Cell-Wall Glycolipids

Identification of the 5-Methylthiopentosyl Substituent in *Mycobacterium tuberculosis* Lipoarabinomannan**

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The search for new and improved drugs against tuberculosis continues to focus on the biosynthesis of the *Mycobacterium tuberculosis* (Mtb) cell wall.[1] Lipoarabinomannan (LAM)[2] is a major component of this cell wall and facilitates entry of Mtb into macrophages. Furthermore, LAM promotes the intracellular survival of Mtb by down-regulating the immune response and providing antioxidative protection.[2] LAM is a lipid-anchored polysaccharide comprising dendritic arabinan chains which emanate from a poly-α(1→6)-mannosyl core bearing α(1→2)-mannosyl substituents (Figure 1). In Mtb, the arabinan chains are capped with several further mannosyl residues, which form the nonreducing termini. We recently discovered[3] a novel substituent of these mannosyl caps, which, through a combination of NMR spectroscopic and

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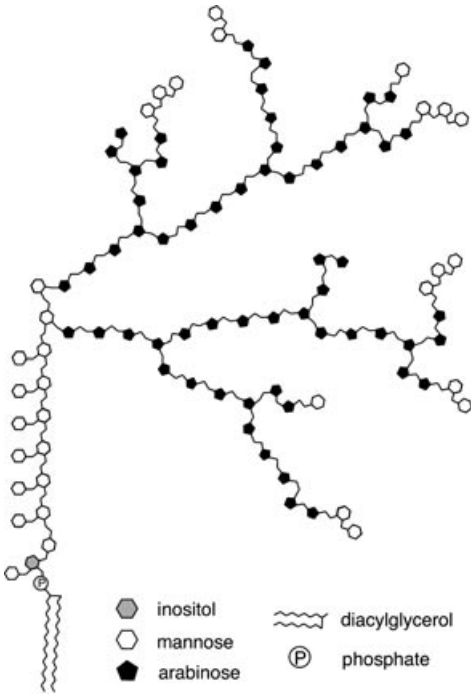
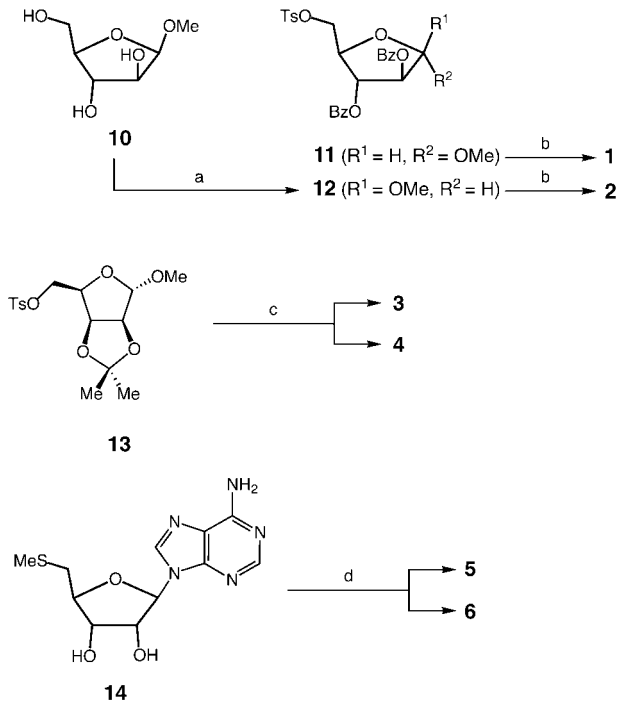


Figure 1. Cartoon representation of LAM, a phosphatidylinositol-anchored polysaccharide from *Mycobacterium tuberculosis*. It is not known how many arabinan side chains are attached to the branched mannan core of LAM. The novel 5-deoxy-5-methylthiopentofuranosyl residue was found to be attached to one of the short oligomannosyl chains capping the arabinan side chains.



