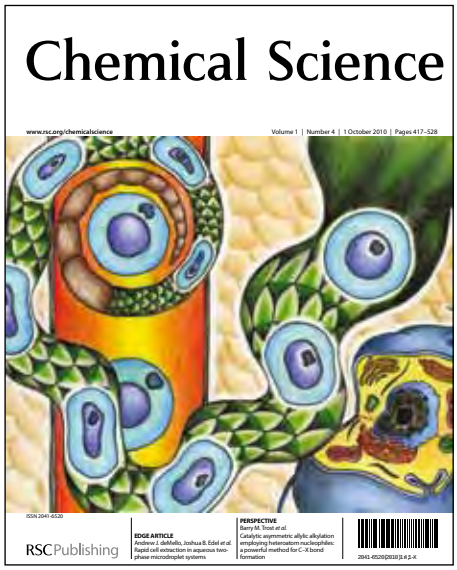
Chemical Science

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the RSC Publishing peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, which is prior to technical editing, formatting and proof reading. This free service from RSC Publishing allows authors to make their results available to the community, in citable form, before publication of the edited article. This *Accepted Manuscript* will be replaced by the edited and formatted *Advance Article* as soon as this is available.

To cite this manuscript please use its permanent Digital Object Identifier (DOI®), which is identical for all formats of publication.

More information about *Accepted Manuscripts* can be found in the **Information for Authors**.

Please note that technical editing may introduce minor changes to the text and/or graphics contained in the manuscript submitted by the author(s) which may alter content, and that the standard **Terms & Conditions** and the **ethical guidelines** that apply to the journal are still applicable. In no event shall the RSC be held responsible for any errors or omissions in these *Accepted Manuscript* manuscripts or any consequences arising from the use of any information contained in them.

View Online

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Total Synthesis, Stereochemical Elucidation and Biological Evaluation of Ac_2SGL; a 1,3-Methyl Branched Sulfoglycolipid from *Mycobacterium tuberculosis*

Danny Geerdink,[a] Bjorn ter Horst,[a] Marco Lepore[b], Lucia Mori[b,e], Germain Puzo[c,d], Anna K. H. Hirsch,[a] Martine Gilleron,[c,d] Gennaro de Libero[b,e] and Adriaan J. Minnaard[a*]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), continues to represent a challenging pathogen causing many deaths. A reason for the persistence of this pathogen is the cell envelope composition, which consists of long-tailed (glyco)lipids, involved in the modulation of the host immune response. Diacylated sulfoglycolipid Ac_2SGL (**1**), found in the cell envelope, is a potent antigen that stimulates the immune response towards TB. This observation suggests the application of **1** as part of a vaccine. Here, we report the first asymmetric total synthesis of **1**. Two approaches were developed for the synthesis of hydroxyphthioceranic acid **4**, its polypropionate part, thereby establishing the absolute stereochemistry of the C-17 hydroxy function to be of (*R*)-configuration. Subsequently, **4** was regioselectively connected to the trehalose core and after selective sulfation and a final fourfold deprotection step, pure **1** was obtained. The identity of synthetic and natural **1** was confirmed by NMR and mass analysis, Furthermore, synthetic **1** shows identical biological function to **1** and activates CD1b-restricted and Ac_2SGL-specific T cells that are highly sensitive to minimal structural modifications of **1** with the same potency. A modeling study is presented to point out the structural features of **1** that are important for binding to the antigen-presenting molecule CD1b and to the T-cell receptor.

Introduction

Tuberculosis (TB) is a disease that remains difficult to cure as it requires the administration of antibiotic drugs for long periods of time. Active TB has a clinical course with bad prognosis and untreated patients have a death rate exceeding 50%. The estimated annual death rate of TB patients is approximately 1.7 million, predominantly in developing countries.[1-2]

Mycobacterium tuberculosis is the causative agent of TB and mostly localizes to the lungs. It is a highly infectious pathogen transmitted with sputum and aerosol droplets from infected individuals. Although only a small part of the population will develop clinically active TB, it is estimated that one-third of the world's population is currently infected with a dormant or latent form of *M. tuberculosis*. With the emergence of multidrug-resistant and extensively drug-resistant mycobacterial bacilli, there is a great need to enhance our understanding of how mycobacteria achieve a dormant and immunologically neutral state and how the immune system provides efficient protection. [3] There is broad consensus that the development of novel vaccines capable of limiting bacilli growth and eradicating intracellular latent forms would represent an important achievement.[4]

One of the main reasons why *M. tuberculosis* is so elusive, is impermeability of the cell-envelope to most antibiotics. The outer membrane consists of a variety of complex, long-tailed (glyco)lipids, creating a hydrophobic barrier against antibiotics.[5-7] Two of these glycolipids, which are found exclusively in *M. tuberculosis*, are $acyl_2$sulfoglycolipid (Ac_2SGL, **1**) and sulfolipid-1 (SL-1 / Ac_4SGL, **2**) (Figure 1). SL-1, a constituent of the cell envelope, was discovered by Goren in the 1970s and was indicated as a virulence factor.[8-10] The role played by SL-1 in the mycobacterial cell wall remains unclear, and different studies have provided contradicting results as to whether SL-1 has an influence on the *in vivo* virulence of mycobacteria.[11-16]

Ac_2SGL, and possibly other SL-1 intermediates, might also modulate *M. tuberculosis* pathogenesis. A ΔmmpL8 mutant, which accumulates Ac_2SGL but lacks SL-1, shows diminished virulence in mice. By contrast, a Δpks2 mutant, which lacks both Ac_2SGL and SL-1, is indistinguishable from wildtype *M. tuberculosis* in mice and guinea pigs.[17-19] Recent studies have also described that lack of SL-1 facilitates intracellular survival of *M. tuberculosis*.[20] These findings were observed when human macrophages and not mouse macrophages were infected, indicating a species-specific effect probably associated with differences in antimycobacterial effector mechanisms of macrophages.

