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PERSPECTIVE

Methylthioxylose – a jewel in the mycobacterial crown?†

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Ten years ago an unusual sugar was discovered in a cell wall polysaccharide of *Mycobacterium tuberculosis*. Structural elucidation revealed the presence of the first thiosugar in a bacterial polysaccharide. Synthetic studies have helped to define its relative and absolute configuration as α-D-methylthioxylofuranosyl. While its biosynthetic origins remain the subject of speculation, work has begun to define its possible biological roles.

Introduction

Mycobacterium tuberculosis (Mtb) is the pathogen responsible for tuberculosis (TB), a lung disease that affected around 9 million people and led to 1.5 million deaths in 2010.[1] Once inhaled, the bacterium enters alveolar macrophage cells, whose purpose is to identify and destroy invading pathogens. However, Mtb can survive the challenging environment within macrophage cells on account of the bacterium having a very robust cell wall formed of complex glycolipids.[2–4] The mycolylarabinogalactan complex provides Mtb with a waxy and impermeable layer that restricts small molecules from entering the bacterium.[5] The lipoarabinomannan (LAM) glycolipid facilitates the bacterium's entry into macrophage cells, by binding to the macrophage mannose receptor,[6] and then down-regulates the immune response by affecting cytokine production in the macrophage.[7] Considering the biological importance of the cell wall polysaccharides, it is not surprising that most front-line anti-tuberculosis drugs target enzymes in cell wall biosynthesis.[8–10] As multi-drug resistant strains of Mtb have appeared over recent decades, research into cell wall structure and biosynthesis has intensified. Ten years ago one such study led to the unexpected discovery of a novel thiosugar attached to LAM.[11] This Perspective article will focus on the discovery and structural elucidation of this unusual monosaccharide, and outline the current thinking on its biosynthesis and biological role.

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Bruce Turnbull was born in Edinburgh, Scotland in 1973. After gaining his BSc from the University of St Andrews in 1995, he stayed on to study for a PhD with Prof. Rob Field. He then held a Wellcome Trust International Prize Travelling Research Fellowship with Prof. Sir Fraser Stoddart at the University of California, Los Angeles and with Prof. Steve Homans at the University of Leeds. Since 2005, he has been a Royal Society University Research Fellow in the School of Chemistry, University of Leeds. His research interests include oligosaccharide synthesis, protein–carbohydrate interactions and their potential application in synthetic biology.

Susanne Stalford

Susanne Stalford was born in Salford, England in 1981. She graduated in 2004 with a master's degree in Chemistry from the University of Leeds. She completed her PhD in Chemistry at the same university under the guidance of Dr Bruce Turnbull in 2008 investigating the synthesis and properties of methylthioxylofuranose with respect to its presence in Mycobacterium tuberculosis. *Since completing her studies, Susanne has been working at Lhasa Limited, a not-for-profit organisation which provides* in silico *prediction software, where she works in the field of computational toxicology.*

Lipoarabinomannan (LAM)

Lipoarabinomannan is a phosphatidylinositol-anchored glycolipid that is believed to be imbedded in both the bacterium's inner and outer membranes.[7,10,12] Attached to the inositol ring is an extended α(1→6)-linked mannosyl core, that is substituted at the 2-position with single α-mannosyl residues (Fig. 1). Emanating from the mannose core are one or more α(1→5)/α(1→3) dendritic chains of arabinofuranose residues. These arabinan chains are exposed on the surface of the bacterium where they are thought to have a role in the immunopathogenesis of tuberculosis.[13]

LAM is not unique to *M. tuberculosis*, as it is also found in other mycobacterial species.[14] LAM is also found in other actinomycetes, but the structure can be quite distinct from mycobacterial LAM.[15,16] The structure of LAM also differs between different mycobacteria; three different classes of LAM have been found to date, which are defined by the capping structures present at the ends of branches of the arabinan chains (Fig. 2). ManLAM has capping motifs of manno-oligosaccharide units and is commonly found in slow growing mycobacteria, including Mtb. ManLAM has also been found in *M. bovis*, *M. avium* and *M. leprae*.[17,18] The main differences between the LAM in these mycobacteria is the amount of mannose capping, which can also vary between different strains of the mycobacterium species in question.[14] *M. leprae* averages only one mannose cap motif per molecule of LAM, while *M. avium* can have up to 10 motifs per molecule; some strains have nearly equal numbers of monomannosyl and dimannosyl caps, while in others, only single mannosyl residues are found.[18] Mtb ManLAM is capped with on average seven to nine mannose capping motifs per LAM molecule, the most abundant being dimannosyl caps.[19] No correlation has been found between the amount of capping and the virulence of the strains of mycobacteria, however differences observed in the amount of capping could arise from how the cells were grown *i.e. in vivo* or *in vitro*.[14]

The second and third types of LAM are known as PILAM and AraLAM. In PILAM the arabinan chains are capped with phospho-*myo*-inositol. Two types of mycobacteria are so far known to this type of capping motif, *M. smegmatis* and *M. fortuitum*,[14,20] both being fast growing mycobacteria. The LAM in these mycobacteria typically have only one phosphoinositide cap per molecule of LAM. AraLAM is devoid of any capping motifs on its terminal arabinose residues.

Discovery of methylthiopentose in LAM

Structural studies on mycobacterial polysaccharides are very challenging. Slow growing mycobacteria such as Mtb may take weeks to propagate sufficient cells for the experiments.[18] The lipopolysaccharides must then be extracted from the cells in a multi-step procedure, and then subjected to reversed phase and/or gel filtration chromatography which provides the purified material in limited quantities.[11,18] Unlike bacterial proteins which would be homogeneous in structure, polysaccharides still display microheterogeneity in which each molecule will have different numbers of each type of monosaccharide. While NMR spectroscopy studies are frequently performed on the intact lipopolysaccharides, partial degradation of the polysaccharide with specific glycosidases is often used to prepare smaller fragments that are amenable to mass spectrometry. These further manipulations provide valuable structural information, but also lead to even smaller quantities of material which may be contaminated