Scheme 1. Reagents: a) 1. $TrCl/C_5H_5N$; 2. $BzCl/C_5H_5N$; 3. 80% AcOH (aq)/△; 4. $TsCl/C_5H_5N$ (48%); b) 1. MeSNa/[18]crown-6/DMF; 2. NaOMe/MeOH (**1**: 94%, **2**: 90%); c) 1. MeSNa/[18]crown-6/DMF; 2. $SrCl_2$/MeOH/TFA/△ (**3**: 7%, **4**: 41%); d) MeOH/TFA/△ (**5**: 15%, **6**: 40%). Tr = triphenylmethyl, Bz = benzoyl, Ts = 4-toluenesulfonyl, TFA = trifluoroacetic acid.

mass spectrometric techniques, was identified as having a 5-deoxy-5-methylthiopentofuranosyl (MTP) structure. The presence of an oxidized form of this residue, 5-deoxy-5-methylsulfoxypentofuranosyl (MSP), was also observed. Such monosaccharide residues have never previously been identified in an oligosaccharide. Herein, we use a combination of chemical synthesis and NMR spectroscopy to reveal the configuration of this novel component of the Mtb cell wall.

The fluctional conformations of furanose rings[4] make the identification of their relative configuration from ^3J coupling constants more difficult than for pyranose sugars. Consequently, we chose to synthesize all four isomers of 5-deoxy-5-methylthio-D-pentofuranose, each as their α- and β-methyl glycosides (see Figure 2, compounds **1**–**8**). Only the *xylo*-configured sugars **7** and **8** have been reported previously, albeit as a mixture of α- and β-anomers.[5] Although it is not possible to distinguish compounds **7** and **8** by thin layer chromatography, they were separated readily by using an anion exchange chromatography column (Dowex 1-X2, OH^- form) and eluting with water. The α-*arabino* isomer **1** was synthesized (Scheme 1) from the known[6] tosylate **11** by reaction with MeSNa and subsequent removal of the benzoyl groups under Zemplén conditions. The analogous β isomer **2** was prepared in a similar fashion from methyl glycoside **10**[7] via tosylate **12**.

Whereas the synthesis of α-lyxofuranose derivatives is relatively straightforward, the β anomers are notoriously difficult to prepare as a consequence of having all four substituents pointing toward the same face of the furanose ring.[8] However, Angyal et al. discovered that the proportion of the β-furanose isomer increased from trace quantities to about 30% when the acid-catalyzed preparation of methyl glycosides of lyxose was conducted in the presence of strontium chloride.[9] Consequently, following the displacement of the Ts group in compound **13**[10] with MeSNa, the acetonide protecting group was removed by acid-catalyzed methanolysis in the presence of anhydrous $SrCl_2$, with concomitant anomerization. Isomers **3** and **4** were thus obtained in 7 and 41% yield, respectively, after chromatography on a Sr^{2+} ion exchange column (Dowex 50WX2-400)[9] and eluting with water. Finally, the *ribo*-configured derivatives **5** and **6** were prepared by methanolysis of 5′-methylthioadenosine (**14**), which is commercially available. In this case, the two isomers were found to be separable by careful silica-gel chromatography. Analytical data for compounds **1**–**8** are presented in Table 1.

The HSQC spectra for compounds **1**–**8** are shown in Figure 2.[11] The signals of the anomeric proton (Figure 2a) are grouped together in two main clusters with ^{13}C chemical shifts of about $\delta = 105$ ppm for the 1,2-*cis* glycosides and about $\delta = 110$ ppm for the 1,2-*trans* glycosides. These groups are further divided into pairs of C3 epimers, with the β anomers located upfield of the α anomers in the ^1H dimension. The remaining signals for the ring atoms are more randomly spread, providing a fingerprint region for comparison with the spectra of MTP-containing LAM.

Table 1: Chemical shifts[a] and optical rotations[b] for compounds **1**–**9** and MTP/MSP-LAM.