In 2004, Gilleron et al. reported the isolation and structure of a diacylated sulfoglycolipid (Ac_2SGL, **1**),[21] exclusively found in *M. tuberculosis*.[22] Ac_2SGL **1** also features a trehalose 2'-sulfate core, but is diacylated with either a palmitic or stearic acid residue at the 2-position and hydroxyphthioceranic acid at the 3-position**.** Additionally, it was proven that **1** functions as a potent

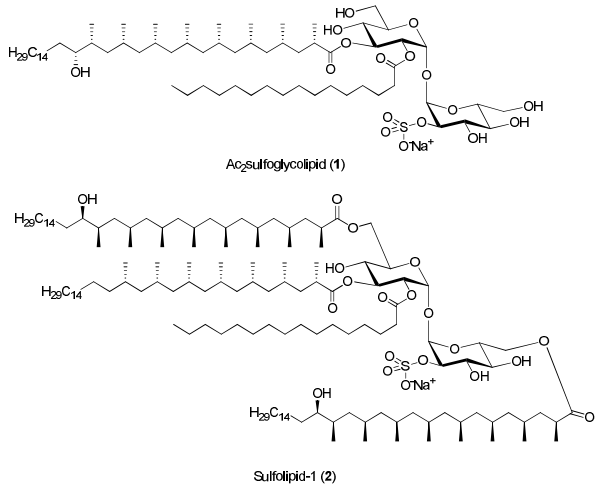


Figure 1 Structures of sulfoglycolipids Ac_2SGL (**1**) and SL-1 (**2**) as found in the cell wall of *M. tuberculosis*.

antigen of CD1b restricted ab T cells in humans infected by *M. tuberculosis*.[23] T cells activated by **1** release interferon-γ, recognize *M. tuberculosis*-infected cells, and kill intracellular mycobacteria. In light of these properties, **1** has been proposed as a component of a new vaccine against tuberculosis.

Comparison of the antigenicity of a panel of synthetic analogues of Ac_2SGL **1**, sharing the same trehalose-sulfate polar headgroup but differing in the structure of their acyl tails, showed that the number of C-methyl substituents on the chain, the configuration of these chiral centres, and the respective location of the two different acyl chains on the trehalose moiety govern T-cell receptor (TCR) recognition and T lymphocyte activation. A direct correlation was observed between the ability of SGLs to stimulate Ac_2SGL-specific T cells and the number of methyl-branched groups on the aliphatic chain at the 3-position of the trehalose. SGLs esterified with saturated methyl-branched acids required at least two 1,3-methyl substituents to stimulate Ac_2SGL-specific T cells at all. Although most synthetic analogues showed activation of Ac_2SGL specific T cells, at low concentrations (<100 nM ~ 0.1 μg/mL) their antigenicity was significantly decreased compared to **1**.[24,25]

Because of their important role in the human immune response and their painstaking purification from pathogenic *M. tuberculosis*,[18] it might appear remarkable that **1** (as well as **2**) has escaped total synthesis to date. All the more so because the application of **1** as part of a vaccine-development program would heavily depend on the access to synthetic material. The molecular complexity of **1** is, however, impressive. The 1,3-array of eight methyl substituents in hydroxyphthioceranic acid (**4**) is unprecedented in synthesis and the presence of a hydroxy function at C17 of **4**, vicinal to a methyl group and with unknown stereochemistry, thwarts a straightforward synthesis even further. At the outset, it was not fully obvious whether this stereochemistry could be unambiguously established by synthesis, also because of the limited amount of natural reference material, available only as component of a complex cell wall extract. Additional challenges in the synthesis of **1** are the regioselective functionalization, including sulfation, of C_2-symmetric trehalose[25] and the necessity to limit the number of synthetic steps after attachment of precious **4** to trehalose.

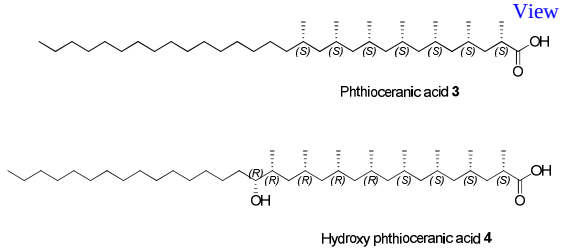
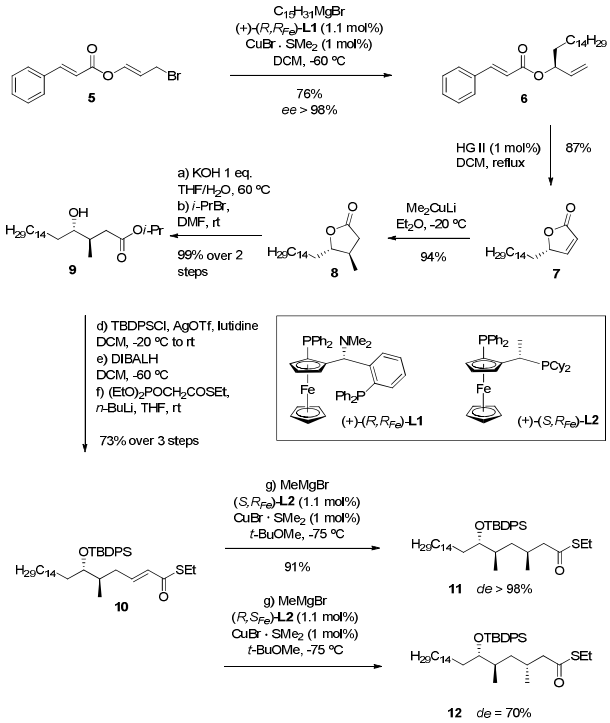


Figure 2 Phthioceranic (**3**) and hydroxyphthioceranic (**4**) acid and the absolute configuration of their stereocenters.

We have been involved in the synthesis of (glyco)lipids from *M. tuberculosis* for several years,[26] and an efficient catalytic iterative protocol for the synthesis of deoxypropionates has been developed and applied to the synthesis of **3**, among other polyketides.[27-30] Taking advantage of this knowledge, the synthesis of **4** has been attempted with the final goal of generating a synthetic sample of **1**. To elucidate the stereochemistry of the C17 stereocenter of **4,** an initial synthetic route has been devised. A second route to **4** has been developed to optimize its synthesis and here we report our results for the complete asymmetric synthesis of **1** and the elucidation of its stereochemistry.