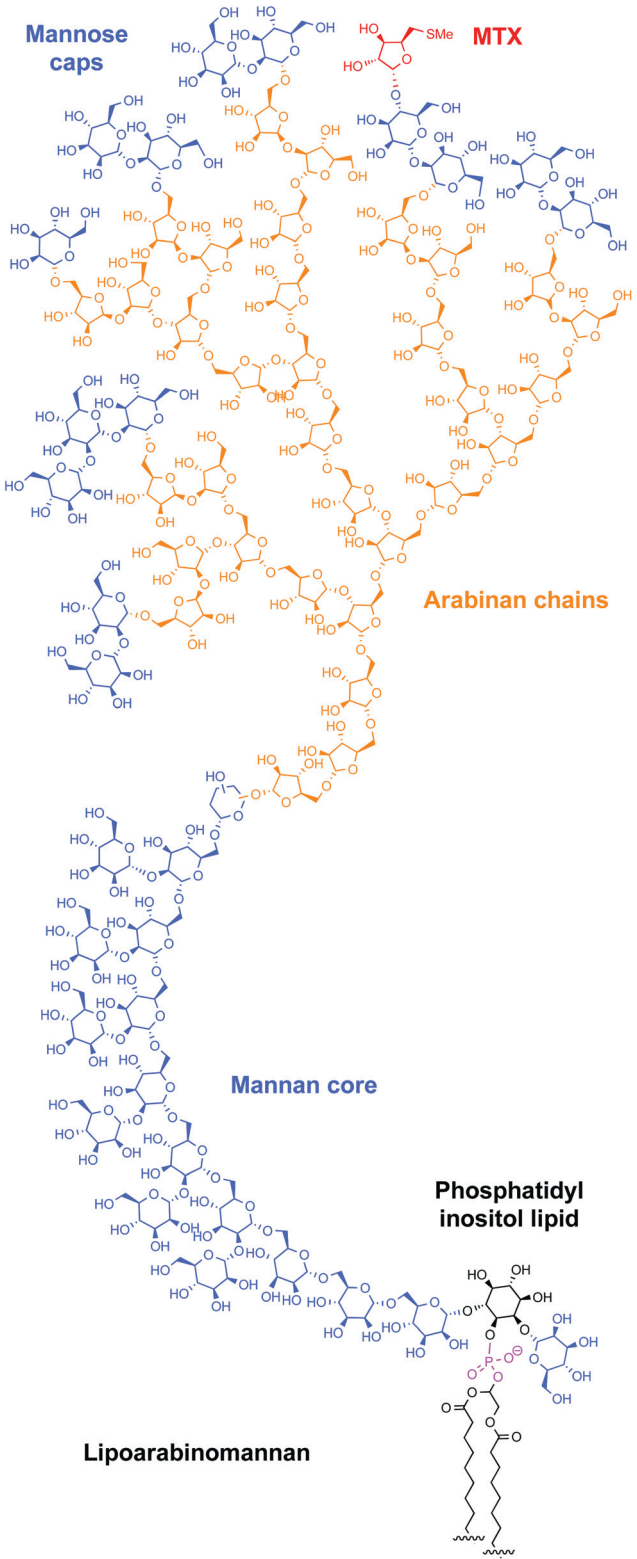


Fig. 1 Representative structure for lipoarabinomannan (LAM) based on the typical carbohydrate composition of Mtb ManLAM.

with the glycosidase enzymes used in the degradation procedures.

In order to enhance the sensitivity of ^{1}H-^{13}C-HSQC (heteronuclear single-quantum coherence) NMR experiments, Treumann *et al.* chose to prepare samples of Mtb LAM that were enriched in ^{13}C, by culturing the bacteria in the presence of ^{13}C-enriched glucose.[11] This strategy also allowed the researchers to employ more sophisticated NMR experiments that are more commonly used in protein NMR studies such as HCCH-TOCSY (total correlation spectroscopy in which the magnetisation is transferred through the carbon backbone from one proton to another).[21]

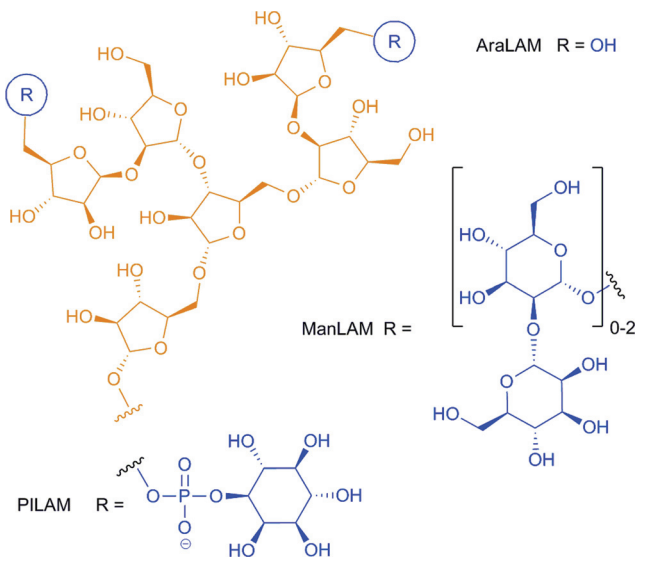


Fig. 2 Capping motifs found in AraLAM, ManLAM and PILAM.

Using these techniques, Treumann *et al.* were able to assign most of the anomeric ^{1}H-^{13}C signals to the known mannosyl and arabinosyl components of LAM.[11] However, there were additional signals present around 5.40–5.45 ppm that could not be attributed to either mannose or arabinose sugars (Fig. 3). HCCH-TOCSY experiments revealed that these peaks were part of three coupling systems that had features similar to pentofuranose systems; however, the C-5 and H-5 signals were shifted significantly up-field indicating that C-5 was bonded to an element other than oxygen.

The ^{13}C-enriched LAM was digested with an *endo*-arabinanase enzyme that can cleave α(1→5)-linkages within the arabinan chains to produce disaccharide fragments. Separation of the resulting fragments, and analysis by NMR spectroscopy revealed that the novel substituents were situated somewhere in the outer arabinan chains of LAM, but not present in its mannan core. The same digestion process was used on unlabelled LAM for mass spectrometry (MS) analysis. Fragmentation of molecular ions at *m/z* 1049 (M + H) and 1071 (M + Na) indicated that the novel sugar had a mass of 196 Da and could lose a 64 Da fragment that is indicative of methylsulfinyl substituent. High resolution FTICR-MS confirmed the presence of sulfur in the novel sugar. It was proposed that the ions corresponded to a 5-deoxy-5-methylsulfinyl-pentofuranose (MSP) attached to a Man_2Ara_4 fragment of LAM (Fig. 4). As the sulfoxide could exist as a pair of diastereoisomers, such a structure is consistent with the NMR spectra for the substituents with anomeric protons at 5.44 and 5.45 ppm. The other sugar (H-1 = 5.40 ppm) could thus be the analogous methylthiopentose (MTP). As the MSP-Man_2Ara_4 oligosaccharide proved to be resistant to digestion by Jack Bean α-mannosidase, the location of MSP/MTP was confirmed as being on the terminal mannosyl residue. Treumann *et al.* thus concluded that MTP/MSP was a novel capping structure in Mtb LAM, typically appearing on only one mannosyl cap per LAM molecule. While MTP/MSP were initially identified in the