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	MTP[c]	MSP[c]
H-1	4.93	4.89	4.95	4.90	5.00	4.90	**5.03**	4.88	5.06	**5.40**	5.44
									5.06		5.45
H-2	4.06	4.13	4.17	4.23	4.20	4.08	**4.17**	4.14	4.18	**4.21**	4.20
									4.18		4.22
H-3	3.95	4.05	4.26	4.22	4.02	4.19	**4.24**	4.17	4.31	**4.26**	4.34
									4.31		4.34
H-4	4.14	3.98	4.34	4.26	4.22	4.13	**4.37**	4.45	4.62	**4.38**	4.65
									4.60		4.61
H-5	2.80	2.77	2.74	2.74	2.73	2.74	**2.69**	2.76	3.29	**2.68**	3.28
									3.13		3.12
H-5′	2.88	2.86	2.82	2.86	2.83	2.88	**2.79**	2.85	3.11	**2.80**	3.08
									3.13		3.12
OCH_3	3.41	3.42	3.46	3.40	3.44	3.41	3.46	3.40	3.46	n.a.[d]	n.a.
									3.46		
SCH_3	2.18	2.17	2.17	2.18	2.17	2.19	**2.17**	2.19	2.80	**2.21**	2.84
									2.79		2.83
C-1	110.5	104.8	110.5	104.9	105.8	110.5	**105.0**	111.4	105.0	**105.2**	105.4
									105.0		105.4
C-2	83.5	79.5	79.6	74.7	73.9	77.2	**79.6**	82.5	79.6	**79.4**	79.4
									79.6		79.3
C-3	82.4	80.6	74.2	72.8	75.0	76.8	**78.4**	77.8	78.7	**78.3**	78.5
									78.7		78.5
C-4	85.3	83.1	82.1	82.4	85.6	84.3	**80.4**	84.3	76.0	**80.5**	76.5
									75.5		75.6
C-5	38.6	40.6	35.0	36.7	39.1	40.5	**35.8**	35.9	55.4	**35.8**	55.6
									57.0		57.1
OCH_3	57.7	58.2	59.2	58.3	58.3	58.2	58.7	58.3	58.4	n.a.	n.a.
									58.4		
SCH_3	17.6	17.4	17.3	17.3	17.6	17.4	**17.4**	17.4	40.2	**17.4**	39.9
									40.5		40.2
$[\alpha]_D$	102.3	−60.1	56.7	−72.1	113.6	−5.4	119.6	−38.2	n.d.[e]	n.a.	n.a.

[a] Chemical shifts [ppm] relative to 3-trimethylsilylpropionate in D_2O. [b] Optical rotations recorded at the sodium D line at 22 °C in H_2O have units of $deg\,cm^3\,g^{-1}\,dm^{-1}$. [c] Values for MTP-LAM and MSP-LAM are taken from reference [3]; values in bold to aid comparison between **7** and MTP. [d] Not applicable. [e] Not determined, as **9** is a mixture of diastereomers.

Similarly, the upfield region (Figure 2b) shows that the methyl-group resonances have near identical chemical shifts and that those for the methylene group attached to the methylthio moiety are somewhat more dispersed.

Comparison of these spectra with TOCSY (Figure 2c) and HSQC (Figure 2d and e) spectra for LAM clearly demonstrates that only the resonances for the α-*xylo* compound **7** are comparable to those for MTP. Indeed, the ^{13}C resonances all agree within $\Delta\delta = 0.2$ ppm with those of MTP and the ^1H resonances within $\Delta\delta = 0.04$ ppm, with the exception of that for the anomeric proton. It is perhaps not surprising that there is disagreement between the anomeric signals when one considers that MTP is attached in LAM to an underlying mannosyl residue and not to a methyl group as in compounds **1**–**8**. The extent of the downfield shift for the anomeric proton ($\Delta\delta = 0.4$ ppm) is nevertheless significant and might indicate steric effects constraining the conformation of the MTP–mannosyl linkage. To seek further confirmation for the α-*xylo* configuration, an aqueous solution of compound **7** was treated with hydrogen peroxide to convert the thioether into a 1:1 diastereomeric mixture of sulfoxides **9**. The HSQC spectrum of compound **9** compares extremely well with that for MSP-LAM (Figure 2d, e), again with the sole exception of the anomeric proton resonance. Furthermore, the distinct downfield shift for the atoms adjacent to the sulfur atom, following oxidation to **9**, demonstrates that compounds **1**–**8** are free from oxidation products.