Synthesis and stereochemical elucidation of hydroxyphthioceranic acid: A first approach

Our initial approach toward **4** was based on the copper-catalyzed asymmetric allylic alkylation of **5**, which in turn is prepared from cinnamoyl bromide and acrolein (Scheme 1).[31,32] Addition of pentadecylmagnesium bromide, using CuBr•SMe_2 and **L1**, provided **6** in 76% yield and 98% *ee*. As the stereochemistry of

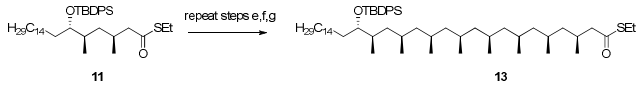


Scheme 1 First approach to the synthesis of hydroxyphthioceranic acid (**4**).

the hydroxyl group in natural **4** was unknown, we deliberately chose the *S*-configuration, assuming that this could be adapted at a later stage via a Mitsunobu reaction should this be necessary. Ring-closing metathesis of **6** using 1 mol% of second-generation Hoveyda-Grubbs catalyst (HG II) afforded α,β-unsaturated lactone **7** in 87% yield. Using substrate control, 1,4-addition with the Gilman reagent Me_2CuLi gave *anti*-**8** as a single diastereomer in 94% yield.

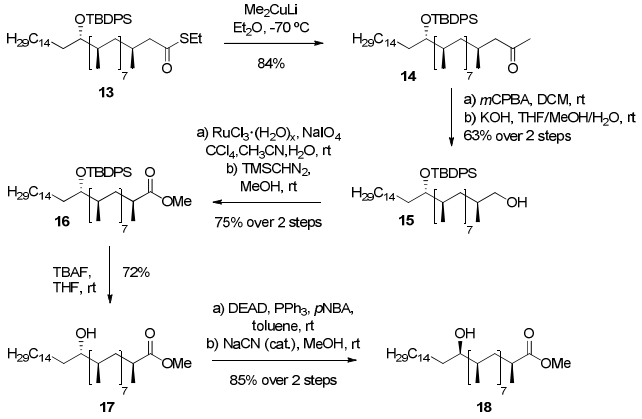
Initially, we attempted to reduce the lactone to the corresponding lactol, followed by Wittig or Horner-Wadsworth-Emmons (HWE) olefination to prepare compound **10**, after silylation. Although reduction of **7** with DIBALH afforded the lactol quantitatively, this compound resisted olefination. Initially, ring opening and esterification of the resulting acid were hampered by the strong tendency to cyclize again. Treatment with one equivalent of KOH in THF/water, followed by addition of an excess of *iso*-propyl bromide in DMF, however, allowed the isolation of ester **9** in near-quantitative yield. The secondary hydroxyl group of **9** was protected as its silyl ether, after which the ester moiety was reduced to the corresponding aldehyde with DIBALH. Subsequent HWE olefination afforded α,β-unsaturated thioester **10** in 73% yield over three steps. With **10** in hand, we were curious what the influence of the substrate would be on the diastereoselectivity of the copper-catalyzed 1,4-addition of MeMgBr using (+)-(S,R_{Fe})- or (–)-(R,S_{Fe})-Josiphos (**L2**) as the ligand. Analysis of both reactions by ^{1}H-NMR spectroscopy showed in the case of (+)-(S,R_{Fe})-Josiphos a clear preference for the desired *syn* product **11**, which was isolated in high yield and a *de* higher than 98%. The *anti*-product **12** was obtained with (–)-(R,S_{Fe})-Josiphos in an acceptable but markedly lower *de* of 70% (Figure 1SI).

The preparation of **11** set the stage for the introduction of all subsequent methyl substituents applying an iterative protocol following the sequence of DIBALH reduction/HWE olefination/asymmetric conjugate addition.[28,29] Repetition of steps e, f and g (Schemes 1 and 2) led to thioester **13** with all eight methyl substituents in a 1,3-array as present in natural **4**. The 21 steps, starting from **10**, were performed in an excellent 14% overall yield, affording **13** as a single stereoisomer without formation of *anti*-product (for the ^{1}H-NMR spectrum, see SI).



Scheme 2 Synthesis of the all-*syn* 1,3-methyl deoxypropionate array.

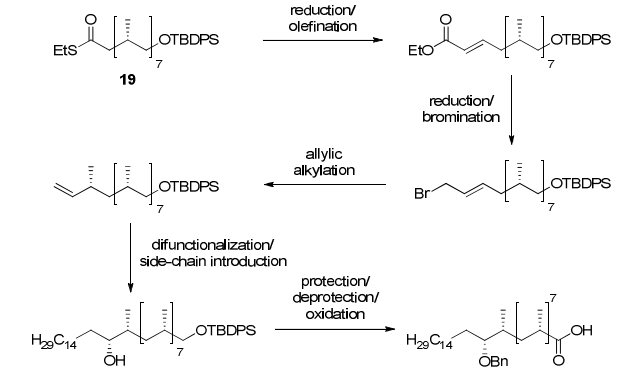
To install the carboxylic acid function of **4** at the correct position starting from **13**, one carbon had to be removed (Scheme 3). To do so, the β-substituted thioester was converted into the corresponding methyl ketone using Me_2CuLi, affording **14** in 84% yield.[33] This ketone was subsequently submitted to a regioselective Baeyer–Villiger oxidation employing *m*-chloroperbenzoic acid. To overcome partial hydrolysis of the acetate formed in the Baeyer–Villiger oxidation, the mixture obtained was hydrolyzed to the primary alcohol **15** in 63% yield over two steps. Oxidation of the primary alcohol and deprotection of the secondary hydroxyl group would directly afford the target compound. However, we realized that in order to compare the synthetic material with that of natural origin, it would be more



