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Synthesis of a key *Mycobacterium tuberculosis* biosynthetic phosphoinositide intermediate ☆

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Abstract—Regioselective mannosylations of a myoinositol acceptor diol are readily achieved by Lewis acid mediated iodinolysis of *n*-pentenyl *ortho*-esters. The procedure affords a psuedotrisaccharide to which the phosphoglyceryl and other lipid residues are added leading to the key biosynthetic intermediate of *Mycobacterium* species.

Recent reviews[1] draw attention to the protracted ravages of tuberculosis. Estimated to infect eight million new subjects per year, the disease has now spread to one-third of the world's population. Primary tuberculosis develops two years after initial infection, and eventually results in two million deaths annually.[2] Equally devastating are the challenges to the patients' immune status,[3] this being a major concern in the case of HIV infected patients.[4] The persistence factors, which are varied and devious, are tied to the pathogen's distinctive ability to survive, and indeed flourish inside macrophages,[5] thereby interfering with intercellular trafficking. The depredation caused by drug-resistant strains has focussed attention on the causative parasite, *Mycobacterium tuberculosis*, with the result that several elegant structure–activity studies have recently emerged.[6]

Of special interest is the parasite's cell wall, which, in spite of its formidable intricacy, has yielded discrete foci of attention. Primary among these is the lipoarabinomannan (LAM) component,[7,8] the covalent structure of which has been deduced in several laboratories.[9] Impressive advances have been made in elucidating LAM biosynthesis,[10] and these studies will profit from the recent decoding of the parasite's complete genome sequence.[11]

The chronology PI → [PIMs] → LM → LAM has been recently established.[12] The abbreviation [PIMs] refers to a sub-set of intermediates shown within the brackets in Scheme 1. Biosynthesis begins with the phosphatidyl inositol (PI), **1**, which undergoes mannosylation at O2 to give the phosphoinositide, **2a**, (Ac_2PIM_1). Acylation of the primary-OH to **2b** (Ac_3PIM_1) follows as a mandatory step, which precedes further mannosylation at O6 to give the pseudotrisaccharide, **3**, (Ac_3PIM_2). Elaboration of the mannan array then begins, leading to **4** and thence to the linear mannan (LM), **5**, the value of *n* being approximately 10. Addition of the arabinan complex then proceeds to afford the lipoarabinomannan (LAM).

However, timely investigations by McConville and co-workers[13] involving cell-free experiments with *M. smegmatis,* now indicate that addition of the third mannose, that is **3** → **4**, is 'an important step in regulating the size of' the PIMs, and that the 'mannosyltransferase that catalyzes this step may be more labile—or—present at very low levels'. In view of this speculation, the heavily lipidated dimannosylated phosphoinositide, **3**, is a crucial intermediate, and its identification, in two independent studies,[12,13] as a mass spectral