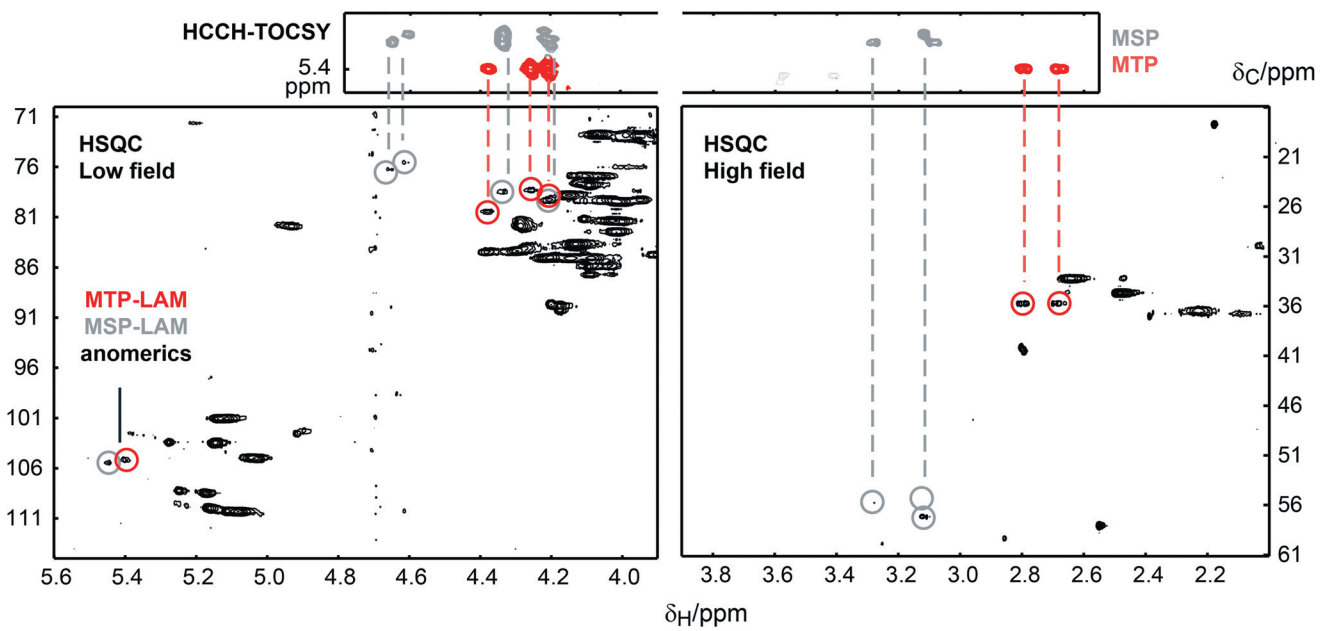


Fig. 3 HCCH-TOCSY and ^{1}H-^{13}C-HSQC spectra for Mtb ManLAM showing the position of the MTP and MSP resonances.

M. tuberculosis H37Ra strain, it has subsequently been identified in several other laboratory strains and clinical isolates, *e.g.*, H37Rv, CSU20 and MT103.[11,22] MTP/MSP was also identified in opportunistic pathogen *M. kansasii*, but in this case Guérardel *et al.*,[23] concluded that the MTP/MSP residue was attached to the mannose core of both LAM and lipomannan.

Synthetic approaches for the structural elucidation of MTP-Man

While the NMR and MS experiments were effective in identifying the presence of the MTP/MSP substituents, they were unable to determine either the configuration of the sugar, or the position of its linkage to the underlying mannose residue. Pyranose rings often have very distinctive patterns of coupling constants, however, the more flexible furanose rings are much more difficult to distinguish by coupling constants alone.[24–26]

There are four distinct aldopentose sugars (arabinose, lyxose, ribose and xylose), each of which can exist as either D- or L-entantiomers. In the furanose ring form, an additional stereocentre is created at the anomeric carbon which can be either α- or β-configured. Each sugar could then be attached at one of four positions on the terminal mannose residue, giving rise to 64 possible stereoisomers. In situations where NMR spectroscopic analysis alone is unable to resolve different structures easily, it can be useful to synthesise candidate compounds for comparison with the NMR data.[27]

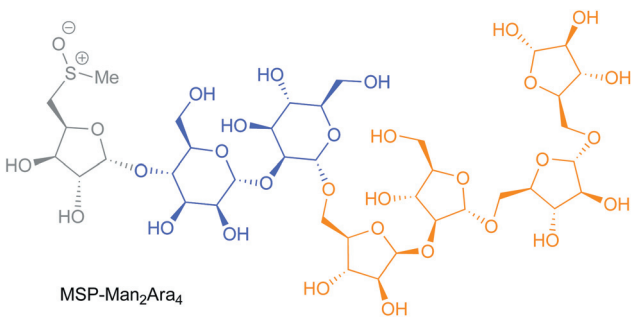


Fig. 4 MSP-Man_2Ara_4 fragment detected by mass spectrometry.

In an attempt to simplify this problem, Turnbull *et al.*, chose to synthesise the eight possible D-configured MTP isomers as methyl glycosides.[28] The HSQC spectrum of each MTP sugar was compared with that for the naturally occurring LAM polysaccharide. Of these, only the α-xylo-configured compound **1** was a good match to the original LAM data (Fig. 5). Oxidation of the α-xyloside **1** to form a 1 : 1 mixture of the sulfoxides **2** gave HSQC signals that similarly matched those for MSP. Therefore, MTP and MSP were henceforth renamed methylthioxylose (MTX) and methylsulfinylxylose (MSX).

A similar strategy was adopted by Joe *et al.*, to determine absolute configuration of MTX and its linkage position to the underlying mannosyl residue.[29] They were able to infer from the HMBC data published by Treumann *et al.*, that the MTX residue was more likely to be attached to a secondary position on the mannose residue. Therefore they elected to synthesise the six possible D/L-MTX-D-Man disaccharides (Fig. 6). Their synthetic strategy involved enantiomeric xylofuranosyl thioglycoside donors bearing a tosyl group at C-5 **3-D**/**3-L** which would allow the methylthio group to be introduced late in the synthesis. They were thus able to make each of the target disaccharides **4–6** for comparison with the published NMR data. Only the D-configured MTX attached to C-4 of the mannose residue **6-D** matched the ^{1}H and ^{13}C NMR data for the natural polysaccharide. This result was further confirmed by excellent correlation of the MSX-α(1,4)Man-αOMe **7-D** NMR data with those for MSX–LAM.