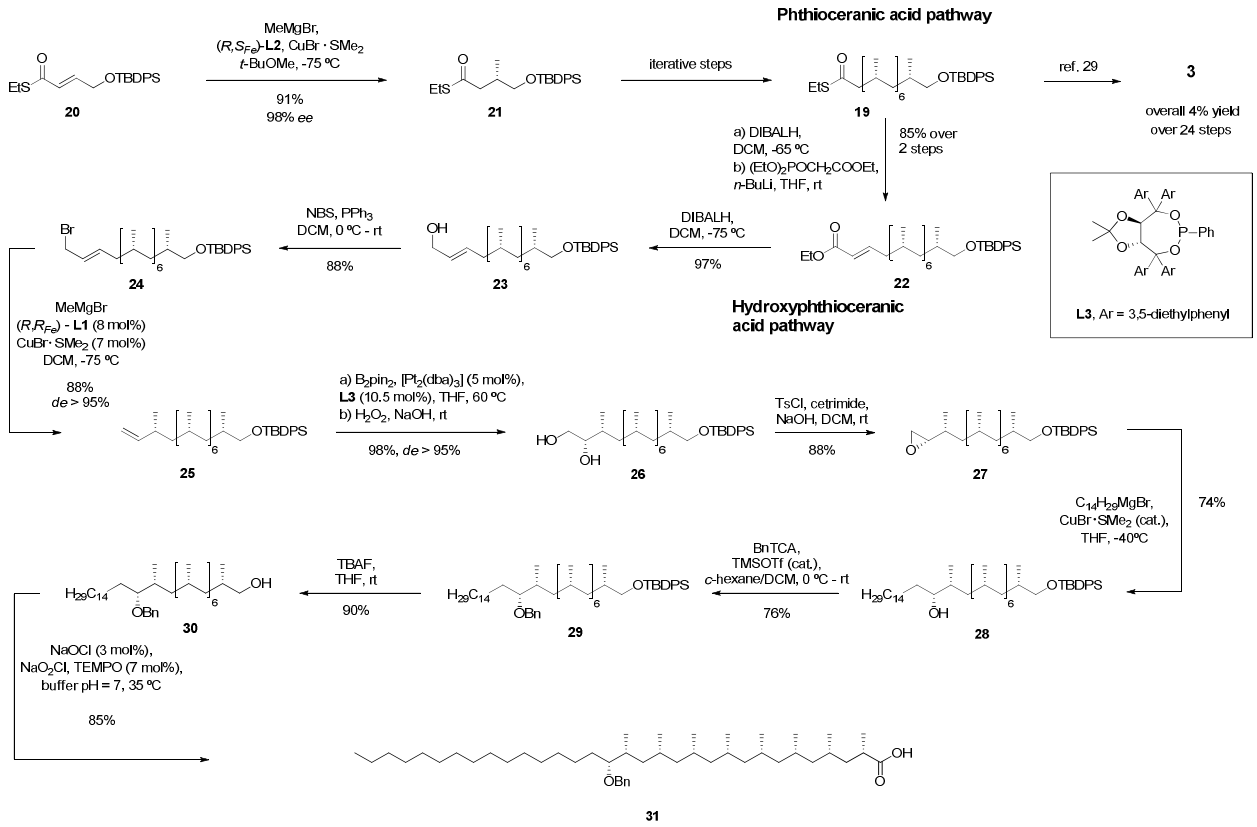
Scheme 3 Synthesis of C17 *syn*- and *anti*-hydroxyphthioceranic acid methyl esters.

convenient to have **4** available as its methyl ester. For this reason, alcohol **15** was oxidized using $RuCl_3\cdot(H_2O)_x$ and $NaIO_4$ and immediately treated with trimethylsilyl diazomethane to give methyl ester **16** in 75% yield over two steps. Deprotection with TBAF afforded the methyl ester of **4** (**17**) with the secondary alcohol at C17 in an *anti* configuration with respect to the methyl group at C16. To unambiguously assign the stereochemistry of C17 in natural **4**, **17** was also converted into its C17-OH epimer (**18**) by Mitsunobu reaction with *p*-nitrobenzoic acid. Trans-esterification with NaCN in MeOH finally afforded **18** in 85% yield over two steps. With the current synthetic route, **4** was obtained in 1.4% overall yield over 32 steps starting from **5**.

The optical rotation of **17** and **18** was virtually identical ([α] = +16.0 for **17** and [α] = +16.4 for **18**, both in $CHCl_3$), and in turn close to that reported in the literature for a mixture of homologues ([α] = +23).[34] An extract of cell-wall lipids containing sulfolipid-1 (**2**), prepared following the procedure of Goren, was trans-esterified with NaCN in MeOH to give a complex mixture of polypropionate methyl esters.[8] The presence of **17** or **18** in the natural sample was proven by mass spectrometry. Analysis with HPLC-ELSD revealed a significant difference in retention time between **17** and **18** and comparison with the natural sample confirmed that *anti*-**17** was not present in the natural sample, whereas *syn*-**18** was (Figure 2SI). The esterified natural sample was compared to synthetic **17** and



Scheme 4 Proposed synthetic pathway for the synthesis of hydroxyphthioceranic acid (**4**).



Scheme 5 Synthesis of phthioceranic and hydroxyphthioceranic acid.

18 by ^{1}H-NMR spectroscopy. Although the natural sample consists of a mixture of homologues, the chemical shift for the methine-proton next to the OH-group is not obscured. Inspection of the ^{1}H-NMR spectrum of synthetic *anti*-**17** and *syn*-**18** revealed a small but distinct difference (3.47 and 3.50 ppm, respectively). A perfect match with *syn*-**18** was found for the natural sample which provided strong evidence for a *syn* relationship in the natural product (Figure 3SI). Additional and conclusive evidence was obtained by comparing the ^{13}C-NMR spectra of all samples. Both **17** and **18** displayed distinct differences in chemical shift for the carbon atoms in the proximity of the secondary hydroxyl group. The natural product was shown to be identical with *syn*-**18**, whereas *anti*-**17** was not detected (Figure 4SI). The combined data establish that **4**/**18** have the same stereochemistry as natural hydroxyphthioceranic acid.

With the structure of **4** established, a more straightforward synthetic route could be envisioned without the need to invert the stereochemistry of the hydroxyl group. In addition, the synthesis of **3** and **4** from a common intermediate is appealing, because both polypropionates are found in sulfolipid-1 (Figure 1).[9]

Commencing the synthesis with the iterative deoxypropionate synthesis protocol, and subsequent functionalization towards **3** and **4** would streamline their synthesis considerably.

In Scheme 4, the most advanced common intermediate for both **3** and **4** is **19**,[29] with seven methyl branches installed.[27] In order to obtain **4**, we planned to introduce the eighth methyl branch via copper-catalyzed allylic substitution.[35] Unlike conjugate addition, allylic substitution enables subsequent difunctionalization of the resulting alkene in order to introduce the hydroxyl group and to append the required alkyl chain to the terminus. Even though the asymmetric allylic alkylation is a reasonably well explored reaction, we were not certain of its viability in the presence of such an extended array of chiral centres nearby. Moreover, enantio- or diastereoselective difunctionalization of monosubstituted aliphatic terminal olefins is notoriously difficult and only few successful examples have been reported in the literature.[36,37] Finally, as **4** will have to be esterified to the trehalose core, its hydroxyl function needed protection in such a way that deprotection would not be detrimental to the rest of the molecule.

