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# Total synthesis of a fully lipidated form of phosphatidyl-*myo*-inositol dimannoside (PIM-2) of *Mycobacterium tuberculosis*

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ABSTRACT

Using an orthogonal protecting group approach to resolve racemic *myo*-inositol directly as its D-mannoside, we report a [2+*n*]-glycan/phosphatidylation strategy to assemble a bis-palmityl, tuberculostearyl form of phosphatidyl-*myo*-inositol dimannoside (PIM-2).



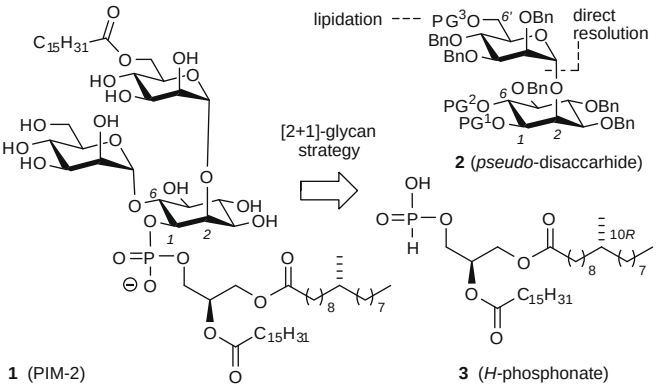
A third of the world's population are infected with the causative organism of tuberculosis (TB), the bacillus *Mycobacterium tuberculosis*.[1] In its active state, TB is responsible for over 2 million deaths every year. Yet the molecular details of TB infection are still not fully understood. One factor involved in the early pathogenicity of infection is the interaction of complex glycolipids on the outer cell wall envelope of the mycobacterium with the mammalian host cell.[2] Ubiquitous amongst the glycolipid core of this lipid envelope are the phosphatidyl-*myo*-inositol mannosides (PIMs) and lipoarabinomannans (LAMs).[3]

To advance our understanding of the biological roles of such glycophospholipids,[4,5] we have embarked on a program to investigate the immuno-pathogenesis of PIMs. To this end, it was important to devise a flexible strategy to synthetic PIMs that would enable the incorporation of a diverse set of lipid and glycan domains. It was further important to achieve domain homogeneity, which typically cannot be achieved (or known with certainty) within biologically derived material. We selected PIM-2 as a pivotal unit for making higher order PIMs and herein describe a convergent total synthesis of PIM-2 (**1**).

Several partial and total syntheses of PIMs in various lipidated forms have been reported.[6–9] Notably, Patil and Hung constructed the bis-stearate form of PIM-2 through a new *myo*-inositol desymmetrization strategy.[10] In order to prepare fully lipidated forms **1** of PIM-2 (Scheme 1),[11] we envisaged a resolvable pseudo-disaccharide **2** that could be coupled to the *H*-phosphonate of a tuberculostearyl/palmityl glyceride **3**. From prior experience,[6,12] we speculated that a D-mannosyl donor bearing a bulky C6′-protecting group ($PG^3$) could allow for the direct resolution of a C2-coupled *myo*-inositide (**2**). Although chiral auxillaries have been attached temporarily to achieve such resolutions,[13] the orthogonal combination of PMB ($PG^1$), allyl ($PG^2$) and TBPDS ($PG^3$) eventually proved successful in utilizing D-mannoside as a permanent resolving unit. Our strategy to **1** was thus designed to allow **2** to be resolved and lipidated en route to glycan and phospholipid attachment.

Having determined appropriate protecting groups,[14] the developed resolution method entailed the direct coupling of the 6-*O*-TBDPS-protected D-mannosyl Schmidt donor **4**[15] with the 1-*O*-PMB/6-*O*-allyl *myo*-inositol (±)-**5**[16] under α-selective TMSOTf conditions (Scheme 2).[12] This afforded a 1:1 mixture of α-pseudo-disaccharides **6**/**7** in 72% yield. While these diastereomers remained inseparable, Pd-mediated removal of the C6-allyl group allowed for silica gel separation by flash chromatography. In this way, the pure α-mannosides **8** and **9** could each be isolated in 30% overall yield from (±)-**5** (i.e., ca. 18% yield from commercial 1,2:4,5-dicyclohexylidene-*myo*-inositol).[17]

Encouraged by this direct resolution study, we turned our attention to developing a viable strategy to construct a fully lipidated D-inositide form of PIM-2 from the pseudo-disaccharide **8**.




