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Synthesis of Mycobacterial Triacylated Phosphatidylinositol Dimannoside Containing an Acyl Lipid Chain at 3-O of Inositol

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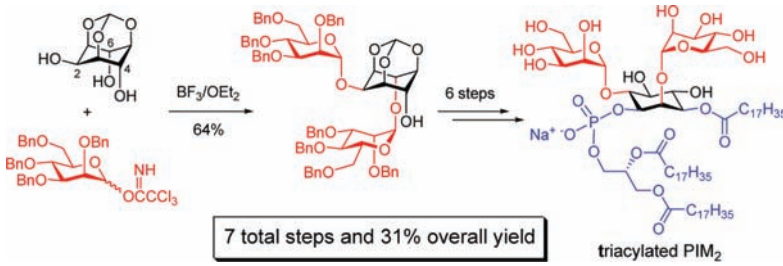
ABSTRACT



A seven-step synthesis of triacylated phosphatidylinositol dimannoside is described from *myo*-inositol 1,3,5-orthoformate. It proceeded in 31% overall yield via a highly regioselective and stereoselective 2,6-di-*O*-D-mannosylation as the key step.

Mycobacterium tuberculosis (*Mtb*), an acid-fast Gram-positive bacterium that persists in infected macrophages for a long-term period, is responsible for the development of the active disease tuberculosis (TB).[1] Although the current BCG vaccine is effective for preventing the incidence of miliary TB in children, it is not effective at protecting adults from TB infection. Due to the spread of multidrug-resistant *Mtb* strains and the increase of patients with lethal HIV-TB co-infection, the development of new drugs or vaccines to combat TB is indeed important.

The *Mtb* cell envelope, consisting of outer capsule, cell wall, and plasma membrane,[2] is responsible for the initial stage of host cell infection. It forms a thick protective barrier to prevent antibiotic penetration[3] and manipulates the host immune system in a way that develops a favorable environment for bacterial survival and growth.[4] The covalently ester-linked mycolic acids and peptidoglycan-arabinogalactans are the major components of cell wall, while phosphatidylinositol D-mannosides (PIMs, Figure 1), lipomannan (LM), lipoarabinomannan (LAM), and mannose-capped lipoarabinomannan (ManLAM) are noncovalently attached to the plasma membrane or outer capsule through their phosphatidylinositol anchor containing palmitic, stearic, and tuberculostearic acid residues. Among these components, PIMs are ubiquitously distributed in both pathogenic and nonpathogenic species of mycobacteria and play significant roles in cell-wall biogenesis[5] and in many immunomodulatory events, including neutralization of potentially cytotoxic O_2 free radicals,[6]

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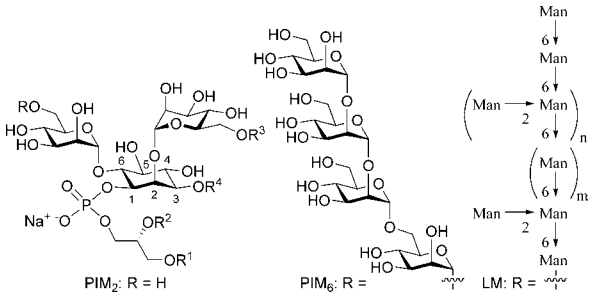


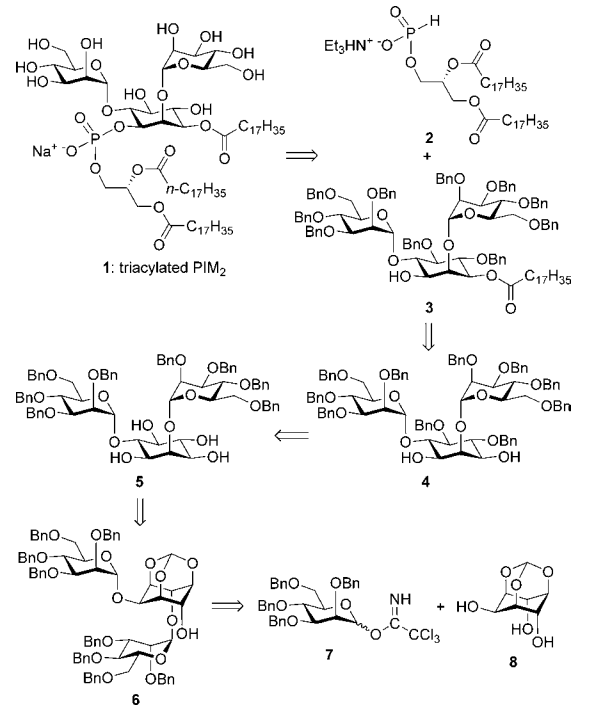
Figure 1. Structures of phosphatidylinositol dimannoside (PIM_2) and phosphatidylinositol hexamannoside (PIM_6). R^1, R^2: palmitic acid, stearic acid, or tuberculostearic acid. R^3, R^4: H, palmitic acid or stearic acid.