While the synthetic strategy of Joe *et al.* was effective in accessing all of their target disaccharides, the use of tosylated glycosyl donor **3-D** necessitated introduction of the methylthio

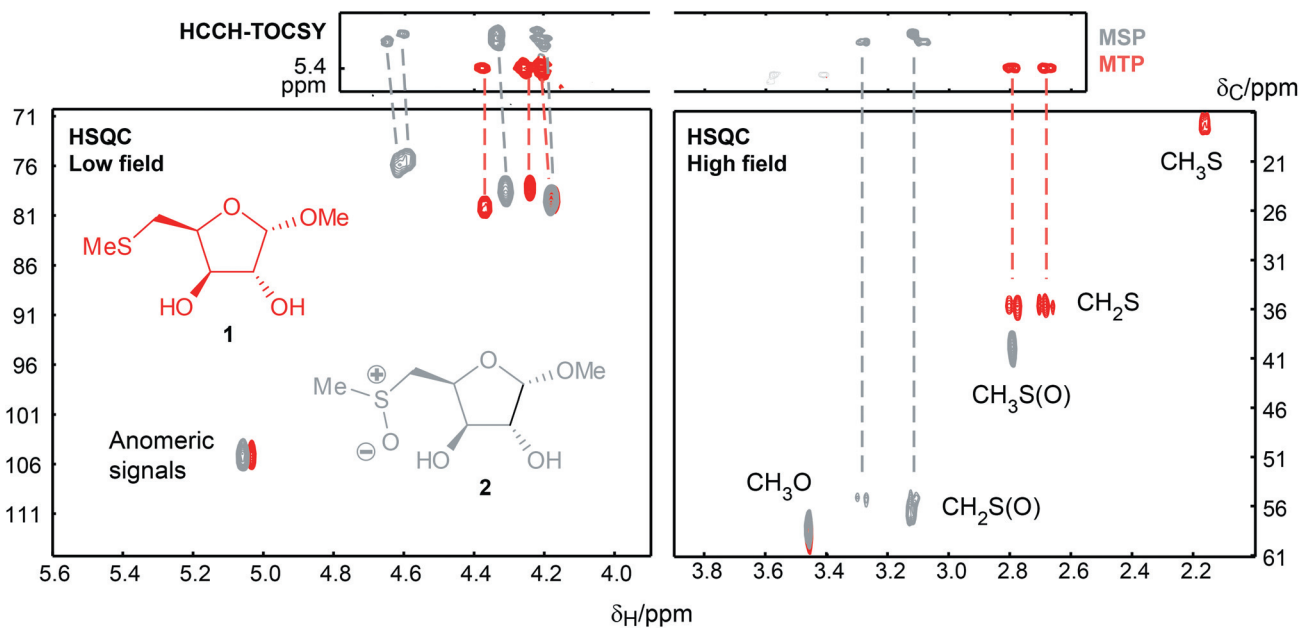


Fig. 5 HCCH-TOCSY spectrum for Mtb ManLAM and ^{1}H-^{13}C-HSQC spectra for xylo-configured MTP and MSP.

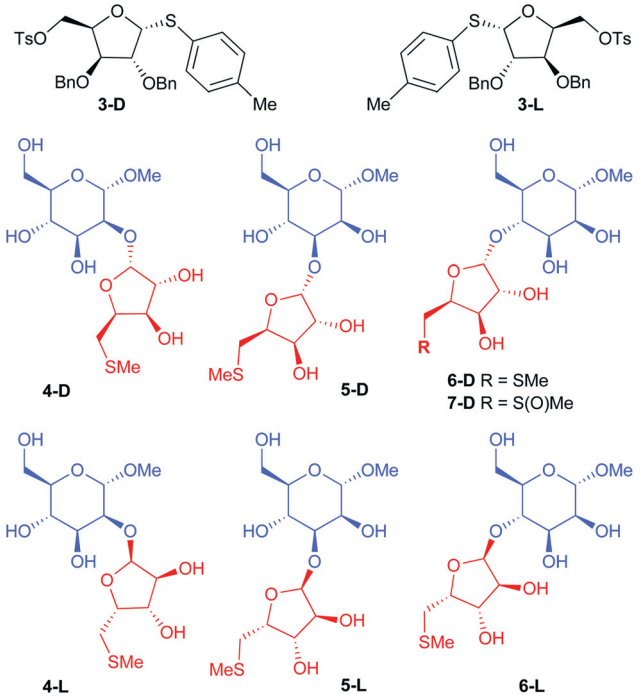
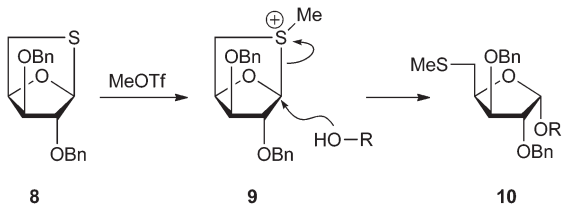


Fig. 6 Six diastereomeric MTX-Man disaccharides and glycosyl donors **3-D** and **3-L**.



Scheme 1 Bicyclic thioglycoside donor.

group as an additional step for each disaccharide produced.[29] An alternative approach would be to include the methylthio group in the glycosyl donor. However, we have found that a wide range of glycosyl donors bearing the methylthio group are quite inefficient as glycosyl donors.[30] A third strategy would be to introduce the methylthio group *during* the glycosylation reaction. To this end Stalford *et al.*, synthesised a bicyclic thioglycoside donor **8** in which the anomeric sulfur atom was also attached to C-5 (Scheme 1).[31] Upon methylation of the sulfur atom, the glycosyl donor would be activated for reaction with an alcohol, such that the leaving group would become the methylthio group in the product. It was anticipated that the bicyclic sulfonium ion intermediate **9** would also act as a participating group to direct the incoming alcohol to the α-face of the sugar. While such strategies have proved effective for pyranose sugars,[32–36] in this case the bicyclic donor was found to be no more stereoselective than the donor prepared by Joe *et al.*[29] Although the bicyclic sulfonium ion was observed as a relatively stable reaction intermediate, it appeared that the reaction proceeded through either an oxacarbenium ion, or more likely, a glycosyl triflate intermediate. Nevertheless, the bicyclic donors were found to offer an alternative concise route for the synthesis of MTX oligosaccharides.[31]

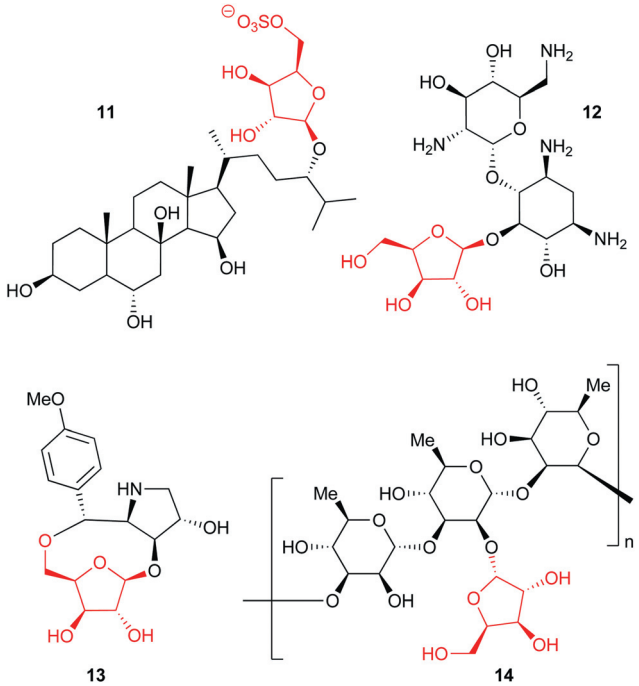


Fig. 7 Natural products incorporating a xylofuranosyl residue.