The discovery of a 5-deoxy-5-methylthio-α-xylofuranosyl (MTX) structure in LAM is remarkable for two reasons. Firstly, there is no precedent, to our knowledge, for a methylthio sugar incorporated into an oligosaccharide. Secondly, there are very few other examples of *xylo*-configured sugars outside the plant kingdom[12] and only one prior example in a bacterial polysaccharide.[12c] Nevertheless, MTX is widespread in strains of Mtb including the well-characterized laboratory strains H37Rv and H37Ra[3] and the clinical isolates CSU20[3] and MT103.[13] Recently MTX was also found in a clinical strain of the nontuberculous opportunistic pathogen *Mycobacterium kansasii*,[14] in which it is attached to the core mannan domain of the related lipomannan and of LAM. This is distinct from LAM of Mtb wherein MTX is attached to the mannosyl caps.

It would be reasonable to predict that MTX is derived biosynthetically from **14**, which is a ubiquitous by-product of polyamine biosynthesis. We would thus tentatively assign the D configuration to MTX, as this would require epimerization of only one stereocenter in **14** (Scheme 2). Indeed, this hypothesis is supported by the remarkable discovery of *xylo*-

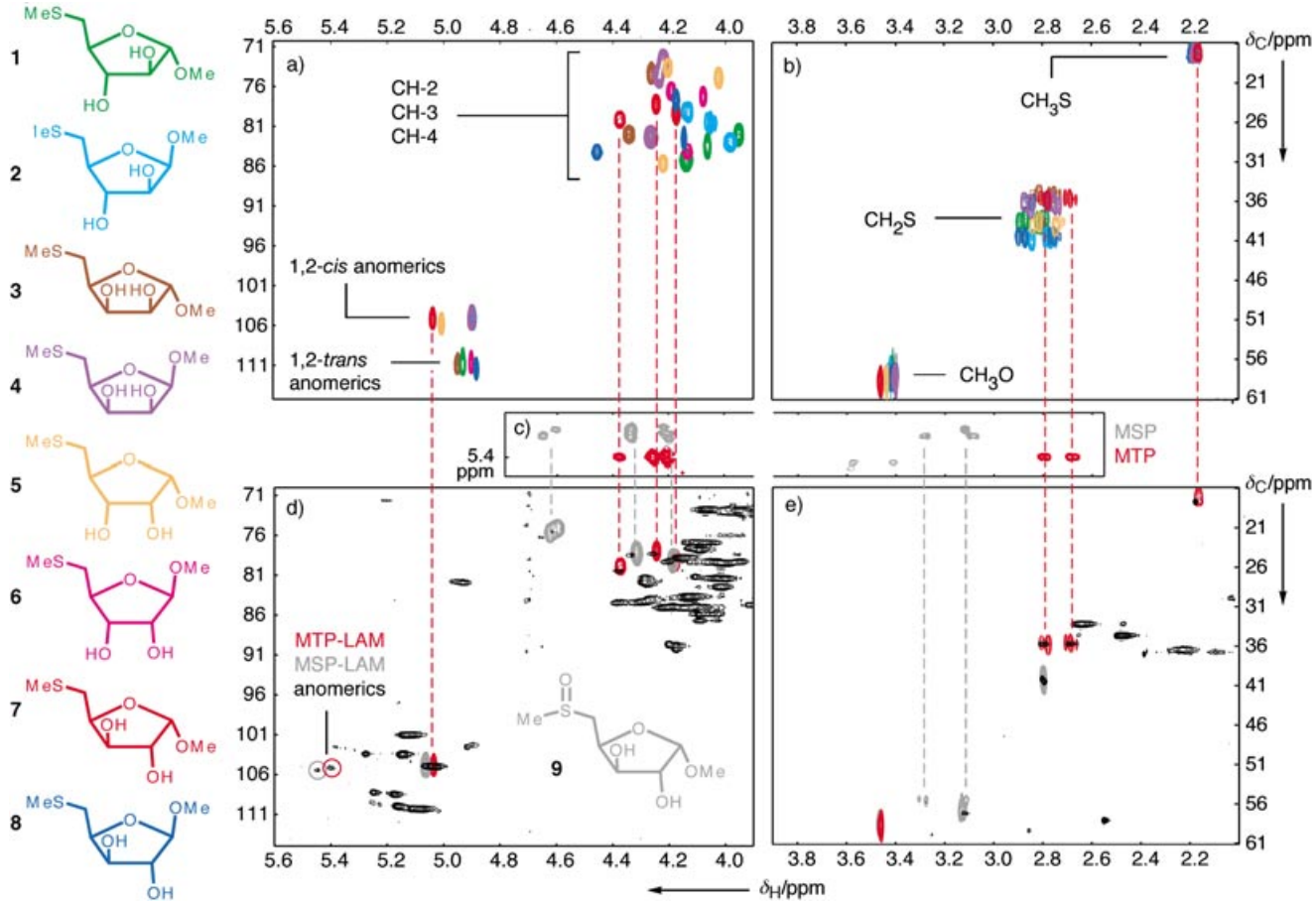
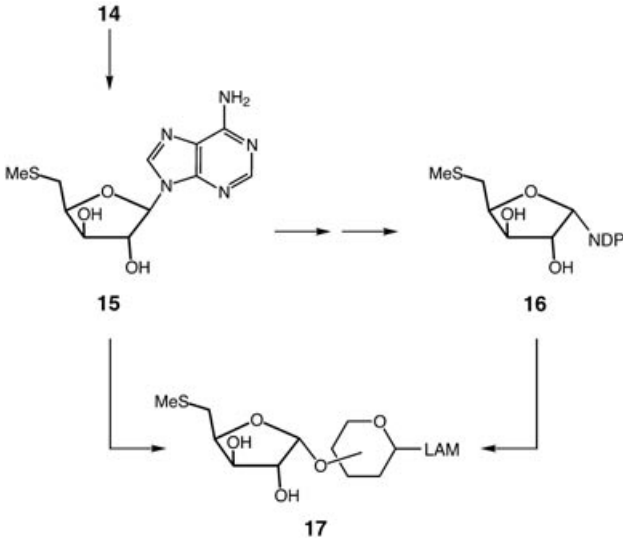