induction of cytokines,[7] phagocytosis of organism by binding with the mannose receptors,[8] and growth of organism in the host macrophages.[9] Different structures of PIMs impact the recognition and response of the host cell and influence the intercellular fate of pathogens.[10] PIMs also interact with VLA-5 on $CD4^+$ T lym-phocytes and activate integrins for promoting adhesion to the extracellular matrix glycoproteins.[11] A recent report has revealed that PIM_6 can stimulate the CD1b-restricted T cells, and the partial digestion of the oligomannose moiety assisted by CD1e for this process is essential.[12]

PIM_2, the smallest precursor for the biosynthesis of highly D-mannosylated molecules, has a diacylated phosphatidyl lipid moiety at the 1-O position of *myo*-inositol and two α-linked D-mannose sugars at the 2-O and 6-O positions. Elongation at the 6′-O position of the 6-O-linked D-mannosyl unit by an α1→6 D-mannosyl core, branched with α1→2-linked D-mannose, forms the higher PIMs and LM, which can be further extended by an arabinan domain to give LAM and ManLAM. The degree of acylation could vary the structures and functions of PIMs, LM, LAM, and ManLAM.[13] Acylations at the primary hydroxy group of the 2-*O*-linked D-mannosyl residue and/or at the 3-O position of *myo*-inositol residue leads to the triacylated and tetraacylated derivatives.

Alkaline hydrolysis of PIMs and LAM abolishing many immunoregulatory effects has clearly indicated the significance of the acyl lipid chains.[14] Owing to the structural complexity and potential immunotherapeutics,[15] the synthesis of diacylated and triacylated PIMs and LM with a lipid chain at 6′-O of the D-mannosyl ring has been documented in the literature.[15a,16] No report has addressed the preparation of triacylated and tetraacylated PIMs or LM containing an acyl chain at the 3-O position of *myo*-inositol. In continuation of our interest in the biosynthesis of PIMs and the development of anti-TB vaccines, we herein report the first synthesis of the triacylated PIM_2 **1**.

Scheme 1. Retrosynthesis of the Triacylated PIM_2 **1**



The generation of optically pure *myo*-inositol derivatives with appropriate protecting groups is a challenging task.[17] Typical procedures include multistep synthesis from D-glucose via Ferrier reaction,[18] resolution of racemic *myo*-

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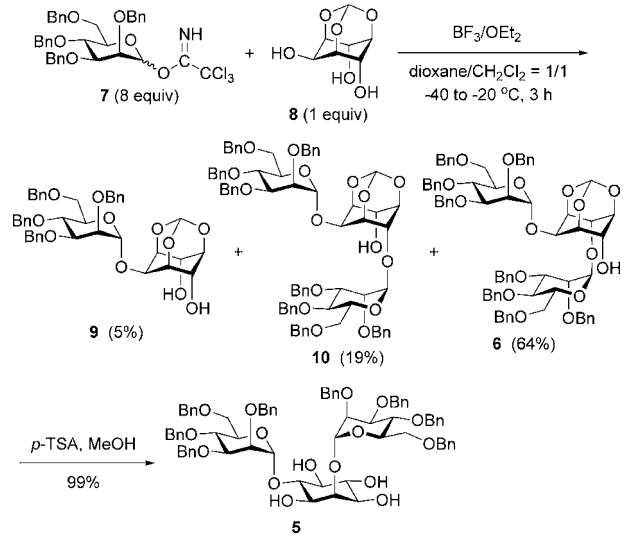
inositol compounds using chiral auxiliaries,[19] intramolecular olefin metathesis of the 1,7-octadiene derivatives followed by dihydroxylation,[20] and desymmetrization of *myo*-inositol employing peptide-based catalysts.[21] We recently found that sugars were useful chiral agents for desymmetrization in natural product synthesis.[16k,22] Conceptually, two D-mannosyl units could be simultaneously installed at the 2-O and 6-O positions of the meso *myo*-inositol-derived 2,4,6-triol. This desymmetrized approach would straightforwardly afford the chiral pseudotrisaccharide core. Scheme 1 illustrates our retrosynthetic design for the triacylated PIM_2 **1**. The target molecule **1** could be obtained by coupling of the *H*-phosphonate **2** with the 1-alcohol **3** followed by global deprotection of all benzyl groups. Due to the steric effect created by the bulkiness of two adjacent 2-*O*- and 6-*O*-linked D-mannosyl rings, the environment of the 1-*C*-hydroxyl group in the *myo*-inositol ring of 1,3-diol **4** is more hindered than the hydroxyl at the 3-C position. The stearyl group could be regioselectively introduced at the 3-*C*-hydroxyl of **4** to get the 1-alcohol **3**. In addition, the 2-*O*-attached mannosyl ring and the 3-*C*-hydroxyl orient toward the same β-face and the 4-*C*- and 5-*C*-hydroxyls are more reactive because of the steric effect. Regioselective 4,5-di-*O*-benzylation of 1,3,4,5-tetraol **5** would yield the desired diol **4**. The preparation of tetraol **5** could be carried out via hydrolysis of the orthoformate group in the 4-alcohol **6** under mild acidic conditions. Regioselective and stereoselective coupling of the D-mannosyl trichloroacetimidate **7**[23] with commercially available *myo*-inositol 1,3,5-orthoformate **8** would furnish the pseudotrisaccharide **6** in a one-pot manner. Theoretically, the first D-mannosyl unit could be assembled at the 2-*C*-equatorial hydroxyl followed by desymmetric installation of the second D-mannosyl ring at the 6-*C*-axial hydroxyl.