Starting from α,β-unsaturated thioester **20**, intermediate **19** was obtained in good yield with seven methyl substituents following our iterative 1,4-addition procedure (Scheme 5).[38] The aliphatic tail was introduced by reduction of the thioester, transformation of the alcohol formed into a leaving group and substitution using the desired Grignard reagent. Subsequently, the silyl ether was removed and the resulting primary alcohol was oxidized to afford **3** in yields corresponding to those reported.

Towards the synthesis of benzylether-protected **4**, intermediate **19** was reduced with DIBALH and the aldehyde was submitted to a HWE olefination, affording oxo-ester **22** in 85% yield over two steps. Reduction of **22** with DIBALH furnished allylic alcohol **23**, which was, in turn, converted to allylic bromide **24**, using NBS/PPh_3 in 88% yield. We were pleased to see that the copper-catalyzed asymmetric allylic alkylation of **24** with (R,R_{Fe})-**L1** and MeMgBr afforded terminal olefin **25** in a remarkable yield of

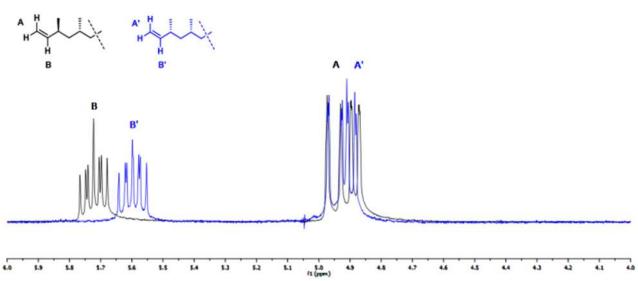
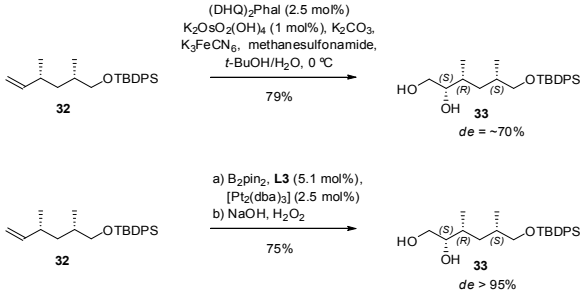


Figure 3 Highly diastereoselective allylic alkylation affording *syn* and *anti* products.

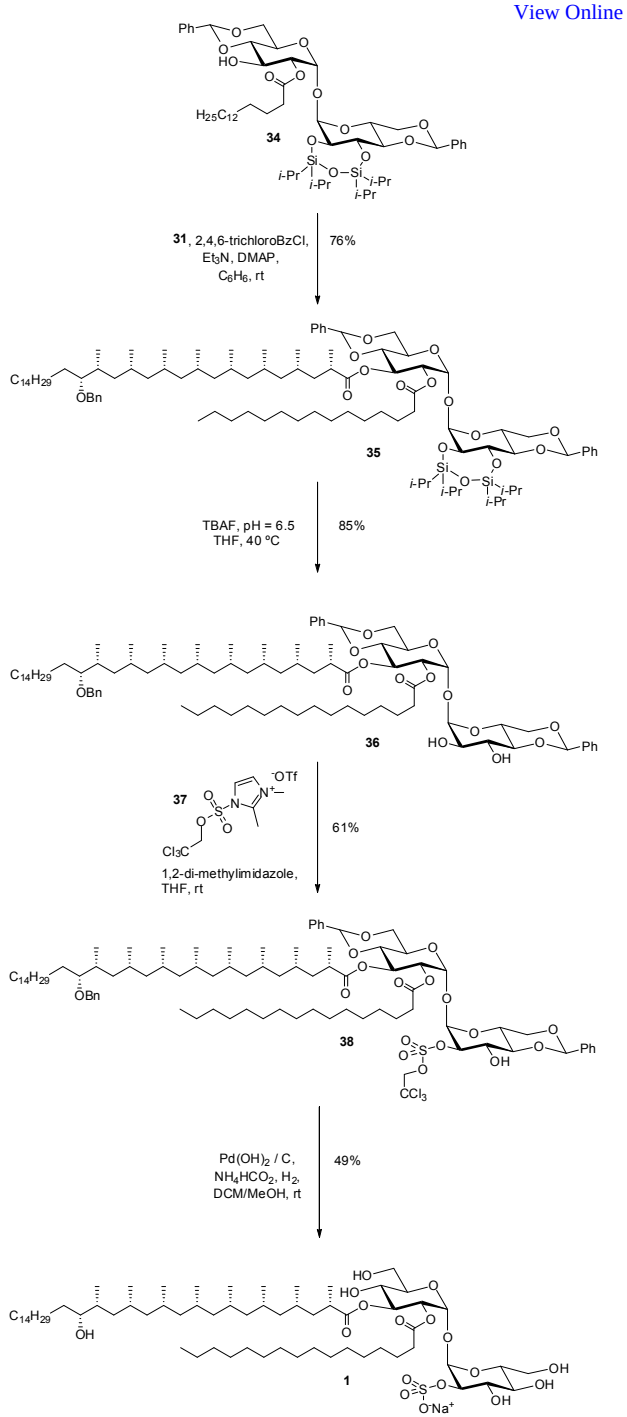
88%. Preparation of the corresponding *anti* product with the enantiomer of **L1** confirmed that both *syn* and *anti* products can be obtained in a *de* >95%. Figure 3 shows that the olefinic protons B and B' display a small but distinct difference in chemical shift in the ^{1}H-NMR spectrum. Comparison of both products with related compounds reported earlier, demonstrated the correct assignment of the relative and absolute configurations.[39,40]

With terminal olefin **25** in hand, the Sharpless dihydroxylation reaction was explored for the introduction of the diol moiety. Although the yield of the dihydroxylation was satisfactory, the 5:1 *syn*:*anti* ratio obtained on model substrate **32** having two methyl substituents was somewhat disappointing (Scheme 6).[41] In 2009, an intriguing enantioselective diboration reaction has been reported, employing bis(pinacolato)diboron (B_2pin_2) and a combination of [$Pt_2(dba)_3$] and phosphonite **L3** as the catalyst.[42] Oxidation of the diboronate with H_2O_2 afforded diols in high enantioselectivities. As the reaction was reported to be applicable to unfunctionalized aliphatic terminal olefins as well, this alternative approach was employed.



Scheme 6 Dihydroxylation of terminal olefins using procedures by Sharpless and Morken.

