *J* = 6.0, 11.9 Hz, H1′a), 4.27 (1 H, d, $J_{4,5}$ = 10.0 Hz, H5), 4.43 (1 H, dd, *J* = 3.7, 11.9 Hz, H1′b), 4.45, 4.74 (2 H, 2 × d, *J* = 10.8 Hz, CH_2Ph), 4.62, 4.77 (2 H, 2 × d, *J* = 12.0 Hz, CH_2Ph), 4.76 (1 H, d, $J_{1,2}$ = 3.5 Hz, H1), 4.80, 4.94 (2 H, 2 × d, *J* = 10.8 Hz, CH_2Ph), 5.16 (2 H, s, CH_2Ph), 5.24 (1 H, m, H2′), 7.12−7.14 (2 H, m), 7.25−7.35 (18 H, m, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3 (2 C, CH_2CH_3), 19.8 (1 C, $CHCH_3$), 22.8, 25.0, 27.2, 29.26, 29.29, 29.47, 29.49, 29.52, 29.66, 29.72, 29.81, 29.85, 30.17, 30.20, 32.1, 32.9, 34.2, 34.4, 37.3 (30 C, palmityl and tuberculostearyl CH + CH_2), 62.5 (1 C, C1′), 66.9 (1 C, C3′), 69.7 (1 C, C2′), 70.9 (1 C, C5), 67.4, 73.5, 75.2, 76.0 (4 C, 4 × CH_2Ph), 79.7 (2 C, C2,4), 81.2 (1 C, C3), 98.2 (1 C, C1), 127.8, 127.9, 128.0, 128.1, 128.4, 128.5, 128.57, 128.65, 128.7, 135.2, 138.0, 138.2, 138.6 (24 C, Ph), 169.6 (1 C, C6), 173.1, 173.5 (2 C, $OCOCH_2$); HRMS (ESI$^+$) calcd for $C_{72}H_{106}NaO_{11}$ [M + Na]$^+$ m/z 1169.7627, found 1169.7628.

1-*O*-Palmityl-2-*O*-((*R*)-10-tuberculostearyl)-*sn*-glyceryl α-D-glucopyranosiduronic Acid (1b). Pd(OH)$_2$−C (20%, 30 mg) was added to a degassed solution of **27** (37 mg, 32 μmol) in MeOH/THF (3:2, 5 mL) containing AcOH (100 μL). The mixture was stirred under a H_2 atmosphere for 2 h. The mixture was filtered through a layer of Celite, rinsed with MeOH/THF (3:2), and then concentrated under reduced pressure. The residue was dissolved in minimal $CHCl_3$/AcOH and purified by silica gel chromatography (99:1 $CHCl_3$/AcOH then 7:2:0.1:0.1 $CHCl_3$/MeOH/H_2O/AcOH) to give **1b** (23 mg, 90%) as a colorless powder: $[\alpha]_D$ +33.5 (*c* 0.48 in DMSO); ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.80 (3 H, d, *J* = 6.5 Hz, $CHCH_3$), 0.84, 0.84 (6 H, 2 × t, *J* = 7.0 Hz, 2 × CH_2CH_3), 1.04−1.49 (55 H, 3 × m, palmityl and tuberculostearyl CH + CH_2), 2.25−2.29 (4 H, m, 2 × $OCOCH_2$), 3.22 (1 H, dd, $J_{1,2}$ = 3.5, $J_{3,4}$ = 9.6 Hz, H2), 3.54 (1 H, dd, *J* = 5.6, 11.0 Hz, H3′a), 3.69 (1 H, dd, *J* = 4.9, 11.0 Hz, H3′b), 3.76 (1 H, d, $J_{4,5}$ = 10.1 Hz, H5), 4.16 (1 H, dd, *J* = 6.6, 12.0 Hz, H1′a), 4.32 (1 H, dd, *J* = 2.1, 12.0 Hz, H1′b), 4.69 (1 H, d, $J_{1,2}$ = 3.5 Hz, H1), 5.11 (1 H, m, H2′); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.9 (1 C, 2 × CH_2CH_3), 19.6 (1 C, $CHCH_3$), 22.1, 24.42, 24.44, 26.4, 26.5, 28.4, 28.7, 28.76, 28.79, 28.95, 29.02, 29.1, 29.36, 29.39, 31.3, 32.1, 33.4, 33.5, 36.43, 36.46 (30 C, palmityl and tuberculostearyl CH + CH_2), 62.2 (1 C, C1′), 65.5 (1 C, C3′), 69.6 (1 C, C2′), 71.4, 71.7, 71.8, 72.6 (4 C, C2,3,4,5), 99.4 (1 C, C1), 171.0 (1 C, C6), 172.3, 172.5 (2 C, CO_2CH_2); HRMS (ESI$^+$) calcd for $C_{44}H_{82}NaO_{11}$ [M + Na]$^+$ m/z 809.5749, found 809.5743.