Although the strategy for direct glycosidation of 2,4,6-triol **8** is promising,[24] its low solubility in most common organic solvents, e.g., dichloromethane, ethyl ether, acetonitrile, toluene, and nitromethane, is a big concern. Compound **8** is slightly soluble in THF and completely dissolves in 1,4-dioxane. Since the melting point of dioxane is 11 °C and the temperature for most sugar coupling reactions is below −20 °C, a mixed solvent of dioxane with dichloromethane or tetrahydrofuran was selected for further investigation. As outlined in Scheme 2, BF_3•OEt_2-activated coupling of **8** with 1.2 equiv of the D-mannosyl donor **7** in a 1:1 ratio of 1,4-dioxane and dichloromethane at −40 °C for 1 h gave the 2-*O*-mannosylated 4,6-diol **9**, the 2,6-di-*O*-mannosylated 4-alcohol **6**, and its diastereoisomer **10** in 67%, 5%, and 3% isolated yields, respectively. This fact has revealed that the 2-*C*-equatorial hydroxy group of **8** is more reactive than the others at 4-C and 6-C. When 2 equiv of the donor **7** was used in the reaction, the yield of **6** slightly increased, but the mono-D-mannosylated compound **9** was acquired as the major adduct. To realize the lower reactivity of the axial hydroxyls, excess donor and higher reaction temperature were employed to improve the results. The optimized 64% yield of **6**, having identical spectral data with a previous report,[16k] was obtained by treatment of **8** with 8 equiv of the donor **7** in the presence of BF_3•OEt_2 as the promoter at −40 °C and then warming to −20 °C. Under these conditions, **9** and **10** were isolated in 5% and 19% yields, respectively. Different promoters (AgOTf and TMSOTf), D-mannosyl donors (thioglycoside, glycosyl phosphate, and glycosyl chloride), and mixed solvent combinations (1,4-dioxane/tetrahydrofuran and pure tetrahydrofuran) were also examined, but the yields of the expected 2,6-di-*O*-mannosylated product **6** were not satisfactory. With the 4-alcohol **6** in hand, removal of the 1,3,5-orthoformate group was further investigated. *p*-Toluenesulfonic acid (*p*-TSA)-catalyzed methanolysis of **6** was carried out at room temperature, and the corresponding 1,3,4,5-tetraol **5** was afforded in excellent yield (99%).

Scheme 2. Desymmetrization of the Meso Triol **8**



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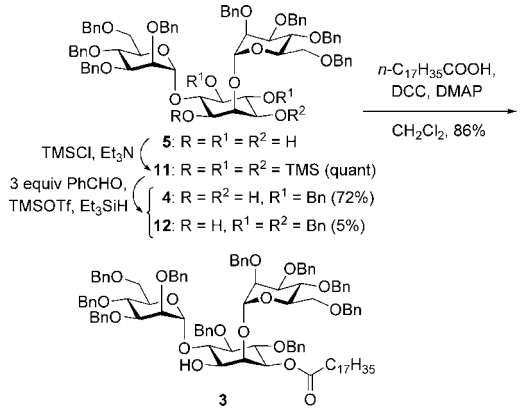
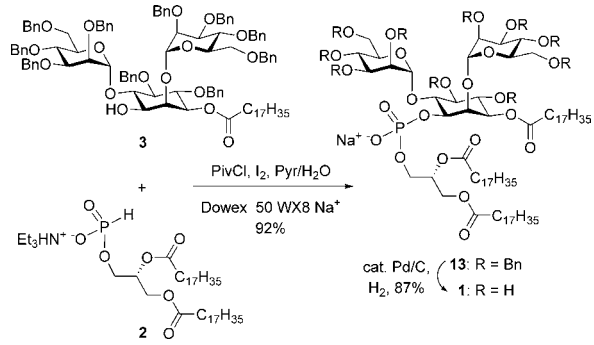
Scheme 3. Regioselective Benzylation and Acylation