When we subjected model substrate **32** to the conditions described in the study of Morken's group [42] we observed the *syn* product with complete selectivity by ^{13}C-NMR spectroscopy (Figure 6SI). As the yield was comparable to that obtained in the Sharpless dihydroxylation reaction, we decided to use the Morken dihydroxylation procedure in our synthesis. However, when the same conditions were applied to substrate **25** with eight methyl groups, only 5–10% of the desired product was obtained. We discovered that with a twofold increase of the catalyst concentration and three equivalents of B_2pin_2, diol **26** was obtained in a gratifying 98% yield and >95% *de*. This example represents the first application of this methodology to the synthesis of a natural product. Epoxidation of **26** under phase-transfer conditions afforded **27** in 88% yield, which was opened



Scheme 7 Functionalization towards Ac_2SGL (**1**)

with tetradecylmagnesium bromide, employing copper catalysis. Analysis of alcohol **28** by ^{13}C-NMR spectroscopy provided strong evidence for the formation of the *syn* alcohol (Figure 5SI). Its chemical shifts match those of *syn*-**18**. Whereas we were unable to protect alcohol **28** under basic conditions, Lewis acidic conditions, in particular TMSOTf with benzyl 2,2,2-trichloroacetimidate, afforded benzyl ether **29** in 76% yield. The silyl ether of **29** was cleaved with TBAF to afford primary alcohol **30** in high yield. Finally, oxidation with TEMPO, NaOCl and $NaClO_2$ gave **31** in an excellent 90% yield.[43] Overall **31** was obtained in 3.0% yield over 32 steps.

Synthesis of diacylsulfoglycolipid: combining hydroxyphthioceranic acid and the trehalose core

To attach **31** regioselectively to the trehalose moiety, **34** was prepared according to a literature procedure (Scheme 7).[25] Several acylation strategies were investigated, but the procedure developed by Yamaguchi proved to be most effective in our system, affording **35** in 76% yield. Using a buffered TBAF solution, the bis(diisopropylsilyl) ether was removed to provide **36** in 85% yield.

For the introduction of the sulfate group in a protected form, we relied on a procedure reported recently by Taylor et al., employing sulfuryl imidazolium salts to regioselectively introduce protected sulfates.[44,45] The 2,2,2-trichloroethyl (TCE) group was considered to be a suitable protecting group as deprotection can be achieved under hydrogenolysis conditions, identical to those planned for the removal of the benzylidene acetals. It was shown that the TCE sulfate moiety could be introduced using the readily prepared imidazolium salt **37** with a remarkable regioselectivity for 4,6-*O*-benzylidene acetals of α- and β-glucosides, affording the 2-OH and 3-OH substituted products, respectively. By taking advantage of this observation, we were pleased to find mainly the desired 2-sulfated product. Although traces of 2,3-disulfated product were observed as well, **38** was obtained in 61% yield.

Given that the 4,6-O-benzylidene acetals, the benzyl ether, and the TCE group all are labile under hydrogenolysis conditions, we envisioned complete deprotection in one step, which would avoid the purification of very polar intermediates. Using ammonium formate and $Pd(OH)_2/C$ under a hydrogen atmosphere, complete deprotection was indeed achieved. Importantly, **1** was obtained as its sodium salt in 49% yield as confirmed by a comparison of the ^{1}H-NMR spectra of natural and synthetic **1**. Even though the natural product has been isolated as a mixture of homologues, the chemical shifts and multiplicities of the distinctive CH protons on the carbohydrate core are unambiguous.[22]

Biological activity

The synthetic sulfoglycolipid **1** was compared with natural **1** for its ability to activate the Ac_2SGL-specific T cells as reported.[19] Human dendritic cells expressing CD1b protein were incubated with varying amounts of natural or synthetic antigen, before addition of specific T cells. T-cell response was quantified by measuring the amounts of GM-CSF and IFN-γ cytokines released by activated T cells in an antigen dose-dependent manner (Figure 4). In these very sensitive assays, the activity of sulfoglycolipid **1** was remarkably comparable to that of purified natural Ac_2SGL.

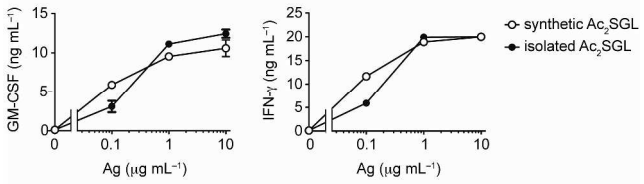


Figure 4 Activity profiles comparing the cytokine release of synthetic and natural Ac_2SGL.

Modeling studies

To investigate how the natural product **1** might be bound by CD1b, the CD1b–diacylsulfoglycolipid co-crystal structure (PDB code: 3T8X)[46] was used as a template to model the natural product into the deep binding pocket. **1** was manually docked into the binding groove, and the energy of the system minimized using the MAB force field as implemented in the computer program MOLOC,[47] while keeping the protein coordinates fixed. Given that the chain at C3 of **1** is considerably longer than the corresponding substituent of the diacylsulfoglycolipid found in the co-crystal structure with CD1b, we decided not to include the model of the endogenous spacer during the energy minimization.[46]

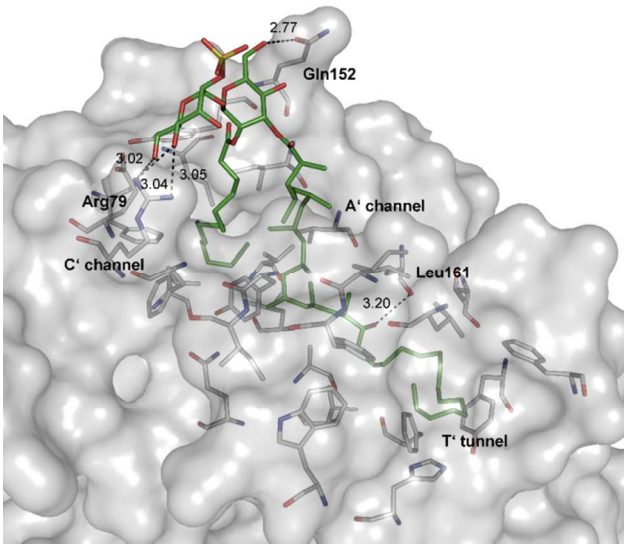


Figure 5 MOLOC-generated molecular model of **1** in the hydrophobic binding pocket of human CD1b (PDB code: 3T8X).[45] Color code: protein skeleton: C: gray; inhibitor skeleton: C: green; O: red; S: yellow. Hydrogen bonds are represented as dashed lines. Distances between heavy atoms are given in Å. Image generated with Pymol.[49]

Analysis of the computed binding mode of **1** to CD1b revealed no repulsive interactions, numerous van-der-Waals interactions and four hydrogen bonds between the terminal trehalose moiety and the side chains of Asp79 and Gln152. In addition, a weak hydrogen bond between the C17 hydroxyl group and the backbone carbonyl group of Leu161 was observed inside the hydrophobic binding pocket. According to the modeled binding pose, this hydroxyl group – that can now be modeled with the correct stereochemistry – not only contributes to the binding energy but could also play an important role in positioning the chain in the tunnel.