Xylofuranose – an unexpected configuration and its possible biosynthetic origins

Sugars in their furanose ring form are typically less stable than in the pyranose form; nevertheless, there are many examples of furanosides throughout nature.[37] Mycobacteria, for example, can make galactofuranose-containing polysaccharides and employ a specific mutase enzyme to transform UDP-galactopyranose into UDP-galactofuranose which may then be incorporated into polysaccharides.[38,39] Of course, in the case of MTX, the pyranose ring form is not possible as C-5 has a thioether substituent.

While the discovery of the first methylthio sugar in a polysaccharide was in itself surprising, to find that it had a xylofuranose structure was perhaps even more remarkable. While xylose is a major component of plant cell walls, there are relatively few examples of naturally occurring xylo-configured sugars outside the plant kingdom, in particular in the furanose ring form. Nevertheless, their structures are quite varied (Fig. 7). The starfish *Astropecten scoparius* and *Certonardoa semiregularis* produce saponins **11** bearing xylofuranosyl residues.[40,41] An aminoglycoside antibiotic **12** with a xylofuranose ring is produced by *Baccillus* sp.[42] *Streptomyces platensis* produces an insecticide substance **13** that has a xylofuranose residue locked in a nine-membered ring.[43] *Citrobacter freundii* produces a rhamnosyl polysaccharide **14** with pendant xylofuranosyl residues.[44]

Most intriguing, however, are several reports of xylofuranosyl compounds with sulfur substituents attached to C-5 (Fig. 8). The Mediterranean nudibranch mollusc *Doris verrucosa* makes a xylo-configured analogue **16** of the ubiquitous metabolite methylthioadenosine (MTA **15**)[45] which is produced during polyamine biosynthesis. *S*-Adenosylmethionine is first decarboxylated before its aminopropyl group is transferred onto

putrescine to form spermidine, leaving MTA as a byproduct. MTA is toxic to the cells and it is converted to adenine and 5-methylthio-ribose-1-phosphate **17** by the enzyme methylthioadenosine phosphorylase. The phosphate and adenine can then be recycled to form methionine and ATP respectively, which may be used in this metabolic pathway again.

Cimino *et al.* first discovered the xylo-configured analogue of MTA (xylo-MTA **16**) in *Doris verrucosa*,[45] which was subsequently shown to be present at concentrations two orders of magnitude higher than MTA.[46] It was initially thought that xylo-MTA could provide some chemical defence mechanism for the sea slug, but was found to be inactive against fungi, bacteria and viruses.[47] So why does *D. verrucosa* accumulate this metabolite? One possible reason could be that its formation is an alternative pathway for avoiding the toxic effects of MTA. While MTA is known to inhibit aminopropyl transferase enzymes, *e.g.*, spermidine and spermine synthetases, xylo-MTA has no inhibitory effects.[48] Porcelli *et al.*, also demonstrated that xylo-MTX was neither a substrate nor an inhibitor of MTA phosphorylase.[48] This observation accounts for its accumulation in *D. verrucosa* as it is untouched by the enzyme that would usually degrade MTA. These authors also demonstrated that MTA was the biosynthetic precursor of xylo-MTA, and a subsequent study employing MTA tritiated at the 3′-position provided compelling evidence that the epimerisation at C-3 occurs through oxidation and subsequent reduction at that carbon.[49] More recently, Peng *et al.*, have reported the discovery of a disulfide analogue of xylo-MTA **18** in the marine sponge *Trachycladus laevispirulifer*.[50] The authors speculated that this compound could be derived from xylo-MTA by demethylation and thiol oxidation. Although they were unable to find evidence to support this biosynthetic route, its occurrence again indicates a pathway involving epimerisation of an adenosyl nucleotide.

So could a similar pathway be active in Mtb for the biosynthesis of MTX? Most oligosaccharides are prepared from nucleotide sugar donors; however, in the case of mycobacterial LAM, polyprenolphosphoryl sugars are also involved.[27,51,52] Therefore, the biosynthesis of MTX-LAM presumably requires the production of either an MTX-nucleotide sugar, *e.g.*, UDP-MTX **19**, or a lipid-liked donor, *e.g.*, decaprenylphosphoryl MTX **20**. The precursor for either glycosyl donor would typically be a sugar phosphorylated at the anomeric centre, and such a species could be formed from xylo-MTA by a phosphorylase enzyme. However, in *D. verrucosa*, xylo-MTA is not a substrate for the native MTA-phosphorylase.[48] Therefore, a biosynthetic route *via* xylo-MTA would require either the Mtb phosphorylase to be more promiscuous than the one in *D. verrucosa*, or an additional enzyme specifically for this purpose.

An alternative route could involve conversion of MTA into methylthio-ribose-1-phosphate **17**, with subsequent epimerisation and glycosyl donor synthesis. When one considers that there are many examples of epimerases that operate on nucleotide sugars,[53] it may be more likely that epimerisation of C-3 to give the xylo-configured sugar donor would be the final step before glycosylation of ManLAM. Indeed, the arabinofuranosyl donor decaprenylphosphoryl arabinofuranose is prepared from the corresponding ribo-configured phospholipid by epimerisation at C-2;[51] therefore epimerisation within a lipid-linked donor also has precedent.

One further possibility would be a route based on the biosynthesis of decaprenylphosphoryl ribofuranose and decaprenylphosphoryl arabinofuranose that are already known to occur in mycobacteria.[51,52] In this case the methylthio group would need to be introduced late in the synthesis, perhaps in a manner analogous to methionine biosynthesis which uses cysteine as the sulfur source. Although such a route is also feasible, we consider a route starting from MTA **15** to be most likely.

While these biosynthetic hypotheses await testing, some authors have speculated about putative genes that could be involved in the biosynthetic pathway,[54] and comparative genomic methods may present other likely candidates.[55] Identification of the MTX biosynthetic enzymes will help complete the story of its existence, but may also help to shed light on its biological role.