Octyl α-D-Glucopyranoside (37). Acetyl chloride (6.59 g, 84.0 mmol) was slowly added to a mixture of D-glucose (6.29 g, 34.9 mmol) and octanol (54.6 g, 419 mmol) in DMF (25 mL) at 60 °C, resulting in the complete dissolution of D-glucose. The mixture was heated at 60 °C for 6 h, and then $NaHCO_3$ (7.1 g) was added to quench the reaction. The mixture was filtered, and the filtrate was purified by flash chromatography (100% petroleum spirits then 9:1 EtOAc/EtOH). The mixture was recrystallized from water, twice, to yield **37** as white needles (3.06 g, 30%): mp 71−72 °C (lit.[44] mp 73−74.4 °C); $[\alpha]^{25}_D$ +141 (*c* 1.05 in MeOH) (lit.[44] $[\alpha]^{25}_D$ +120.0); ^{1}H NMR (499.7 MHz, CD_3OD) δ 0.92−0.95 (3 H, m, CH_3), 1.34−1.45 (10 H, br m, 5 × CH_2), 1.62−1.72 (2 H, m, CH_2), 3.30−3.34 (1 H, m, H4), 3.40−3.43 (1 H, dd, $J_{1,2}$ 3.5, $J_{2,3}$ 9.5 Hz, H2), 3.45−3.49 (1 H, m, OCH_2), 3.58−3.62 (1 H, m, H5), 3.65−3.78 (3 H, m, H3,6,OCH_2), 3.81−3.84 (1 H, dd, $J_{5,6'}$ 2.5, $J_{6,6'}$ 12.5 Hz, H6′), 4.79−4.80 (1 H, d, $J_{1,2}$ 4.0 Hz, H1); ^{13}C NMR (125.6 MHz, CD_3OD) δ 14.4, 23.7, 27.3, 30.4, 30.56, 30.61, 33.0 (7 C, octyl), 62.7, 69.1, 71.8, 73.57, 73.59, 75.2 (6 C, C2,3,4,5,6,C1′-octyl), 100.0 (C1).

Octyl α-D-Glucopyranosiduronic Acid (38). A solution of **37** (640 mg, 2.20 mmol), TEMPO (68 mg, 0.4 mmol), and KBr (260 mg, 2.2 mmol) was dissolved in $NaHCO_3$/Na_2CO_3 solution (10 mL, pH 9.5). The mixture was cooled to 0 °C, and $Ca(OCl)_2$ (629 mg, 4.4 mmol) was added. The mixture was stirred under N_2 for 40 min, and then $Na_2S_2O_3$ was added to quench the reaction. The pH was adjusted to pH 2 by dropwise addition of 2 M HCl. The mixture was extracted with 1-butanol (5 × 10 mL), and the organic layers was combined and evaporated under reduced pressure. The residue was purified by flash chromatography (7:2:1 EtOAc/MeOH/H_2O + 0.5% AcOH) to give **38** as a colorless paste (607 mg, 90%): mp 71−72 °C (lit.[62] mp >250 °C); $[\alpha]_D^{25}$ +120 (*c* 1.02 in MeOH); ^{1}H NMR (499.7 MHz, CD_3OD) δ 0.92−0.95 (3 H, m, CH_3), 1.34−1.45 (10 H, br m, 5 × CH_2), 1.62−1.72 (2 H, m, CH_2), 3.54−3.81 (5 H, m, H2,3,4,OCH_2), 4.06 (1 H, d, $J_{4,5}$ 9.5 Hz, H5), 4.97 (1 H, d, $J_{1,2}$ 3.5 Hz, H1); ^{13}C NMR (125.6 MHz, CD_3OD + CF_3CO_2H) δ 14.4, 23.7, 27.3, 30.4, 30.5, 33.0 (7 C, octyl), 69.8, 72.9, 73.1, 73.4, 74.6 (6 C, C2,3,4,5,C1′-octyl), 100.6 (C1), 173.4 (C=O).