**Scheme 1.** Retrosynthetic disassembly of PIM-2 (1).

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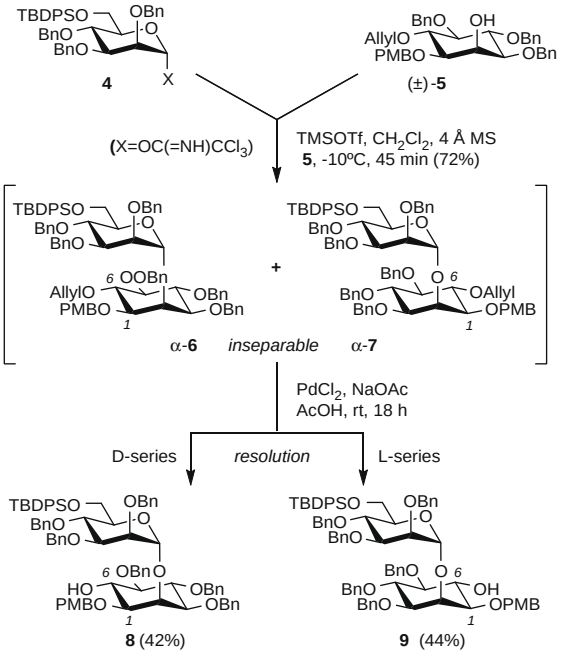