In close analogy to the binding mode of diacylsulfoglycolipid, **1** was modeled to bind with the sugar moieties at the T-cell-receptor (TCR) recognition surface and the long, aliphatic chains extending into a deep cleft, consisting of a series of three hydrophobic channels and a tunnel at the core of the $\alpha_1\alpha_2$ domain, referred to as A', C', and F' channels and T' tunnel, respectively.[48] The chain at C2 occupies the C' channel nearly fully and extends into the lateral opening. The chain at C3 is accommodated in the A' and T' channels, only partially filling the latter. The F' channel remains unoccupied (Figure 5 and

Figure 7SI). The proposed binding mode shows that the first and third methyl group are surface-exposed, enabling them to be involved in recognition of the TCR, whereas the remaining methyl groups are buried in the A' channel. The additional methyl branches of **1** could enhance the strength of the interaction with CD1b and also have an influence on the conformation of the whole molecule, in particular the surface-exposed methyl groups that might interact with the TCR. This is also supported by structure-functional studies performed using analogues of **1** with less methyl groups.[24]

Given the presumed degree of flexibility in the CD1b binding groove, the modeling was repeated while leaving the side chains lining the pocket flexible. Only a few amino-acid side chains moved to a small extent, illustrating how CD1b is capable of accommodating lipids with different numbers, patterns and types of substituents (amino-acid residues in question shown as sticks in Figure 5). This observation is in agreement with the results reported in the literature and suggests that T-cell activation by CD1b is controlled by its three-dimensional structure as well as the way in which the polar head group and some of the methyl groups of **1** are presented to the TCR.[50-52]

Conclusions

Hydroxyphthioceranic acid **4** has been prepared, for the first time, via two different routes. The most efficient route afforded the product in 3.0% yield over 32 steps and its stereochemistry was established by comparison with that of the natural product. The highest-yielding synthesis is based on a very efficient catalytic iterative protocol for the stereoselective introduction of methyl substituents, a highly stereoselective catalytic allylic substitution and a extremely stereoselective platinum-catalyzed diboration reaction. This result was used for the first synthesis of Ac_2SGL, a complex glycolipid isolated from *M. tuberculosis*. The synthesis is based on a regioselective functionalization of trehalose, and a carefully devised protecting-group strategy. Ac_2SGL was prepared in seven steps from trehalose with an overall yield of 4.1% based on **4**, and its structure was identical to that of the natural product. Biological evaluation revealed that the antigenic potency of synthetic **1** is identical to that of the natural product. Together with earlier studies on model compounds, this implies that the precise structure and stereochemistry of the hydroxyphthioceranic acid part in **1** are important for its antigenic activity. As Ac_2SGL (**1**) is an extremely potent antigen, studies on its application as a vaccine against tuberculosis are of utmost importance and can now be performed with the pure synthetic material available. Modeling studies of natural Ac_2SGL give an indication on the influence of altering the complex side chain of Ac_2SGL on binding. This approach could be valuable for the development of future analogues.

Acknowledgements

We thank Dr. Laura Kliman from the group of Prof. Morken for her advice on the asymmetric diboration/dihydroxylation. Financial support from The Netherlands Organization for Scientific Research (NWO-CW) is acknowledged.

Notes and references

[a]*Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, NL-9747 AG Groningen, The Netherlands. E-mail: a.j.minnaard@rug.nl*
[b]*Experimental Immunology, Department of Biomedicine, University Hospital Basel, Hebelstrasse 20, CH-4031, Basel, Switzerland*
[c]*CNRS, IPBS (Institut de Pharmacologie et de Biologie Structurale), 205 route de Narbonne BP 64182, F-31077 Toulouse, France*
[d]*Université de Toulouse, UPS, IPBS, F-31077 Toulouse, France*
[e]*Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Biopolis, Singapore.*

† Electronic Supplementary Information (ESI) available: HPLC traces, spectroscopic characterization, modeling studies and experimental details. See DOI: 10.1039/b000000x/