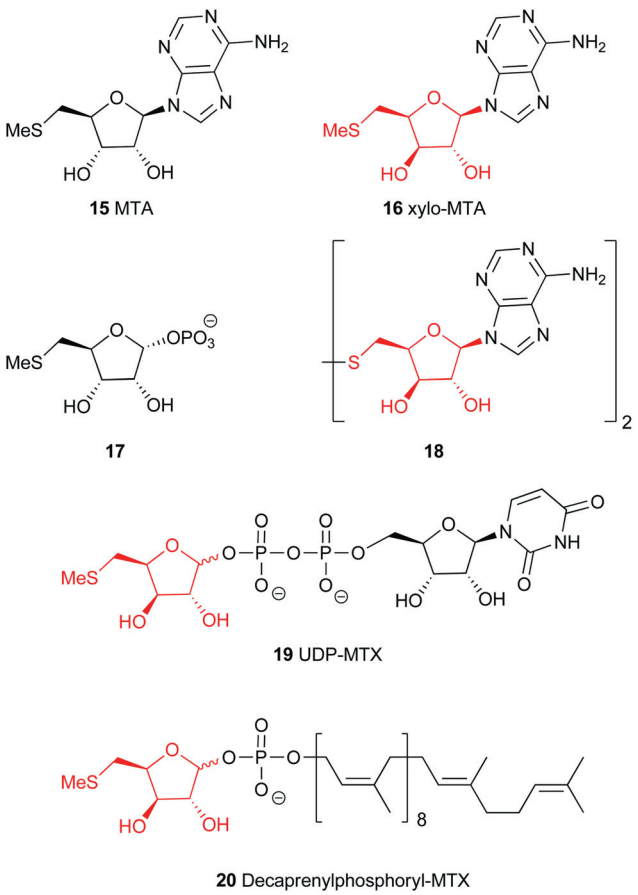


Fig. 8 Xylo-methylthioadenosine **16** and related compounds.

Potential biological functions of MTX-LAM: inhibition of cytokine production and anti-oxidative properties

When one considers that Mtb must invest significant biosynthetic effort to prepare and attach MTX to its surface, it is reasonable to expect that this substituent is in some way beneficial for the bacterium. In most instances, cell surface carbohydrates exert their biological function through interactions with proteins, and this observation is especially valid for sugar residues located at

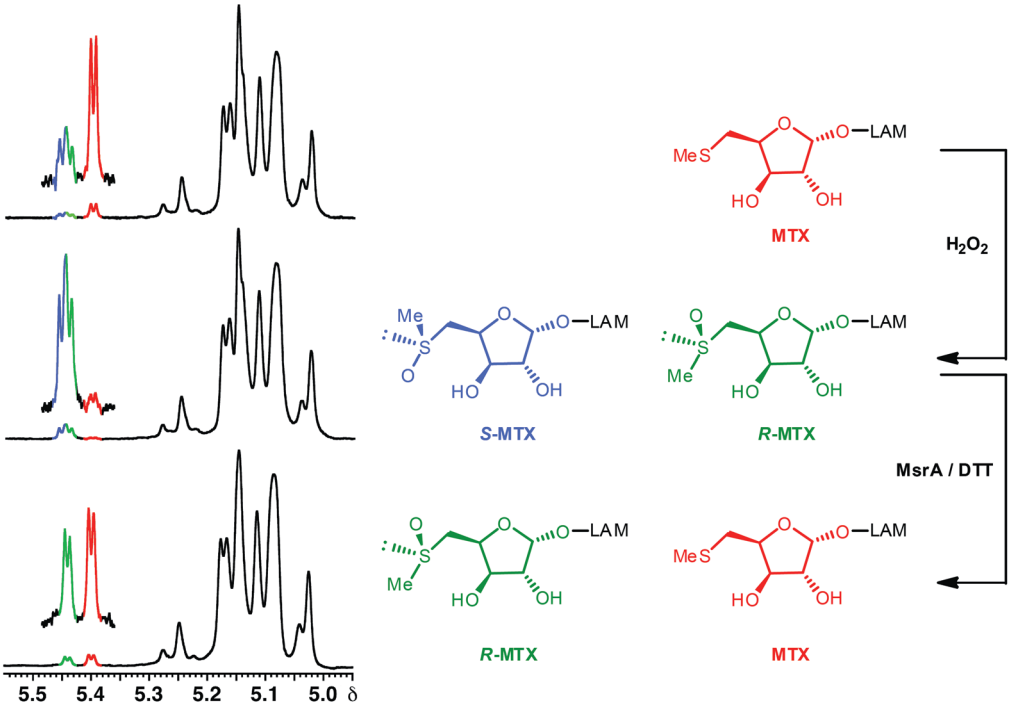


Fig. 9 Oxidation of MTX-LAM by hydrogen peroxide and stereoselective enzymatic reduction my Mtb methionine sulfoxide reductase (MsrA).

the non-reducing termini of glycans. Therefore, MTX/MSX is in the ideal location to contribute to the biological properties of LAM.

Lipoarabinomannan has been shown to have a range of biological functions. The mannose caps of Mtb LAM are thought to mediate binding to the macrophage mannose receptor which leads to entry into the macrophage.[6] However, recent studies have suggested that capsular polysaccharides may be responsible for cell entry.[7] Once inside the macrophage cells, LAM can modulate the immune response by inhibiting phagosome maturation, the production of pro-inflammatory cytokines, and Mtb-induced apoptosis.[7,14]

Joe *et al.*, evaluated MTX-Man and MSX-Man disaccharides for their ability to induce or suppress the formation of cytokines TNF-α or IL-12p70.[29] They found that while neither disaccharide induced cytokine production, MTX-Man **6-D** had a similar inhibitory potency on a molar basis to ManLAM. This inhibition was reduced upon oxidation to the sulfoxide **7-D**. The effect on IL-12p70 inhibition was substantially lower.

It has also been reported that LAM is able to scavenge oxygen based free radicals *in vitro*.[56] Experiments employing ESR spectroscopy and colorimetric assays showed that the polysaccharide could scavenge superoxide radicals effectively. Hydroxyl radical concentration was also reduced, but it was not clear if this was a direct result of scavenging hydroxyl radicals or the superoxide precursor. This evidence suggests that LAM may protect the bacterium from oxidative cytocidal mechanisms that would otherwise result in apoptosis.[56] It was not clear from these studies which part of the oligosaccharide would act to sequester the reactive oxygen species. In our own studies we have failed to detect any superoxide sequestering by the monosaccharide components of which LAM is constituted. However, we have observed sequestering of hydroxyl radicals by methyl glycosides of mannose, arabinose, MTX and MSX. Curiously, MSX was found to be the most effective scavenger.[30]

Hydrogen peroxide is a reactive oxygen species that is produced during a respiratory burst in macrophage cells,[57] but this species was not evaluated in the original study by Chan *et al.*[56] Peroxide is known to be the cause of protein oxidation *in vivo*, in particular for the oxidation of methionine residues. When one considers that MTX has the same methylthio functional group as methionine, it is reasonable to presume that peroxide should also lead to production of MSX.