Octyl 4,6-*O*-Benzylidene-α-D-glucopyranoside (41). A mixture of **37** (3.38 g, 12.0 mmol), benzaldehyde dimethyl acetal (4.40 g, 16.0 mmol), and *p*-toluenesulfonic acid (100 mg) in $CHCl_3$ (50 mL) was refluxed under Dean−Stark conditions for 20 min. The mixture was neutralized by addition of K_2CO_3 (5 g) and was stirred for 1 h. The

mixture was filtered, and the filtrate was concentrated under reduced pressure to give a yellow oil. The oil was purified by flash chromatography (1:1 EtOAc/petroleum spirits) to give **41** as a white solid (3.79 g, 83%): mp 101−102 °C (lit.[63] mp 74 °C); $[\alpha]_D^{25}$ +120 (*c* 1.05 in $CHCl_3$); ^{1}H NMR (499.7 MHz, $CDCl_3$) δ 0.88−0.91 (3 H, m, CH_3), 1.28−1.37 (10 H, br m, 5 × CH_2), 1.60−1.66 (2 H, m, CH_2), 2.37 (1 H, br s, OH), 3.00 (1 H, br s, OH), 3.43−3.49 (2 H, m, H4,OCH_2), 3.59−3.60 (1 H, m, H2), 3.70−3.75 (2 H, m, H6,OCH_2), 3.78−3.83 (1 H, m, H5), 3.91 (1 H, t, *J* 9.5 Hz, H3), 4.28 (1 H, dd, $J_{5,6'}$ 4.5, $J_{6,6'}$ 10.0 Hz, H6′), 4.85−4.86 (1 H, d, $J_{1,2}$ 4.0 Hz, H1), 5.52 (1 H, s, PhCH), 7.35−7.51 (5 H, m, Ar−H); ^{13}C NMR (125.6 MHz, $CDCl_3$) δ 14.4, 22.8, 26.2, 29.3, 29.5, 29.6, 31.9 (7 C, octyl), 62.7, 69.0, 69.2, 72.1, 73.2, 81.2 (6 C, C2,3,4,5,6,C1′-octyl), 98.9, 102.1 (C1, PhCH), 126.5, 128.5, 129.4, 137.3 (Ar).

Octyl 2,3-*O*-Acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (42). A mixture of **41** (3.58 g, 6.78 mmol) and acetic anhydride (18 mL, 190 mmol) in pyridine (35 mL) was stirred for 3 h and then was diluted with ice−water (200 mL) and the organic phase extracted with EtOAc (3 × 150 mL). The extract was washed with 2 M HCl (2 × 100 mL), satd $NaHCO_3$ (3 × 150 mL), and water (3 × 100 mL). The extract was dried ($MgSO_4$) and the solvent evaporated under reduced pressure to afford **42** as a white solid (2.87 g, 91%): mp 86−87 °C; $[\alpha]_D^{25}$ +104 (*c* 1.03 in $CHCl_3$); ^{1}H NMR (499.7 MHz, $CDCl_3$) δ 0.87−0.90 (3 H, m, CH_3), 1.28−1.36 (10 H, br m, 5 × CH_2), 1.57−1.63 (2 H, m, CH_2), 2.06 (1 H, s, Ac), 2.07 (1 H, s, Ac), 3.37−3.42 (1 H, m, OCH_2), 3.63 (1 H, t, *J* 10.0 Hz, H4), 3.59 (1 H, m, H2), 3.67−3.72 (1 H, m, OCH_2), 3.75 (1 H, t, *J* 10.0 Hz, H6), 3.93−3.98 (1 H, m, H5), 4.29 (1 H, dd, $J_{5,6'}$ 5, $J_{6,6'}$ 10.5 Hz, H6′), 4.86 (1 H, d, $J_{1,2}$ 5.0, $J_{2,3}$ 10.0 Hz, H2), 5.05 (1 H, d, $J_{1,2}$ 4.0 Hz, H1), 5.50 (1 H, s, PhCH), 5.60 (1 H, t, *J* 9.5 Hz, H3), 7.33−7.46 (5 H, m, Ar-H); ^{13}C NMR (125.6 MHz, $CDCl_3$) δ 14.0, 20.6, 20.8, 22.6, 26.0, 29.18, 29.24, 29.3, 31.8 (9 C, octyl, CH_3CO × 2), 62.3, 68.6, 68.8, 69.0, 71.7, 79.3 (6 C, C2,3,4,5,6,C1′-octyl), 96.5, 101.4 (C1, PhCH), 126.1, 128.1, 130.0, 136.9 (Ar), 169.7, 170.4 (C=O × 2). Anal. Calcd for $C_{25}H_{36}O_8$: C, 64.64; H, 7.81. Found C, 64.69; H, 7.90.

