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Total Synthesis of Phosphatidylinositol Dimannoside: A Cell-Envelope Component of *Mycobacterium tuberculosis*

Pratap S. Patil[a] and Shang-Cheng Hung*[a, b]

Dedicated to Professor Chun-Chen Liao on the occasion of his 65th birthday

Mycobacterium tuberculosis, a gram-positive bacteria, has a strong ability to infect mammalian cells and survive in host tissues for a long-term period. Among the two billion people currently infected, 5–10% of individuals are estimated to develop the active disease, tuberculosis (TB).[1] TB has become a major public health problem due to the emergence of drug-resistant strains and the combination of TB and HIV infection.[2] The development of new anti-TB drugs or vaccines is indeed an urgent issue.[3]

The mycobacterial cell envelope possesses three structural components: plasma membrane, wall, and outer layer or capsule.[4] The plasma membrane contains a class of phospholipids (Figure 1), including the di- (R, R^1), tri- (R, R^1, R^2), and tetra-*O*-acylated (R, R^1, R^2, R^3) phosphatidylinositol mannosides (PIMs) and lipoarabinomannan (LAM) consisting of a lipomannan (LM) core. PIMs and LAM play an important role in the integrity and survival of the pathogen by associating with many immunomodulatory events occurring in the progression of disease, including suppression of immunity, neutralization of potentially cytotoxic O_2 free radicals, induction of cytokines, phagocytosis of the organism by binding with the mannose receptor, and growth of the organism in the host macrophage.[5] Due to their structural complexity and critical roles in TB studies, some groups have reported the preparation of PIM_2[6] and its analogues,[7] PIM_6,[8] as well as LM components.[9] In these strategies, the optically pure *myo*-inositol derivatives with appropriate protecting groups are, however, obtained by multistep routes from D-glucose through Ferrier rearrangement[10] or by resolution from *myo*-inositol by using chiral auxiliaries in a 1:1 ratio.[11] To tackle these problems, we report herein a straightforward synthesis of PIM_2 **1**, a basic molecule toward the preparation of higher PIMs, LM, and LAM, employing direct 6-*O*-mannosylation of the *myo*-inositol-derived *meso*-4,6-diol as a key step.

The synthetic challenges of PIM_2 **1** include the efficient generation of chiral *myo*-inositol-derived compounds, the highly regioselective distinction of C-1, C-2, and C-6 hydroxyl groups from the others, and the installation of two mannosyl units and a lipid chain at the desired positions. Our retrosynthetic plan for PIM_2 **1** is illustrated in Scheme 1. Coupling of the 1-alcohol **2** with the *H*-phosphonate **3**[8] followed by removal of the permanent protecting groups (PG)

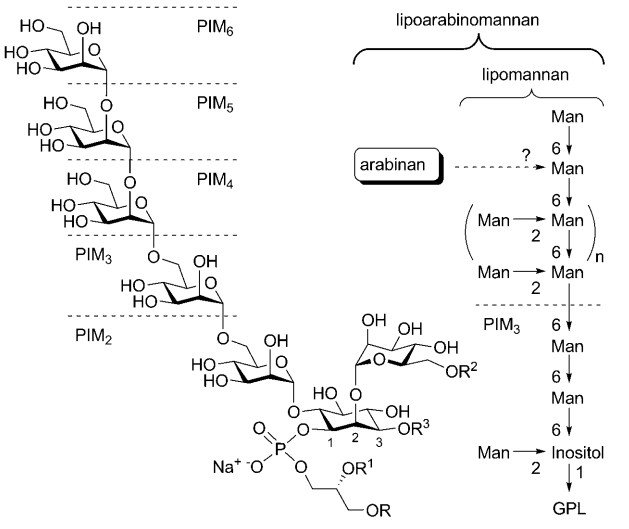

Figure 1. Structures of phosphatidylinositol mannosides (PIMs), lipomannan, and lipoarabinomannan (R, R^1: palmitic acid, stearic acid or tuberculostearic acid; R^2, R^3: H, palmitic acid or tuberculostearic acid).