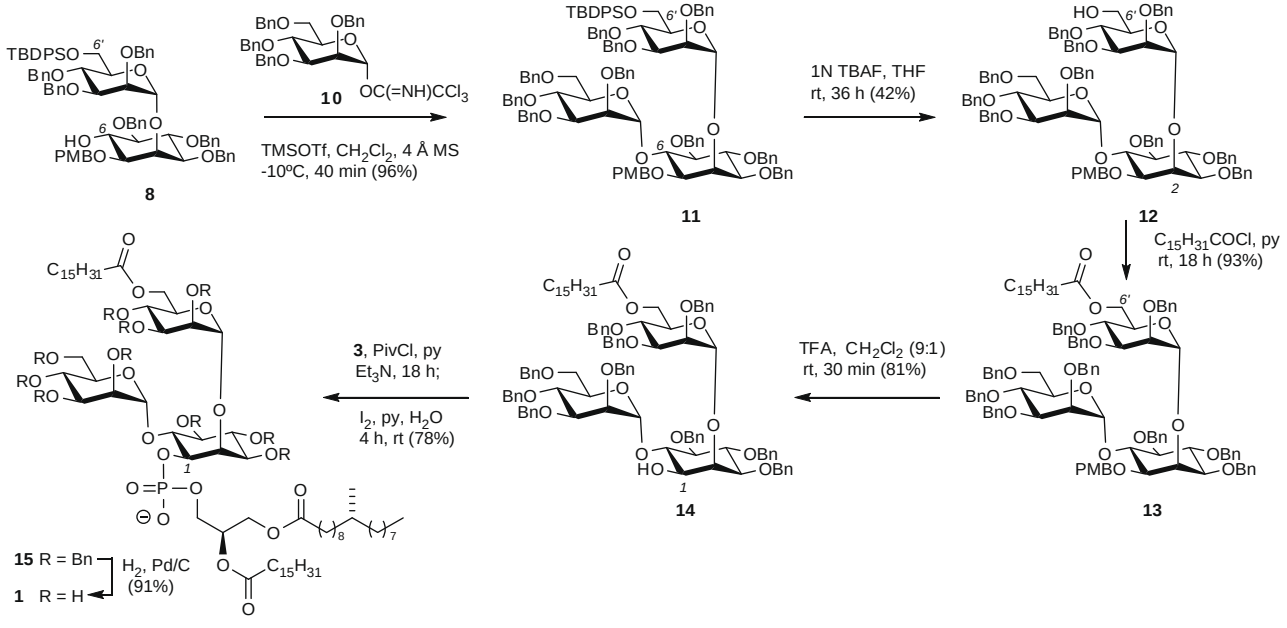

**Scheme 2.** Direct α-mannoside resolution of *myo*-inositol.

(Scheme 3). First, we determined suitable conditions to form a higher order [2+1] glycan domain. Under the established TMSOTf-promoted conditions,[12] α-mannosidation of the D-isomer **8** with 2,3,4,6-tetra-*O*-benzyl-α-D-manno-pyranosyl trichloroacetimidate **10** occurred smoothly at the 6-position to give the α,α′-dimannoside **11** of D-*myo*-inositol in excellent yield. Removal of the TBDPS-ether group in **11** proved to be problematic. A number of acidic and fluoride-based desilylation conditions were screened, but the yield of **12** could not be improved beyond 42% in a clean fashion. Conceivably, the 6-*O*-α-linked mannose hindered the cleavage of the TBDPS ether of the 2-*O*-α-linked mannoside, an observation which has some precedence.[18] Importantly, this glycosylation strategy has potential to be expanded to higher order [2+*n*] glycan domains by using oligosaccharide donors akin to **10**.

Subsequent attachment of the palmitate ester by reacting palmitoyl chloride with **12** in pyridine afforded **13** in good yields. Again, somewhat surprisingly, standard oxidative conditions (pH-buffered DDQ or CAN) to remove the 1-*O*-PMB-protecting group in **13** proved problematic. Care was also needed to prevent cleavage of the newly installed fatty ester side-arm of the 2-*O*-mannoside. Eventually we succeeded in the selective removal of the 1-*O*-PMB group with TFA/$CH_2Cl_2$ (9:1) to yield the known glycan **14**.[8]

Important to future biological work was the differential selection of lipids associated with TB. We thus prepared 10*R*-methyloctadecanoic acid, commonly known as tuberculostearic acid (TBSA),[19] and subsequently constructed the desired diacyl *H*-phosphonate **3**[8] using established methods.[12] Initial attempts to couple the phospholipid moiety **3** with the palmitylated glycan **14** were unsuccessful; for example, mixing **3** and **14** in the presence of pivaloyl chloride and pyridine over 12 h at room temperature, typically gave no reaction.[20] We reasoned that the TBSA and palmitate esters might hinder nucleophilic attack of the C1-alcohol of **14** onto the mixed anhydride intermediate derived from **3**. We thus added a stronger base ($Et_3N$) to promote the reaction. Under these conditions, the *H*-phosphonate diester formed reliably over 18 h, which was subsequently oxidized in situ with iodine in wet pyridine to afford the phosphate diester **15** in 78% yield (Scheme 3).[21] Compound **15** was isolated as its triethylammonium salt (HR-MS found 2314.906 and 2314.902; calcd 2314.370 for $C_{142}H_{194}O_{24}P$) and correlated well with that reported by the Seeberger group.[8] Subsequent controlled global hydrogenolysis of the benzyl-protecting groups over Pd/C ($H_2$, EtOAc/THF, $^i$PrOH, $H_2O$, rt, 3 days) gave **1** in 91% yield, in fully lipidated D-*myo*-inositide form. For the first time, the exact mass of *m/z* 1413.9016 (calcd *m/z* of 1413.9003 for $C_{72}H_{134}O_{24}P^-$) was confirmed by FT-MS-ESI analysis of **1** from a 1:1 solution of $CH_3CN/H_2O$ (Thermo Scientific LTQ-Orbitrap XL, negative mode).

In summary, under a program to provide homogenous materials for glycolipid-based immunomodulators and carbohydrate-based vaccines against TB, we have reported the synthesis of a fully lipidated, tuberculostearate form **1** of the phosphatidyl-*myo*-inositol dimannoside (PIM-2) of *M. tuberculosis*. Our approach implemented an orthogonal set of protecting groups to identify the optimal




**Scheme 3.** Assembly of PIM-2 (**1**) via [2+1]-glycan construction, lipidation and phosphatidylation.

conjugate **2** between racemic *myo*-inositol and D-mannose, which could be directly resolved by standard silica gel chromatography. Using the differentially protected pseudo-disaccharide of D-inositol (**8**), we established a convergent assembly of the C6′-*O*-palmitylated phosphatidyl-glycan domain **13**. While providing unnatural L-inositides (**9**) for biological study, the total synthesis of **1** from the racemic *myo*-inositol **5** was achieved in 6% overall yield. Our [2+*n*] glycan strategy is capable of providing a versatile range of higher order, lipidated PIM-congeners. A full account of our investigations on the synthesis of higher order, lipidated PIMs in both D- and L-inositide forms will be reported elsewhere.

## Acknowledgements

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## Supplementary data

Experimental procedures, characterization data and scanned spectra for key compounds and intermediates are given. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.07.109.

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