Octyl 2,3-*O*-Acetyl-6-*O*-benzyl-α-D-glucopyranoside (43). Trifluoroacetic acid (3.29 g, 28.5 mmol) was added dropwise to a solution of **42** (2.64 g, 5.68 mmol), triethylsilane (3.31 g, 28.5 mmol), and 4 Å molecular sieves (5 g) in CH_2Cl_2 (30 mL) at 0 °C. When the addition was complete (5 min), the mixture was warmed to room temperature and stirred overnight. The mixture was filtered through Celite and poured into ice-cold satd $NaHCO_3$ (100 mL). The organic phase was extracted with CH_2Cl_2 (3 × 30 mL), and the combined extract was dried ($MgSO_4$) to give **43** as a pale yellow oil (2.21 g, 83%): $[\alpha]_D^{25}$ +81 (*c* 0.97 in $CHCl_3$); ^{1}H NMR (499.7 MHz, $CDCl_3$) δ 0.88 (3 H, t, *J* 6.8, CH_3), 1.23−1.33 (10 H, br m, 5 × CH_2), 1.54−1.60 (2 H, m, CH_2), 2.04 (1 H, s, Ac), 2.08 (1 H, s, Ac), 2.87 (1 H, br s, OH), 3.36−3.41 (1 H, m, OCH_2), 3.66−3.86 (5 H, m, H4,5,6,6′,OCH_2), 4.57 4.62 (2 H, 2 d, *J* 12.9, PhCH_2), 4.82 (1 H, dd, $J_{1,2}$ 3.5, $J_{2,3}$ 10.0 Hz, H2), 5.01 (1 H, d, $J_{1,2}$ 3.5 Hz, H1), 5.32 (1 H, dd, $J_{3,4}$ 9.0, $J_{2,3}$ 10.0, H3), 7.25−7.35 (5 H, m, Ar−H); ^{13}C NMR (125.6 MHz, $CDCl_3$) δ 14.0, 20.7, 20.9, 26.0, 29.2, 29.2, 31.8 (9 C, octyl, CH_3CO × 2), 68.4, 69.5, 70.0, 70.7, 70.9, 73.4, 73.6 (7 C, C2,3,4,5,6,PhCH_2,C1′-octyl), 95.6 (C1), 127.6, 127.7, 128.4, 137.8 (4 C, Ar), 170.3, 171.6 (2 C, C=O). Anal. Calcd for $C_{25}H_{38}O_8$: C, 64.36; H, 8.21. Found: C, 64.39; H, 8.20.

Octyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-α-D-glucopyranoside (45). A suspension of the trichloroacetimidate **44**[47] (2.03 g, 4.12 mmol), acceptor **43** (1.28 g, 2.74 mmol), and 4 Å molecular sieves (3 g) in dry CH_2Cl_2 (20 mL) was stirred at room temperature for 30 min and then cooled to −40 °C. TMSOTf (98 μL, 0.12 g, 0.55 mmol) was slowly added, and the mixture was stirred at −40 °C for 2 h. The mixture was warmed to room temperature, stirred for a further 2 h, neutralized with Et_3N, and filtered through Celite. The filtrate was reduced to dryness and purified by flash chromatography (3:7 EtOAc/petroleum spirits) to give **45** as a pale yellow oil (1.97 g, 90%): $[\alpha]_D^{25}$ +89 (*c* 1.07 in $CHCl_3$); ^{1}H NMR (499.7 MHz, $CDCl_3$) δ 0.86−0.89 (3 H, m, CH_3), 1.24−1.35 (10 H, br m, 5 × CH_2), 1.57−1.63 (2 H, m, CH_2), 1.97, 2.02, 2.03, 2.04, 2.08, 2.12 (18H, 6 × s, Ac), 3.34−42 (1 H, m, OCH_2), 3.65−3.94 (7 H, m, H4,5,5′,6,6,6′,OCH_2), 4.11 (1 H, dd, $J_{5',6'}$ 4.5, $J_{6',6'}$ 12.0 Hz, H6′), 4.53 (1 H, dd, *J* 11.2 Hz, PhCH_2), 4.59 (1 H, dd, *J* 11.2 Hz, PhCH_2), 4.70 (1 H, dd, $J_{1,2}$ 3.5, $J_{2,3}$ 10.5 Hz, H2), 4.95−4.96 (2 H, m, H1,1′), 5.03−5.04 (1 H, m, H2′), 5.18−5.23 (2 H, m, H3′,4′), 5.49−5.53 (1 H, m, H3), 7.25−7.35 (5 H, m, Ar−H); ^{13}C NMR (125.6 MHz, $CDCl_3$) δ 13.9, 20.37, 20.44. 20.5, 20.57, 20.65, 22.4, 25.8, 29.0, 29.1, 31.6 (13 C, octyl, CH_3 × 6), 62.2, 65.8, 68.3, 68.4, 69.3, 69.5, 69.6, 71.37, 71.40, 73.2, 77.1 (12 C, C2,2′,3,3′,4,4′,5,5′,6,6′,PhCH_2O,C1″-octyl), 95.43, 99.19 (C1,1′), 127.3, 127.4, 128.2, 137.8 (4 C, Ar), 169.3, 169.4, 169.6, 169.8, 170.1, 170.2 (6 C, C=O). Anal. Calcd for $C_{39}H_{56}O_{17}$: C, 58.78; H, 7.08. Found: C, 58.72; H, 7.11. Evidence for the exclusive formation of the α-anomer in this glycosylation was obtained through examination of the $^1J_{C,H}$ coupling constants for the anomeric carbons of the disaccharide. Each constant was >170 Hz (175 and 171 Hz), thereby showing that all *O*-glycosidic linkages formed in this work are α.[64]

Octyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-D-glucopyranoside (46). A mixture of **45** (2.31 g, 2.90 mmol) and $Pd(OH)_2$ (10%, 200 mg) in EtOAc/EtOH (2:1, 30 mL) was stirred under H_2 overnight. The mixture was filtered through Celite and the solvent evaporated to give a residue, which was purified by flash chromatography (1:1 EtOAc/petroleum spirits) to afford **46** as a pale yellow oil (1.87 g, 91%): $[\alpha]_D^{25}$ +85 (*c* 1.02 in $CHCl_3$); ^{1}H NMR (499.7 MHz, $CDCl_3$) δ 0.86−0.89 (3 H, m, CH_3), 1.24−1.34 (10 H, m, 5 × CH_2), 1.56−1.61 (2 H, m, CH_2), 1.98, 2.03, 2.03, 2.06, 2.08, 2.12 (18H, 6 × s, Ac), 3.36−3.40 (1 H, m, OCH_2), 3.63−3.67 (1 H, m, OCH_2), 3.77−3.86 (3 H, m, H5,6,6), 3.92 (1 H, t, $J_{3,4}$ 9.5, $J_{4,5}$ 9.5 Hz, H4), 4.08 (1 H, ddd, $J_{4,5}$ 9.5, $J_{5,6}$ 2.4, 6.9 Hz, H5), 4.12−4.15 (1 H, dd, $J_{5',6'}$ 2.5, $J_{6',6'}$ 12.0 Hz, H6′), 4.20−4.23 (1 H, dd, $J_{5',6'}$ 6.3, $J_{6',6'}$ 12.0 Hz, H6′), 4.72 (1 H, dd, $J_{1,2}$ 3.5, $J_{2,3}$ 10.0 Hz, H2), 5.01 (1 H, d, $J_{1,2}$ 3.5 Hz, H1), 5.04−5.06 (2 H, m, H1′,2′), 5.22 (1 H, t, *J* 10.0 Hz, H4′), 5.29 (1 H, dd, $J_{1',2'}$ 2.5, $J_{2',3'}$ 10.0 Hz, H3′), 5.58 (1 H, dd, $J_{3,4}$ 9.3, $J_{2,3}$ 10.0 Hz, H3); ^{13}C NMR (125.6 MHz, $CDCl_3$) δ 13.9, 20.4, 20.46, 20.50, 20.51, 20.6, 20.7, 22.5, 25.8, 29.07, 29.11, 29.13, 31.7 (13 C, octyl, CH_3CO × 6), 61.0, 62.7, 66.2, 68.2, 68.5, 69.6, 69.8, 70.0, 71.47, 71.54, 76.3 (11 C, C2,2′,3,3′,4,4′,5,5′,6,6′,C1″-octyl), 95.5, 99.2 (C1,1′), 169.4, 169.5, 169.6, 169.8, 170.3, 170.4 (6 C, C=O); HRMS (ESI^+) calcd for $C_{32}H_{50}NaO_{17}$ $[M + Na]^+$ *m*/*z* 729.2940, found 729.2933.

Octyl α-D-Mannopyranosyl-(1→4)-α-D-glucopyranoside (39). A mixture of **46** (380 mg, 0.54 mmol) and 0.5 M NaOMe in MeOH (15 mL) was stirred for 4 h. The mixture was reduced to dryness and purified by flash chromatography (17:2:1 EtOAc/MeOH/H_2O). Further purification by reversed-phase chromatography (water/MeOH) afforded **39** as a white powder (177 mg, 72%): $[\alpha]_D^{25}$ +98 (*c* 0.99 in MeOH); ^{1}H NMR (499.7 MHz, CD_3OD) δ 0.92−0.95 (3 H, m, CH_3), 1.33−1.41 (10 H, m, 5 × CH_2), 1.63−1.68 (2 H, m, CH_2), 3.39 (1 H, $J_{1,2}$ 3.4, $J_{2,3}$ 9.3 Hz, H2), 3.42−3.47 (2 H, m, OCH_2), 3.51−3.83 (10 H, m, H2,3,4,5,6,6,3′,4′,5′,6′,OCH_2), 3.86 (1 H, dd, $J_{5',6'}$ 1.5, $J_{6',6'}$ 11.5 Hz, H6′), 3.95 (1 H, dd, $J_{1',2'}$ 1.5, $J_{2',3'}$ 3.0 Hz, H6′), 4.77 (1 H, d, $J_{1,2}$ 3.5 Hz, H1), 5.35 (1 H, d, $J_{1',2'}$ 1.5 Hz, H1′); ^{13}C NMR (125.6 MHz, CD_3OD) δ 14.4, 23.7, 27.3, 30.4, 30.5, 30.6, 33.0 (7 C, octyl), 62.3, 63.0, 68.6, 69.2, 72.2, 72.3, 72.5, 73.7, 75.5, 75.6, 77.3 (11 C, C2,2′,3,3′,4,4′,5,5′,6,6′,C1″-octyl), 99.9, 102.8 (C1,1′); HRMS (ESI^+) calcd for $C_{20}H_{38}NaO_{11}$ $[M + Na]^+$ *m*/*z* 477.2306, found 477.2304.

Octyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-D-glucopyranosiduronic Acid (47). A mixture of **46** (466 mg, 0.66 mmol), TEMPO (62 mg, 0.40 mmol), and BAIB (bisacetoxyiodobenzene) (1.28 g, 3.96 mmol) in CH_2Cl_2−H_2O (2:1, 15 mL) was stirred at room temperature for 3 h. The reaction was quenched with 10% aq $Na_2S_2O_3$ (40 mL), and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic phase was washed with water and dried ($MgSO_4$). After evaporation of the solvent, the residue was purified by flash chromatography (1:1 EtOAc−petroleum spirits then 7:1:0.1 EtOAc−petroleum spirits−AcOH) to give **47** as a colorless paste (428 mg, 90%): $[\alpha]_D^{25}$ +89 (*c* 1.07 in $CHCl_3$); ^{1}H NMR (499.7 MHz, $CDCl_3$) δ 0.84−0.87 (3 H, m, CH_3), 1.25−1.34 (10 H, m, 5 × CH_2), 1.54−1.65 (2 H, m, CH_2), 1.97, 2.01, 2.02, 2.07, 2.10, 2.12 (18H, 6 × s, Ac), 3.38−3.43 (1 H, m, OCH_2), 3.69−3.74 (1 H, m, OCH_2), 3.99−4.02 (2 H, m, H4,5′), 4.10−4.13 (2 H, m, H6′,6′), 4.28 (1 H, d, $J_{4,5}$ 10.0 Hz, H5), 4.78 (1 H, dd, $J_{1,2}$ 3.5, $J_{2,3}$ 10.0 Hz, H2), 5.07 (1 H, d, $J_{1,2}$ 3.5 Hz, H1), 4.99−5.01 (2 H, m, H1,2′), 5.24−5.33 (2 H, m, H3′,4′), 5.51−5.55 (1 H, dd, $J_{3,4}$ 9.5, $J_{2,3}$ 10.0 Hz, H3); ^{13}C NMR (125.6 MHz, $CDCl_3$) δ 14.0, 20.55, 20.58, 20.7, 20.9, 22.6. 25.8, 29.13, 29.14, 29.2, 31.7 (13 C,

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octyl, $CH_3 \times 6$), 62.7, 66.2, 68.3, 68.7, 69.1, 69.8, 69.9, 70.91, 70.94, 78.4 (10 C, C2,2′,3,3′,4,4′,5,5′,6′,C1″-octyl), 96.0, 99.1 (C1,1′), 169.6, 169.7, 169.76, 169.84, 170.0, 170.3, 172.8 (7 C, C═O) HRMS (ESI^+) calcd for $C_{32}H_{48}NaO_{18}$ $[M + Na]^+$ m/z 743.2733, found 743.2733.