All organisms have a system to repair methionine oxidation that comprises a pair of methionine sulfoxide reductase enzymes (named MsrA and MsrB) that can each reduce one of the sulfoxide diasteroisomers.[58,59] It has been proposed by Levine that a redox cycle of methionine oxidation followed by enzymatic reduction of methionine sulfoxide could provide anti-oxidative protection for cells by sequestering reactive oxygen species.[60] Work by Friguet and co-workers has shown that over expression of a methionine sulfoxide reductase enzyme can indirectly reduce irreversible protein oxidation, *e.g.*, carbonyl formation.[61] Furthermore, MsrA has been shown to be important for survival of mycobacteria in macrophage cells.[62]

Stalford *et al.*, demonstrated that when LAM from the Mtb clinical isolate CSU20 was exposed to hydrogen peroxide, MTX became oxidised to MSX (Fig. 9).[63] Two MSX anomeric signals were observed, corresponding to the *R*- and *S*-stereoisomers of the sulfoxide group. No other changes appeared in the LAM NMR spectrum indicating that only MTX can be responsible for any anti-oxidative protection attributed to peroxide reacting with LAM. They then showed that the MsrA enzyme from M. tuberculosis was able to reduce the *S*-configured sulfoxide

isomer to reform MTX. When one considers that MsrA is present in the cell wall of Mtb,[63] this result supports the hypothesis that MTX could potentially provide anti-oxidative protection for the bacterium through a redox cycle of chemical oxidation and enzymatic reduction. By repeating this experiment on synthetic samples of D- and L-configured MSX, Stalford *et al*., were also able to prove that the naturally occurring stereoisomer of MTX has the D-configuration. This result provided independent confirmation for the conclusions of Joe *et al*., on the structure of MTX-LAM.[29]

Conclusions and future perspectives

The story of MTX is intriguing but as yet incomplete. An unusual substituent that had presumably been overlooked in earlier studies has received significant attention over the past ten years. While we are now confident about its structure and location in the LAM polysaccharide, there is still much to be learned about its origins and purpose. Of course, it may transpire that this apparent jewel in the mycobacterial crown is nothing more than a decoration – bacterial bling. If so, then its occurrence will become even more intriguing. Substantial effort must be invested by the bacterium to express the enzymes for the MTX biosynthetic pathway, which presumably requires at least a glycosyl transferase, epimerase and either a nucleotide sugar or polyprenylphosphoryl sugar synthase. Efforts to identify the putative enzymes and test their substrate specificities are now required. Identification of the biosynthetic genes will enable the preparation of knockout strains that are necessary to test the hypotheses for its biological role. Such studies would also be facilitated by the development of specific antibodies for MTX and MSX which would simplify quantitation of these substituents without having to resort to the lengthy isolation and purification of LAM from the bacteria. As oligosaccharide-bound MTX appears to be the exclusive preserve of mycobacteria, antibodies against this structure could even have applications in the diagnosis of tuberculosis and related diseases. In each of these endeavours, the preparation of synthetic, chemically homogeneous compounds will play a vital role for future progress.

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Notes and references

1 WHO, *Global Tubercoluosis Control 2011*, World Health Organisation, Geneva, 2011.
2 P. J. Brennan and H. Nikaido, *Annu. Rev. Biochem.*, 1995, **64**, 29–63.
3 D. Chatterjee and P. J. Brennan, in *Microbial Glycobiology*, eds. A. P. Moran, O. Holst, P. J. Brennan and M. von Itzstein, Elsevier, London, 2009, pp. 147–167.
4 D. Kaur, M. E. Guerin, H. Skovierova, P. J. Brennan and M. Jackson, *Adv. Appl. Microbiol.*, 2009, **69**, 23–78.
5 G. S. Besra, K.-H. Khoo, M. R. McNeil, A. Dell, H. R. Morris and P. J. Brennan, *Biochemistry*, 1995, **34**, 4257–4266.
6 A. Venisse, J.-J. Fournié and G. Puzo, *Eur. J. Biochem.*, 1995, **231**, 440–447.
7 A. K. Mishra, N. N. Driessen, B. J. Appelmelk and G. S. Besra, *FEMS Microbiol. Rev.*, 2011, **35**, 1126–1157.
8 P. J. Brennan and D. C. Crick, *Curr. Top. Med. Chem.*, 2007, **7**, 475–488.
9 L. G. Dover, A. Bhatt, V. Bhowruth, B. E. Willcox and G. S. Besra, *Expert Rev. Vaccines*, 2008, **7**, 481–497.
10 F. E. Umesiri, A. K. Sanki, J. Boucau, D. R. Ronning and S. J. Sucheck, *Med. Res. Rev.*, 2010, **30**, 290–326.
11 A. Treumann, X. Feng, L. McDonnell, P. J. Derrick, A. E. Ashcroft, D. Chatterjee and S. W. Homans, *J. Mol. Biol.*, 2002, **316**, 89–100.
12 S. Pitarque, G. Larrouy-Maumus, B. Payré, M. Jackson, G. Puzo and J. Nigou, *Tuberculosis*, 2008, **88**, 560–565.
13 D. Chatterjee and K. H. Khoo, *Glycobiology*, 1998, **8**, 113–120.
14 J. Nigou, M. Gilleron and G. Puzo, *Biochimie*, 2003, **85**, 153–166.
15 K. J. C. Gibson, M. Gilleron, P. Constant, G. Puzo, J. Nigou and G. S. Besra, *Microbiology*, 2003, **149**, 1437–1445.
16 K. J. C. Gibson, M. Gilleron, P. Constant, G. Puzo, J. Nigou and G. S. Besra, *Biochem. J.*, 2003, **372**, 821–829.
17 J. B. Torrelles, K.-H. Khoo, P. A. Sieling, R. L. Modlin, N. Zhang, A. M. Marques, A. Treumann, C. D. Rithner, P. J. Brennan and D. Chatterjee, *J. Biol. Chem.*, 2004, **279**, 41227–41239.
18 K. H. Khoo, J. B. Tang and D. Chatterjee, *J. Biol. Chem.*, 2001, **276**, 3863–3871.
19 J. Nigou, A. Vercellone and G. Puzo, *J. Mol. Biol.*, 2000, **299**, 1353–1362.
20 M. Gilleron, N. Himoudi, O. Adam, P. Constant, A. Venisse, M. Rivière and G. Puzo, *J. Biol. Chem.*, 1997, **272**, 117–124.
21 K. Zangger, in *New Research on Magnetic Resonance Imaging*, ed. B. C. Castleman, Nova Science Publishers, Inc., Hauppauge, N.Y., 2007, pp. 73–91.
22 P. Ludwiczak, M. Gilleron, Y. Bordat, C. Martin, B. Gicquel and G. Puzo, *Microbiology*, 2002, **148**, 3029–3037.
23 Y. Guérardel, E. Maes, V. Briken, F. Chirat, Y. Leroy, C. Locht, G. Strecker and L. Kremer, *J. Biol. Chem.*, 2003, **278**, 36637–36651.
24 J. G. Napolitano, J. A. Gavin, C. Garcia, M. Norte, J. J. Fernandez and A. H. Daranas, *Chem.–Eur. J.*, 2011, **17**, 6338–6347.
25 J. B. Houseknecht, C. Altona, C. M. Hadad and T. L. Lowary, *J. Org. Chem.*, 2002, **67**, 4647–4651.
26 H. A. Taha, P.-N. Roy and T. L. Lowary, *J. Chem. Theory Comput.*, 2011, **7**, 420–432.
27 B. Cao and S. J. Williams, *Nat. Prod. Rep.*, 2010, **27**, 919–947.
28 W. B. Turnbull, K. H. Shimizu, D. Chatterjee, S. W. Homans and A. Treumann, *Angew. Chem., Int. Ed.*, 2004, **43**, 3918–3922.
29 M. Joe, D. Sun, H. Taha, G. C. Completo, J. E. Croudace, D. A. Lammas, G. S. Besra and T. L. Lowary, *J. Am. Chem. Soc.*, 2006, **128**, 5059–5072.
30 S. A. Stalford, PhD Thesis, University of Leeds, 2008.
31 S. A. Stalford, C. A. Kilner, A. G. Leach and W. B. Turnbull, *Org. Biomol. Chem.*, 2009, **7**, 4842–4852.
32 T. J. Boltje, J.-H. Kim, J. Park and G.-J. Boons, *Nat. Chem.*, 2010, **2**, 552–557.
33 J. H. Kim, H. Yang, J. Park and G. J. Boons, *J. Am. Chem. Soc.*, 2005, **127**, 12090–12097.
34 M. A. Fascione, S. J. Adshead, S. A. Stalford, C. A. Kilner, A. G. Leach and W. B. Turnbull, *Chem. Commun.*, 2009, 5841–5843.
35 M. A. Fascione, C. A. Kilner, A. G. Leach and W. B. Turnbull, *Chem.–Eur. J.*, 2012, **18**, 321–333.
36 M. A. Fascione, N. J. Webb, C. A. Kilner, S. L. Warriner and W. B. Turnbull, *Carbohydr. Res.*, 2012, **348**, 6–13.
37 P. Peltier, R. Euzen, R. Daniellou, C. Nugier-Chauvin and V. Ferrières, *Carbohydr. Res.*, 2008, **343**, 1897–1923.
38 M. R. Richards and T. L. Lowary, *ChemBioChem*, 2009, **10**, 1920–1938.
39 M. B. Poulin and T. L. Lowary, *Methods Enzymol.*, 2010, **478**, 389–411.
40 M. Iorizzi, L. Minale, R. Riccio and H. Kamiya, *J. Nat. Prod.*, 1990, **53**, 1225–1233.
41 W. Wang, F. Li, J. Hong, C.-O. Lee, H. Y. Cho, K. S. Im and J. H. Jung, *Chem. Pharm. Bull.*, 2003, **51**, 435–439.
42 S. Horii, I. Nogami, N. Mizokami, Y. Arai and M. Yoneda, *Antimicrob. Agents Chemother.*, 1974, **5**, 578–581.
43 K. Kanbe, Y. Mimura, T. Tamamura, S. Yatagai, Y. Sato, A. Takahashi, K. Sato, H. Naganawa and H. Nakamura, *J. Antibiot.*, 1992, **45**, 458–464.
44 N. A. Kocharova, Y. A. Knirel, A. S. Shashkov, N. K. Kochetkov, E. V. Kholodkova and E. S. Stanislavsky, *Carbohydr. Res.*, 1994, **263**, 327–331.
45 G. Cimino, A. Crispino, S. S. De, M. Gavagnin and G. Sodano, *Experientia*, 1986, **42**, 1301–1302.