[a] P. S. Patil, S.-C. Hung
Department of Chemistry, National Tsing Hua University
101, Section 2, Kuang-Fu Road, Hsinchu 300 (Taiwan)
Fax: (+886) 3-5711082
E-mail: hung@mx.nthu.edu.tw

[b] S.-C. Hung
Genomics Research Center
Academia Sinica, 128, Section 2, Academia Road
Taipei 115 (Taiwan)

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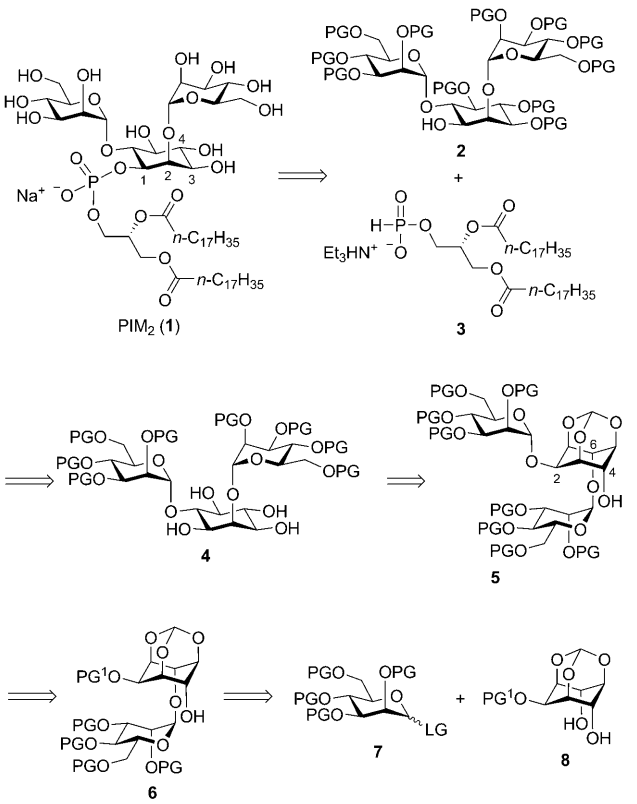
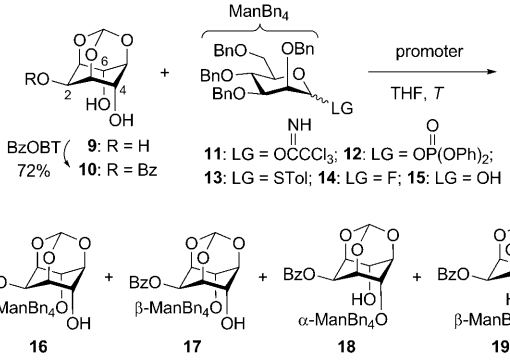


Scheme 1. Retrosynthetic design of phosphatidylinositol dimannoside (PIM_2). PG: permanent protecting group; PG^1: temporary protecting group; LG: leaving group.

would give the target molecule **1**. Conceptually, alcohol **2** could be obtained via regioselective protection of the 1,3,4,5-tetraol **4** at the O-3, O-4, and O-5 positions since the C-1 hydroxy group would be adjacent to both D-mannosyl rings and the axial pyranosyl ring at O-2 would orient toward the same β-face. These steric effects could cause a more hindered environment for the C-1 hydroxy group than other three hydroxyls. Tetraol **4** would be yielded by hydrolysis of the orthoformate **5** under mild acidic conditions. A temporary protecting group (PG^1) would be needed to mask the O-2 position of the inositol unit in **6** that could be consecutively cleaved and D-mannosylated to furnish the 4-alcohol **5**. The preparation of **6** in an optically pure form would be carried out via diastereoselective 6-*O*-glycosidation of the 2,3,4,6-tetra-*O*-protected D-mannosyl donor **7** with the 2-*O*-protected *myo*-inositol-derived 4,6-diol **8**. Conceptually, the chiral sugar **7** could be used for the desymmetrization of the *meso*-compound **8**, which is different from the resolution method employing a racemic mixture of D-glucosamine derivatives.[12] This direct coupling could afford the desired disaccharide moiety and offer an opportunity for the synthesis of PIM_2 in an efficient manner.