Octyl α-D-Mannopyranosyl-(1→4)-α-D-glucopyranosiduronic Acid (40). A mixture of **47** (273 mg, 0.38 mmol) and NaOMe in MeOH (0.5 M, 10 mL) was stirred for 4 h. The solution was acidified with trifluoroacetic acid, and then reduced to dryness, and the residue was purified by flash chromatography (17:2:1 EtOAc−MeOH−H_2O) to give the product as a colorless paste. Further purification by reversed-phase chromatography (water/MeOH) gave **40** as a colorless paste (115 mg, 65%): $[\alpha]^{25}_D$ +89 (*c* 1.07 in MeOH); ^1H NMR (499.7 MHz, CD_3OD) δ 0.92−0.95 (3 H, m, CH_3), 1.34−1.43 (10 H, m, 5 × CH_2), 1.65−1.72 (2 H, m, CH_2), 3.47−3.58 (3 H, m, H2,5′,OCH_2), 3.68−3.83 (7 H, m, H3,4,3′,4′,6′,6′,OCH_2), 3.91 (1 H, dd, $J_{1,2}$ 1.7, $J_{2,3}$ 3.2 Hz, H2′), 4.10−4.12 (1 H, m, H5), 4.83 (1 H, d, $J_{1,2}$ 4.0 Hz, H1), 5.39 (1 H, d, $J_{1',2'}$ 1.0 Hz, H1′); ^{13}C NMR (125.6 MHz, CD_3OD) δ 14.4, 23.7, 27.3, 30.4, 30.5, 30.6, 33.0 (7 C, octyl), 62.4, 68.0, 69.8, 72.3, 72.4, 73.3, 74.6, 75.2, 78.4 (10 C, C2,2′,3,3′,4,4′,5,5′,6′,C1″-octyl), 100.6, 102.2 (C1,1′), 173.1 (C═O); HRMS (ESI^+) calcd for $C_{20}H_{36}NaO_{12}$ $[M + Na]^+$ m/z 491.2099, found 491.2091.

Preparation of Crude Cellular Fractions. *Coyrnebacterium glutamicum* ATCC 13032 was grown in BHI medium (brain heart infusion, Oxoid) until mid log phase (OD_{600} = 3−8) and then harvested. The pellet was washed in 25 mM Hepes, pH 7.4 and resuspended in ice-cold sonication buffer (25 mM Hepes, pH7.4, protease inhibitor cocktail, "Complete, EDTA-free", 1 mM DTT) to 0.25 g wet weight/mL prior to sonication at 20 μm 4 × 45 s, on ice with 1 min interval (Soniprep 150, MSE, UK). The lysate was centrifuged at 4000*g* for 10 min at 4 °C, and the supernatant was used for assay as "lysate". The lysate was further centrifuged at 100000*g* for 1 h at 4 °C. The resulting supernatant was used as the "cytosol" fraction. The pellet was resupended in the sonication buffer to the same volume as cytosol fraction and used as the "membrane" fraction.

Preparation of Lipid Extracts. Total lipid extract of *M. tuberculosis* H37Rv was obtained from the Colarado State University TB Vaccine Testing and Research Materials Center (funded by NIH, NIAD N01-AI-40091).

Coyrnebacterium glutamicum ATCC 13032 was grown in 1 liter BHI (brain heart medium) at 30 °C to midlog phase (OD = 5.9) and harvested. The bacteria pellet was washed in PBS once and its wet weight measured (0.45 g).

M. smegmatis mc^2155 was grown in 10 mL of M7H10 medium supplemented with glycerol (0.2%), DC enrichment (0.2% glucose, 0.085% NaCl, final), at 37 °C for 2 days for a seed culture. The seed culture was diluted to 1/100 and cultured in the same medium as above for 15 h to midlog phase at 37 °C and harvested. The bacteria pellet was washed once using PBS and its wet weight measured (0.25 g).

Both bacterial pellets were treated with 15 mL of chloroform/methanol (2:1 (v/v)) for 2 h, 6 mL of chloroform/methanol (2:1 (v/v)) for 2 h, and 7.8 mL of chloroform/methanol/water (1:2:0.8 (v/v)) overnight. The first two chloroform/methanol extracts were transferred to another tube and dried under a stream of N_2. The third extract was transferred into the same tube, and then 6 mL of chloroform and 2.4 mL of water were added to make a final composition of chloroform/methanol/water 8:4:3 (v/v). The lower organic phase was transferred to a new glass tube and washed twice with chloroform/methanol/water 3:48:47(v/v). The organic phase was dried under a stream of N_2. This yielded the following crude lipid extracts: *C. glutamicum*, 31.5 mg, and *M. smegmatis*, 2.2 mg.

Mannosyltransferase Assays. A 90 μL aliquot of each fraction was mixed with $MgCl_2$ to 7.5 mM, 100 mM substrate (**37**−**40**) to 5 mM final concentration (or as indicated in the figure) or water for a total volume of 100 μL. After preincubation at 37 °C for 5 min, 2 μL of GDP-[2-^{3}H]mannose (6.3 Ci/mmol, final concentration, 170 nCi/reaction) or 100 mM cold GDP-mannose was added to start the reaction. After 2 h, the reaction was stopped by the addition of 375 μL of 1:2 (v/v) chloroform/methanol and vortexing. After the centrifugation at 4000*g* for 10 min, the supernatant was removed, an aliquot of chloroform/methanol/water (1:2:0.8, v/v) was added, and another extraction was performed. The second supernatant was pooled with the first and the total extract dried under a stream of N_2. The dried material was partitioned in a biphasic system of 1-butanol and water (2:1, v/v). After centifugation (16000*g* for 2 min), the lower aqueous phase was washed once with water-saturated 1-butanol and the combined butanol phases back-extracted with I volume of water before being dried in a rotational vacuum concentrator (RVC). The lipid residue was dissolved in 10 μL of water-saturated 1-butanol and analyzed by high-performance thin layer chromatography (HPTLC) using aluminum-backed silica gel 60 sheets. HPTLC sheets were developed in chloroform/methanol/1 M ammonium acetate/13 M ammonium solution/water (180:140:9:9:23, v/v) and then sprayed with En^{3}Hance and exposed to high sensitivity X-ray film (BiomaxMR film) at −80 °C to detect ^{3}H-labeled lipids.