Figure 2. Structural formulas of the eight possible methyl glycosides of MTP (compounds **1**–**8**) and their NMR spectra. a) Low- and b) high-field regions of the HSQC spectra. c) A strip from the TOCSY spectrum of MTP-LAM showing resonances of nuclei correlating with the anomeric protons for MTP and MSP (red and gray, respectively). d) Low- and e) high-field regions of the HSQC spectrum of MTP-LAM (in black) are presented for comparison with those of α-*xylo*-configured compounds **7** and **9** (red and gray, respectively).

configured methylthioadenosine **15** in the mediterranean nudibranch mollusc *Doris verrucosa*,[12d] wherein compound **15** is produced by epimerization at C3′.[15] Whether biosynthesis of MTX-LAM (**17**) proceeds through direct glycosylation with **15**, or, more likely, via a nucleoside diphosphate activated sugar **16** remains to be demonstrated.



Scheme 2. Proposed biosynthesis of MTX-LAM (**17**).

Although no biological activity has yet been observed for **15**,[16] the extra biosynthetic effort that mycobacteria must expend to make MTX-LAM and also its widespread occurrence[3,13,14] would suggest that MTX provides some biological advantage to these pathogens. The relative stability of compounds **1**–**8** would imply that the nonstereoselective oxidation of MTX in LAM might occur as the result of exposure to reactive oxygen species which would be found within macrophage cells in vivo. Although the low concentration of MTX in LAM detracts from the argument that sulfide oxidation may give rise to the antioxidative properties of LAM,[2] it is difficult to imagine how else a polysaccharide could accomplish such protection chemically. Nevertheless, the identification of an unusual *xylo*-configured sugar residue in LAM opens new avenues for research into the role played by LAM in Mtb pathogenesis. If enzymes involved in MTX-LAM biosynthesis turn out to be essential for Mtb, they may be potential novel targets for the future development of antituberculosis drugs.

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