To address our approach, the benzoyl group was selected as a temporary protecting group in the study. Regioselective benzoylation at the C-2 equatorial hydroxy group of commercially available *myo*-inositol 1,3,5-orthoformate **9** with *N*-benzoyloxy benzotriazole (BzOBT) afforded the corresponding 4,6-diol **10** in an improved yield (72%, lit. [13] 60%). Table 1 outlines the conditions and results for the regioselective and stereoselective coupling reactions of the *meso*-4,6-diol **10** with a variety of D-mannose-derived donors **11**–**15** in THF. Four possible diastereoisomers **16**–**19** were separated by column chromatography on silica gel. In entries 1–6, $BF_3{\cdot}OEt_2$-catalyzed coupling of **9** with imidate **11**[14] was tested at different temperatures, and the best yield of the desired 6-*O*-α-mannosylated 4-OH **16** (64%) was obtained when the reaction was initially carried out at −78°C and then gradually warmed up to −20°C. Under these conditions, the other regioisomer **18** was isolated in 12% yield (Table 1, entry 6). Changing the promoter to TMSOTf (Table 1, entry 7) and AgOTf (Table 1, entry 8) furnished **16** in 46 and 20% yield, respectively. In the case of glycosyl phosphate **12** (Table 1, entry 9), only its hydrolyzed derivative **15** was found, without generating any of the expected products. When thioglycoside **13** (Table 1, entry 10), glycosyl fluoride **14** (Table 1, entry 11), and lactol **15** (Table 1, entry 12) were used as donors, the alcohol **16** was obtained in 42, 21, and 22% yield, respectively.

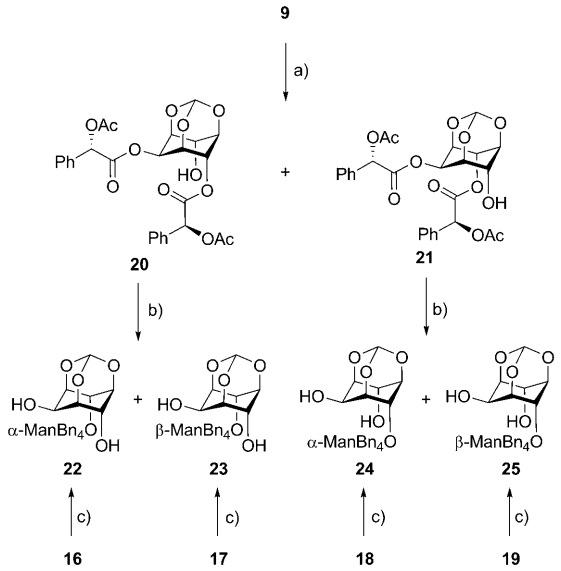
Table 1. Regioselective and stereoselective coupling of the *myo*-inositol-derived 4,6-diol **10** with various D-mannopyranosyl donors.