Scheme 4. Synthesis of PIM_2 **1**



The next challenges included not only regioselective di-*O*-benzylation of the 4,5-dihydroxyls at the *myo*-inositol subunit of **5** but also regioselective introduction of an acyl group at the 3-O position (Scheme 3). Williamson's etherification of **5** under basic conditions (NaH, BnBr), even at low temperature, resulted in a mixture of different *O*-benzylated isomers, which were very difficult to separate and identify. Alternative reagent combinations employing Ag_2O/BnBr (neutral conditions) and $BF_3{\cdot}OEt_2$/$BnOC(=NH)CCl_3$ (acidic conditions) also failed to provide the desired 1,3-diol **4**. Recently, we had developed a two-step procedure to introduce benzyl-type protecting groups on carbohydrates in a highly regioselective manner via per-*O*-trimethylsilylation of the starting alcohol followed by TMSOTf-catalyzed Et_3SiH-reductive etherification.[25] Treatment of compound **5** with trimethylsilyl chloride and triethylamine led to the 1,3,4,5-tetra-*O*-TMS ether **11** (quantitative yield), which was subjected to 3 equiv of benzaldehyde and Et_3SiH in the presence of TMSOTf at −78 °C and then warming to −20 °C, yielding the 4,5-di-*O*-benzylated 1,3-diol **4** and the 3,4,5-tri-*O*-benzylated 1-alcohol **12** in 72% and 5% yields, respectively. The configuration of compound **4** was determined through a series of NMR experiments, including ^1H, ^{13}C, DEPT, $^1H-^1H$ COSY, $^1H-^{13}C$ COSY, and $^1H-^1H$ NOESY spectra (Supporting Information) in $CDCl_3$. All protons on the *myo*-inositol ring contained at least one big *trans*-diaxial coupling constant, except the 2-H proton which had a triplet splitting with a small coupling constant $J = 2.2$ Hz. The chemical shift of this characteristic 2-H signal was easily identified at δ 4.27 ppm in the ^1H spectrum. The 1-H and 3-H protons, which individually exhibited a correlation with the 2-H proton, were found to have couplings with the protons of two hydroxy groups, respectively. In addition, the 2-H and 4-H protons also showed NOE correlation with the anomeric protons of the two mannosyl rings. Regioselective esterification of 1,3-diol **4** with stearic acid via a combination of dicyclohexylcarbodiimide (DCC) and 4-*N*,*N*-dimethylaminopyridine (DMAP) provided the corresponding 1-alcohol **3** as a single diastereoisomer in 86% yield. The high regioselectivity was attributed to the steric effect of two mannosyl moieties that blocked the nucleophilic acylation of the 1-*C*-hydroxyl. The installation of the stearic acyl chain at the 3-O position of compound **3** was confirmed by the correlation of 1-H with the hydroxy proton and the significant downfield shift of the 3-H proton from δ 3.25 (^1H spectrum of **4** in $CDCl_3$) to 4.76 ppm (^1H spectrum of **3** in $CDCl_3$).

Finally, the synthesis of the target molecule **1** is depicted in Scheme 4. Pivaloyl chloride mediated coupling of the 1-alcohol **3** with the *H*-phosphonate **2**[16g] followed by iodine oxidation in situ gave the corresponding phosphate, which underwent counterion exchange on Dowex WX8 Na^+ resin to furnish the fully protected phosphatidyl dimannoside **13** in 92% yield. Cleavage of all benzyl groups in **13** under hydrogenolytic conditions afforded the expected PIM_2 **1** (87%).

In summary, we have developed a straightforward route to prepare the triacylated PIM_2 **1** from *myo*-inositol 1,3,5-orthoformate **8** in seven steps with an overall yield of 31%. The immuno properties of this PIM_2 molecule and its application to the enzymatic biosynthesis of higher D-mannosylated PIMs, LM, and LAM derivatives will be further studied.

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Supporting Information Available: Experimental procedure and ^1H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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