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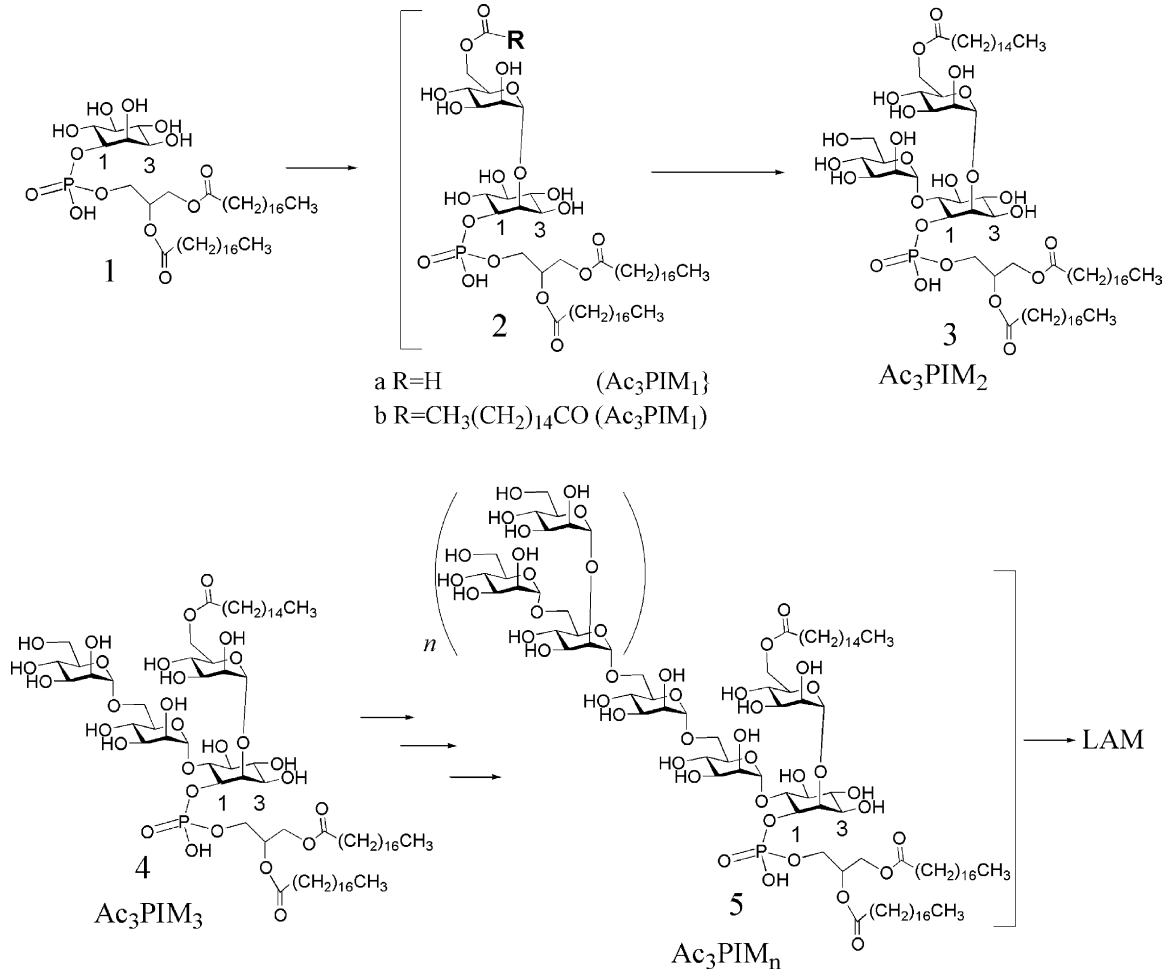


Scheme 1.

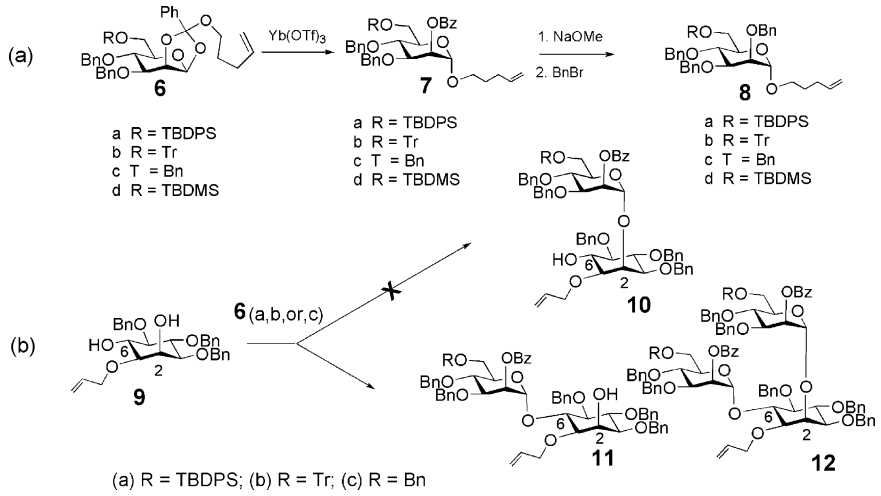
fragment in *M. smegmatis* extracts, enhances its significance. In this manuscript we report the first laboratory preparation of this crucial biosynthetic intermediate.