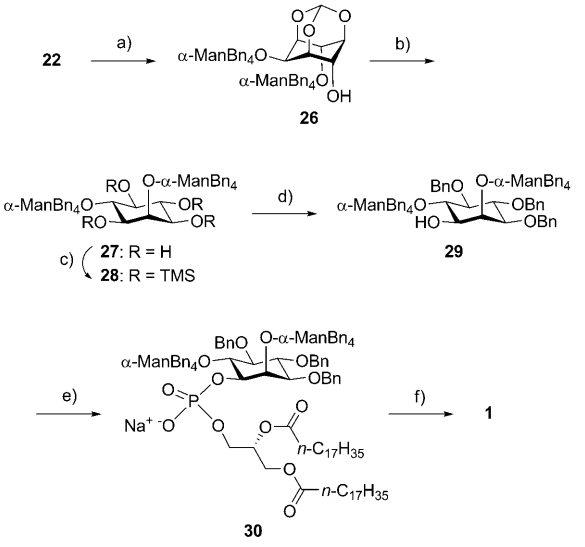
Entry	Donor	Promoter	T [°C]	Yield [%]			
				16	**17**	**18**	**19**
1	11	$BF_3{\cdot}OEt_2$	0	51	0	19	0
2	11	$BF_3{\cdot}OEt_2$	−20	42	4	23	8
3	11	$BF_3{\cdot}OEt_2$	−30	45	9	18	14
4	11	$BF_3{\cdot}OEt_2$	−40	56	5	21	10
5	11	$BF_3{\cdot}OEt_2$	−60	47	5	14	6
6	11	$BF_3{\cdot}OEt_2$	−78→−20	64	0	12	0
7	11	TMSOTf	−78→−20	46	0	15	0
8	11	AgOTf	−78→−20	20	0	10	0
9	12	TMSOTf	−78→−20	0	0	0	0
10	13	TESOTf, NIS	−78→−20	42	0	16	0
11	14	Cp_2HfCl_2, AgOTf	−78→−20[a]	21	8	19	7
12	15	DMS, Tf_2O	0→RT	22	18	0	0

[a] A mixed solvent of THF/CH_2Cl_2 1:5 was used.

The structural determination of four diastereoisomers **16**–**19** was a challenging task since no single crystal for X-ray diffraction analysis could be obtained. A chemical correlation method, as illustrated in Scheme 2, was utilized as the



Scheme 2. a) (*S*)-2-Acetyloxy-2-phenylacetyl chloride, pyridine/CH_2Cl_2 1:1, 0 °C→RT, 10 h, **20**: 15 %, **21**: 39 %; b) 1) TMSOTf, **11**, 3 Å MS, CH_2Cl_2/CH_3CN 3:1, −78→−20 °C, 3 h; 2) NaOMe, MeOH, RT, 5 h; **22**: 56 %, **23**: 13 %, **24**: 45 %, **25**: 9 % in two steps; c) NaOMe, MeOH, RT, 5 h, **22**–**25**: quant. TMSOTf: trimethylsilyl trifluoromethanesulfonate; MS: molecular sieves.



Scheme 3. a) BF_3·OEt_2, **11**, CH_2Cl_2, −60 °C, 3 h, 87 %; b) *p*-TSA, CH_2Cl_2/MeOH 1:1, RT, 20 h, 99 %; c) TMSCl, Et_3N, CH_2Cl_2, 0 °C→RT, 36 h, quant; d) TMSOTf, PhCHO, Et_3SiH, 3 Å MS, CH_2Cl_2, −40→−10 °C, 28 h, 80 %; e) **3**, PivCl, pyridine, RT, 4 h, then I_2, pyridine/H_2O 50:1, RT, 3 h, 82 %; f) 10 % Pd/C, 60 psi H_2, EtOAc/THF/1-PrOH/H_2O 2:1:1:1, RT, 36 h, 52 %. *p*-TSA: *p*-toluenesulfonic acid monohydrate; TMSCl: chlorotrimethylsilane; PivCl: pivaloyl chloride.

solution. Diacylation of *myo*-inositol 1,3,5-orthoformate **9** with (*S*)-2-acetyloxy-2-phenylacetyl chloride, generated from the corresponding carboxylic acid, led to the 6-alcohol **20** and 4-alcohol **21** in 15 and 39 % yield, respectively. The isolation and spectral characterization of both compounds have been reported.[15] TMSOTf-catalyzed coupling of the imidate donor **11** with the 6-alcohol **20** followed by deacylation with sodium methoxide furnished the α-D-mannosylated 2,4-diol **22** and its β-isomer **23** in 56 and 13 % yield, respectively. Similar conditions were applied to the 4-alcohol **21**, and the α-D-mannosylated 2,6-diol **24** (45 %) and its β-isomer **25** (9 %) were individually obtained. Debenzoylation of compounds **16**–**19** with sodium methoxide gave the identical diols **22**–**25** in quantitative yields, respectively.