46 M. Porcelli, G. Cacciapuoti, A. Oliva and V. Zappia, *J. Chromatogr., A*, 1988, **440**, 151–155.
47 A. Pani, M. E. Marongiu, P. Obino, M. Gavagnin and P. La Colla, *Experientia*, 1991, **47**, 1228–1229.
48 M. Porcelli, G. Cacciapuoti, G. Cimino, M. Gavagnin, G. Sodano and V. Zappia, *Adv. Exp. Med. Biol.*, 1988, **250**, 219–228.
49 M. Porcelli, G. Cacciapuoti, G. Cimino, M. Gavagnin, G. Sodano and V. Zappia, *Biochem. J.*, 1989, **263**, 635–640.
50 C. Peng, G. M. K. B. Gunaherath, A. M. Piggott, Z. Khalil, M. Conte and R. J. Capon, *Aust. J. Chem.*, 2010, **63**, 873–876.
51 K. Mikušová, H. Huang, T. Yagi, M. Holsters, D. Vereecke, W. D'Haeze, M. S. Scherman, P. J. Brennan, M. R. McNeil and D. C. Crick, *J. Bacteriol.*, 2005, **187**, 8020–8025.
52 B. A. Wolucka, *FEBS J.*, 2008, **275**, 2691–2711.
53 M. E. Tanner, *Curr. Org. Chem.*, 2001, **5**, 169–192.
54 P. R. Wheeler, N. G. Coldham, L. Keating, S. V. Gordon, E. E. Wooff, T. Parish and R. G. Hewinson, *J. Biol. Chem.*, 2005, **280**, 8069–8078.
55 R. Banerjee, P. Vats, S. Dahale, S. M. Kasibhatla and R. Joshi, *PLoS One*, 2011, **6**, e19280.
56 J. Chan, X. Fan, S. W. Hunter, P. J. Brennan and B. R. Bloom, *Infect. Immun.*, 1991, **59**, 1755–1761.
57 J. A. Imlay, *Annu. Rev. Biochem.*, 2008, **77**, 755–776.
58 S. Boschi-Muller, A. Olry, M. Antoine and G. Branlant, *Biochim. Biophys. Acta*, 2005, **1703**, 231–238.
59 S. J. Sasindran, S. Saikolappan and S. Dhandayuthapani, *Future Microbiol.*, 2007, **2**, 619–630.
60 R. L. Levine, L. Mosoni, B. S. Berlett and E. R. Stadtman, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 15036–15040.
61 C. R. Picot, I. Petropoulos, M. Perichon, M. Moreau, C. Nizard and B. Friguet, *Free Radical Biol. Med.*, 2005, **39**, 1332–1341.
62 T. Douglas, D. S. Daniel, B. K. Parida, C. Jagannath and S. Dhandayuthapani, *J. Bacteriol.*, 2004, **186**, 3590–3598.
63 S. A. Stalford, M. A. Fascione, S. J. Sasindran, D. Chatterjee, S. Dhandayuthapani and W. B. Turnbull, *Chem. Commun.*, 2009, 110–112.