Recent reports from our laboratory[14] have revisited Paulsen's concept of 'matching' donors with acceptors in order to optimize saccharide coupling under nonforcing conditions. This process is facilitated by chemoselective activation of *n*-pentenyl donors by Lewis acid-controlled decomposition of *N*-iodosuccinimide.[15] For example, ytterbium triflate ($Yb(OTf)_3$) activates *n*-pentenyl *ortho*-esters (NPOEs), for example, **6**, which in the absence of an acceptor are quantitatively rearranged to disarmed *n*-pentenyl glycosides, for example, **7**. The armed counterparts, for example, **8** are then easily obtained by routine transformations (Scheme 2a).

Notably, $Yb(OTf)_3$ does not activate **7** or **8**, thereby permitting use of this salt specifically for NPOEs. By contrast, scandium triflate ($Sc(OTf)_3$) and *tert*-butyldimethysilyltriflate (TBDMSOTf) activate armed and disarmed donors.[15] Application of these glycosylation strategies to an appropriate inositol acceptor was an attractive approach. The starting material was the diol **9** (Scheme 2b), which is readily available[16] from methyl α-D-glucopyranoside by the procedure of Bender and Budhu.[17]

Glycosidation with various *n*-pentenyl *ortho*-esters (NPOEs)[18] was tested using a variety of Lewis acids to activate *N*-iodosuccinimide. The most remarkable result in Table 1 is the formation of only one monomannosylation product, regardless of the type, or amount, of Lewis acid used. The structure of the product was unequivocally established by acetylation and ^1H NMR analysis. In view of the parameters of the downshifted proton (5.8 ppm; $J = 2.7$ and 2.4 Hz), the material was identified as **11** rather than **10** in keeping with precedents.[14]

In light of the foregoing, it is further remarkable that, whereas the $Yb(OTf)_3$ catalyzed reactions were exquisitively selective, those with scandium triflate ($Sc(OTf)_3$) and *tert*-butyldimethylsilyltriflate (TBDMSOTf) induced formation of minor amounts of double glycosidation products, **12** (entries i → vi). These data support previous findings in van Boom's[19] laboratory and ours,[20]



Scheme 2.

Table 1. Effect of Lewis acid promoters on NIS-induced mannosylation of acceptor **9** with *n*-pentenyl *ortho*-esters[a]

Entry	Lewis acid	NPOE	Donor (equiv)	Glycosidation products (%)			Yield (%)[a]
				10[a]	**11**[a]	**12**[a]	
i	$Sc(OTf)_3$	**6a**	3	0	65	25	87
ii		**6a**	3	0	70	23	82
iii		**6c**	3	0	68	26	85
iv	TBDMSOTf	**6a**	3	0	67	26	82
v		**6b**	3	0	72	21	62
vi		**6c**	3	0	75	27	84
vii	$Yb(OTf)_3$	**6a**	3	0	100	0	92
viii		**6b**	3	0	100	0	97
ix		**6c**	3	0	100	0	96

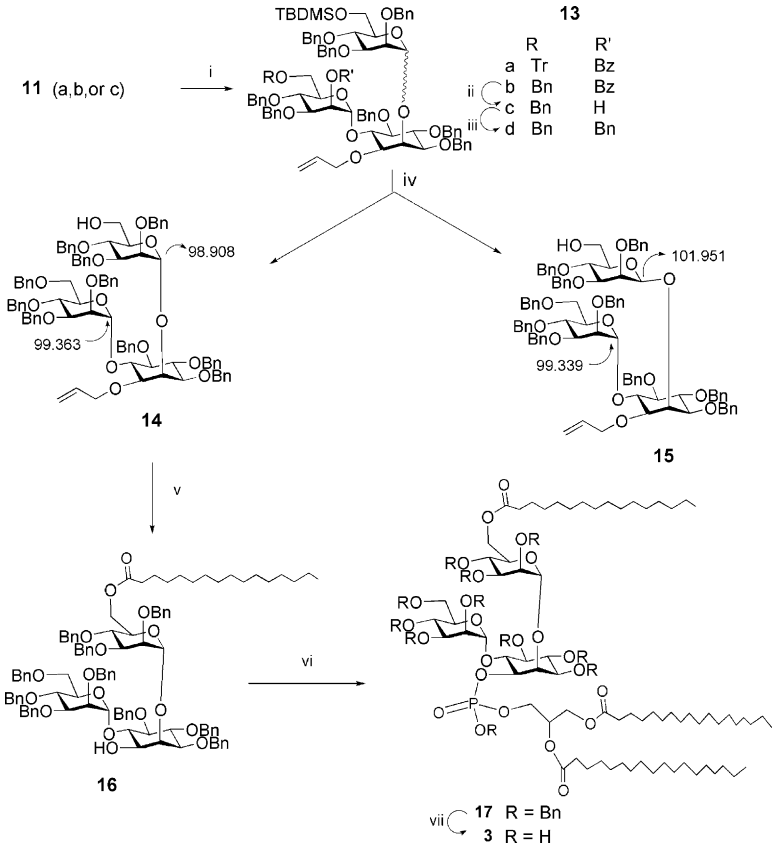
[a] Yields and selectivity were given as an average value of two set of reactions.