With the synthon **22** in hand, the total synthesis of PIM_2 **1** was further investigated (Scheme 3). Since the equatorial C-2 hydroxy group in **22** is more reactive than the axial one at C-4, regioselective and stereoselective coupling of the D-mannosyl donor **11** with **22** in the presence of BF_3·OEt_2 as catalyst provided the desired 4-alcohol **26** (87 %) as a single diastereoisomer. Removal of the orthoformate protecting group in **26** under mild acidic conditions afforded tetraol **27** in almost quantitative yield. The next challenge was the regioselective protection of the hydroxy groups at the C-3, C-4, and C-5 positions. Williamson etherification of **27** (NaH, BnBr) led to a mixture of different *O*-benzylated isomers, which was very difficult to purify and identify. An alternative approach via TMSOTf-catalyzed Et_3SiH-reductive etherification of the per-*O*-trimethylsilylated compound was then pursued.[16] Silylation of **27** yielded the corresponding tetra-*O*-TMS ether **28** (quant.), which was regioselectively benzylated to give the desired 1-alcohol **29**[17] in 80 % yield. The excellent regioselectivity is perhaps induced by the steric hindrance of both O-2- and O-6-mannosyl rings preventing the nucleophilic attack of O-1 to benzaldehyde. Coupling of compound **29** with the *H*-phosphonate **3**[8] by using a combination of pivaloyl chloride, iodine, and pyridine furnished the product **30**[18] (82 %), which was subjected to hydrogenolysis to give the target molecule **1**[18] in 52 % yield. The ^1H and ^{13}C NMR spectra of compounds **29**,[17] **30**,[18] and **1**[18] are identical to the literature reports (see Supporting Information).

In summary, we have developed an efficient and convenient route to synthesize PIM_2 **1** from commercially available *myo*-inositol 1,3,5-orthoformate **9** in nine steps in 13 % overall yield. The *meso*-diol **10** can be D-mannosylated at the O-6 position to yield the corresponding chiral disaccharide **16** in high regioselectivity and stereoselectivity. The stepwise sugar coupling described here allows the introduction of two different D-mannopyranosides at the O-2 and O-6 positions for the synthesis of higher di-*O*-acylated and tri-*O*-acylated PIMs. The Et_3SiH-reductive benzylation using TMSOTf as a catalyst enables the installation of two benzyl groups at O-4 and O-5 by controlling the amount of benzaldehyde that can be applied to prepare the tetra-*O*-acylated PIMs.

Experimental Section

Procedure for BF_3·OEt_2-activated regioselective and stereoselective coupling of 10 with 11: A mixture of the 4,6-diol **10** (0.35 g, 1.2 mmol), man-

nosyl trichloroacetimidate **12** (0.82 g, 1.2 mmol), and freshly dried 3 Å molecular sieves (1.2 g) was stirred in THF (25 mL) at room temperature for 1 h under nitrogen. The reaction flask was cooled to −78 °C, $BF_3{\cdot}OEt_2$ (45 μL, 0.36 mmol) was added to the solution, and the mixture was gradually warmed to −20 °C. After stirring for 1 h, a solution of the imidate **11** (0.82 g, 1.2 mmol) in THF (2 mL) and $BF_3{\cdot}OEt_2$ (45 μL, 0.36 mmol) were consecutively added to the reaction solution, and the mixture was continuously stirred at the same temperature for 2 h. Triethylamine (0.2 mL) was added to quench the reaction, the whole mixture was filtered through celite, and the filtrate was evaporated under reduced pressure. The residue was purified by flash column chromatography (gradient EtOAc/Hex 1:3.25 to 1:2.5) to obtain the products **16** (0.62 g, 64 %) and **18** (0.12 g, 12 %). For the preparation of other compounds, please see the detailed procedure in Supporting Information.

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Keywords: glycosylation · phosphatidylinositol mannosides · total synthesis · tuberculosis

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