For Studying the Effect of Metal Ions. EGTA (to a final concentration of 1 mM) and one each of $CaCl_2$, $CuCl_2$, $FeCl_3$, $MgCl_2$, $MnCl_3$, or $ZnCl_2$ (to a final concentration of 15 mM) were added to the assay mixtures.

For Studying the Effect of Amphomycin. $MgCl_2$ (7.5 mM), 5 μg of amphomycin, and 1 mM DTT were added to the assay mixture for 10 min prior to the addition of GDP-[^{3}H]mannose.

Mannosidase Treatment of Glycolipids. ^{3}H-mannose-labeled total lipids were dried and resuspended in 0.1 M sodium acetate buffer (pH 5.0) and treated with jack bean α-mannosidase (4 units) or 0.1 M citric acid-phosphate buffer (pH 4.5) and treated with snail β-mannosidase (0.5 units) in both cases in the presence of 0.1% taurodeoxycholate. The mixtures were incubated at 37 °C for 48 h. The reaction was stopped by the addition of 3.75 volumes of chloroform:methanol (1:2, v/v, and insoluble material removed by centrifugation (16000*g*, 5 min). The supernatant was dried under a stream of nitrogen, and the lipids were recovered by 1-butanol, water (2:1, v/v) biphasic partitioning as described above. The lipids were separated by HPTLC and detected on X-ray film using fluorography as described previously.

Multistage Mass Spectrometric Analysis of GlcAGroAc$_2$ ($C_{19:0}$/$C_{16:0}$) (1a), GlcAGroAc$_2$ ($C_{16:0}$/$C_{19:0}$) (1b), and *C. glutamicum*, *M. smegmatis*, and *M. tuberculosis* Extracts. Negative-ion multistage tandem mass spectrometry experiments were conducted using a modified Finnigan LTQ quadrupole ion-trap mass spectrometer equipped with a Finnigan electrospray ionization source. Compounds **1a** and **1b** were dissolved in $CHCl_3$ containing 1% AcOH to give 1 mg/mL stock solutions. Mass spectrometry samples were prepared by diluting the stock solutions to 0.01 mg/mL in methanol. Samples were directly injected into the mass spectrometer via the ESI probe at a rate of 5 μL/min. The sheath gas, spray voltage and capillary temperature were adjusted to approximately 5−20 au, 3−4.5 kV, and 330 °C, respectively. The tube lens and capillary voltages were adjusted to maximize the yields of the desired ions. Collision induced dissociation (CID) experiments were performed by mass selecting the desired precursor ion, with a selection window of 1.0−8 m/z (depending on the desired isotope envelope), and then subjecting it to an activation potential of 20−40% and an activation Q of 0.25−0.4 for a period of 30 ms. Crude glycolipid extracts were at 0.1 mg/mL in 1:1 $CHCl_3$/MeOH.

Mass Spectrometric Analysis of 40. The total lipid obtained after incubation of octyl α-D-glucopyranosiduronic acid (**38**) was 10 times diluted with a solution of 10 mM ammonium formate in methanol. The solution was flow injected into an Agilent 6520 Accurate-Mass Q-TOF LC/MS at the rate of 0.2 mL/min in H_2O/methanol/THF (14:20:66, v/v/v). The capillary voltage was set at 4000 V, and the gas temperature and flow rate were set to 250 °C and 7 L/min, respectively. The nebulizer was set at 40 psi. For MS^2 total ion scanning, the fragmentator was set to 135 V in negative mode. MS^2 experiments were conducted using an Agilent 6410 triple quadrupole mass spectrometer in negative mode with a fragmentator voltage of 135 V and collision energy of 20 eV.

■ ASSOCIATED CONTENT

Supporting Information

Figures S1 (additional characterization of MgtA) and S2 (mass spectrometric characterization of octyl ManGlcA **40**). Copies of

the ^{1}H and ^{13}C NMR spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org

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Notes

The authors declare no competing financial interest.

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