about the difficulty of introducing an O2 mannosyl group into a psuedodisaccharide acceptor such as **11**—particularly when a disarmed donor[19] or NPOE[20] is used.

An armed donor is the proper 'match' for mannosylation at O2.[21] The desired candidate had to provide for future acylation of the primary-OH and, at the same time, minimize β-coupling. Several experiments were necessary to determine the best choice. The *tert*-butyldiphenylsilylated acceptor **11a** yielded products only in the 20% range regardless of the Lewis Acid ($Sc(OTf)_3$ or TBDMSOTf) that was used to promote iodonium induced reaction of donor **8a**. The tritylated analogue **11b**, gave acceptable yields of pseudotrisaccharide **13a**; however removal of the trityl group under mild acidic conditions was problematic, because desilylation also occurred.

The best choice involved reaction of acceptor **11c** with armed donor **8d**, which gave compound **13b**. The α/β isomers could not be separated at this stage, nor could the products from saponification and subsequent benzylation, **13c** and **13d**, respectively. However, acid catalyzed desilylation solved the problem, in that two compounds could be separated. Identification of each was based on the ^{13}C NMR chemical shifts of the anomeric carbons in keeping with established trends.[22] Thus one showed well separated signals at 99.339 and 101.951, while the other showed closely related signals at 98.908 and 99.363. The former could therefore be assigned as the α/β diastereomer **15**, and the latter as the α/α counterpart **14**. Acylation with palmitic acid in the presence of DCC and DMAP followed by deallylation gave **16** in 54% yield over two steps (Scheme 3).

The phosphoglycerolipid moiety was attached by use of the phosphoamidite and 1H tetrazole, followed by oxidation with MCPBA[23] to provide psuedotrisaccharide **17** in 70% yield. Cleavage of the benzyl groups was effected by atmospheric hydrogenolysis over 5% palladium on carbon in methanol/chloroform for 12 h, followed by addition of water, then continuing for a further 20 h. Compound **3** was identified by MALDI (1473.9 $M+Na^+$; 1489.6 $M+K^+$), and by 600 MHz ^1H NMR in DMSO, which showed, among other things, anomeric protons at 4.97 and 4.81 ppm,



Scheme 3. Reagents and conditions: (i) **8b**, $Yb(OTf)_3$/NIS, CH_2Cl_2, 92%; (ii) NaOMe, CH_2Cl_2/MeOH, 82%; (iii) BnBr, NaH, DMF, 95%; (iv) TsOH, MeOH/CH_2Cl_2, 72%; (v) a. $CH_3(CH_2)_{14}COOH$, DMAP, DCC, CH_2Cl_2, 24 h; b. HOAc/H_2O, $PdCl_2$, NaOAc, 2 days, 54%; (vi) $C_{17}H_{35}CO$-$OCH_2CH(OCOC_{17}H_{35})CH_2OP(OBn)N(i\text{-}Pr)_2$/1H-tetrazole, −20 °C/MCPBA, 70%; (vii) 5% Pd/C, MeOH (1 mL)/$CHCl_3$ (1 mL), H_2 (balloon), 12 h, H_2O (0.3 mL), 20 h, 40%.

and 6a methyl protons at 2.34–2.21 ppm. Biological studies with **3** will be undertaken and reported in due course.

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