1 A. Koul, E. Arnoult, N. Lounis, J. Guillemont, K. Andries, *Nature*, 2011, **469**, 483.
2 www.who.int/topics/tuberculosis/en/
3 C.E. Barry, J.S. Blanchard, *Curr. Opin. Chem. Biol.*, 2010, **14**, 456.
4 M.J. Brennan, J. Thole, *Tuberculosis*, 2012, **92**, S6.
5 M. Daffé, J.-M. Reyrat, *The mycobacterial cell envelope*; ASM Press: Washington, DC, 2008.
6 D.E. Minnikin, L. Kremer, L.G. Dover, G.S. Besra, *Chem. Biol.*, 2002, **9**, 545.
7 M. Jackson, G. Stadthagen, B. Gicquel, *Tuberculosis*, 2007, **87**, 78.
8 M.B. Goren, *Biochim. Biophys. Acta*, 1970, **210**, 116.
9 M.B. Goren, *Biochim. Biophys. Acta*, 1970, **210**, 127.
10 The synthesis of a sulfolipid-I analogue was reported earlier: C.D. Leigh, C.R. Bertozzi, *J. Org. Chem.*, 2008, **73**, 1008.
11 J.P. Brozna, M. Horan, J.M. Rademacher, K.M. Pabst, M.J. Pabst, *Inf. Immun.*, 1991, **59**, 2542.
12 M.B. Goren, O. Brokl, W.B. Schaeffe, *Infect. Immun.*, 1974, **9**, 142.
13 Y. Okamoto, Y. Fujita, T. Naka, M. Hirai, I. Tomiyasu, I. Yano, *Microb. Pathog.*, 2006, **40**, 245.
14 L. Zhang, M.B. Goren, T.J. Holzer, B.R. Andersen, *Inf. Immun.*, 1988, **56**, 2876.
15 M. Kato, M.B. Goren, *Inf. Immun.*, 1974, **10**, 733.
16 O. Marjanovic, A.T. Iavarone, L.W. Riley, *J. Microbiol.*, 2011, **49**, 441.
17 S.E. Converse, J.D. Mougous, M.D. Leavell, J.A. Leary, C.R. Bertozzi, J.S. Cox, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 6121.
18 P. Domenech, M.B. Reed, C.S. Dowd, C. Manca, G. Kaplan, C.E. Barry, *J. Biol. Chem.*, 2004, **279**, 21257.
19 C. Rousseau, O.C. Turner, E. Rush, Y. Bordat, T.D. Sirakova, P.E. Kolattukudy, S. Ritter, I.M. Orme, B. Gicquel, M. Jackson, *Inf. Immun.*, 2003, **71**, 4684.
20 S.A. Gilmore, M.W. Schelle, C.M. Holsclaw, C.D. Leigh, M. Jain, S.J. Cox, J.A. Leary,C.R. Bertozzi, *ACS Chem. Biol.*, 2012, **7**, 863.
21 Compound **1** was isolated as a mixture of homologues with a chain length varying from 22 to 42 carbons and the number of 1,3-methyl groups varying from 3 to 12. The depicted structure of **1** corresponds to the most abundant SGL.
22 M. Gilleron, S. Stenger, Z. Mazorra, F. Wittke, S. Mariotti, G. Bohmer, J. Prandi, L. Mori, G. Puzo, G. De Libero, *J. Exp. Med.*, 2004, **199**, 649.
23 D.C. Barral, M.B. Brenner, *Nat. Rev. Immunol.*, 2007, **7**, 929.
24 J. Guiard, A. Collmann, L.F. Garcia-Alles, L. Mourey, T. Brando, L. Mori, M. Gilleron, J. Prandi, G. De Libero, G. Puzo, *J. Immunol.*, 2009, **182**, 7030.
25 The following report was used as the guideline for this part: J. Guiard, A. Collmann, M. Gilleron, L. Mori, G. De Libero, J. Prandi, G. Puzo, *Angew. Chem. Int. Ed.*, 2008, **47**, 9734.
26 R.P. van Summeren, D.B. Moody, B.L. Feringa, A.J. Minnaard, *J. Am. Chem. Soc.*, 2006, **128**, 4546.
27 R.D. Mazery, M. Pullez, F. Lopez, S.R. Harutyunyan, A.J. Minnaard, B.L. Feringa, *J. Am. Chem. Soc.*, 2005, **127**, 9966.
28 B. ter Horst, B.L. Feringa, A.J.Minnaard, *Chem. Commun.*, 2007, **5**, 489.
29 B. ter Horst, B.L. Feringa, A.J. Minnaard, *Org. Lett.*, 2007, **9**, 3013.

30 B. ter Horst, B.L. Feringa, A.J. Minnaard, *Chem. Commun.*, 2010, **46**, 2535.

31 K. Geurts, S.P. Fletcher, B.L. Feringa, *J. Am. Chem. Soc.*, 2006, **128**, 15572.

32 The synthesis of **18** via this approach and its comparison with natural **18** have been described in B. ter Horst, PhD thesis University of Groningen, 2010. ISBN 978-90-367-4085-2.

33 R.J. Anderson, C.A. Henrick, L.D. Rosenblum, *J. Am. Chem. Soc.*, 1974, **96**, 3654.

34 M.B. Goren, O. Brokl, B.C. Das, E. Lederer, *Biochemistry*, 1971, **10**, 72.

35 F. Lopez, A.W. van Zijl, A.J. Minnaard, B.L. Feringa, *Chem. Commun.*, 2006, **4**, 409.

36 A.B. Zaitsev, H. Adolfsson, *Synthesis*, 2006, **11**, 1725.

37 A. Francais, O. Bedel, Haudrechy, A. *Tetrahedron*, 2008, **64**, 2495.

38 A.V.R. Madduri, A.J. Minnaard, *Chem. Eur. J.*, 2010, **16**, 11726.

39 A.B. Smith, III, T. Bosanac, K. Basu, *J. Am. Chem. Soc.*, 2009, **131**, 2348.

40 H.M. Ko, C.W. Lee, H.K. Kwon, H.S. Chung, S.Y. Choi, Y.K. Chung, E. Lee, E. *Angew. Chem. Int. Ed.*, 2009, **48**, 2364.

41 The ^{13}C NMR spectrum of the diols obtained via both dihydroxylation procedures can be found in the Supporting Information.

42 L.T. Kliman, S.N. Mlynarski, J.P. Morken, *J. Am. Chem. Soc.*, 2009, **131**, 13210.

43 M.Z. Zhao, J. Li, E. Mano, Z.G. Song, D..M. Tschaen, E.J.J. Grabowski, P.J. Reider, *J. Org. Chem.*, 1999, **64**, 2564.

44 L.J. Ingram, A. Desoky, A.M. Ali, S.D. Taylor, *J. Org. Chem.*, 2009, **74**, 6479.

45 A.Y. Desoky, S.D. Taylor, *J. Org. Chem.*, 2009, **74**, 9406.

46 L.F. Garcia-Alles, A. Collmann, C. Versluis, B. Lindner, J. Guiard, L. Maveyraud, E. Huc, J.S. Im, S. Sansano, T. Brando, S. Julien, J. Prandi, M. Gilleron, S.A. Porcelli, H. de la Salle, A.J.R. Heck, L. Mori, G. Puzo, L. Mourey, G. De Libero, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 17755.

47 P.R. Gerber, K. Müller, *J. Comput.-Aided Mol. Des.*, 1995, **9**, 251.

48 T. Batuwangala, D. Shepherd, S.D. Gadola, K.J.C. Gibson, N.R. Zaccai, A.R. Fersht, G.S. Besra, V. Cerundolo, E.Y. Jones, *J. Immunol.*, 2004, **172**, 2382.

49 The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC.

50 D.C. Young,D.B. Moody, *Glycobiology*, 2006, **16**, 103R.

51 A.G. Kasmar, I. van Rhijn, T-Y. Cheng, M. Turner, C. Seshadri, A. Schiefner, R.C. Kaluthur, J.W. Annand, A. de Jong, J. Shires, L. Leon, M. Brenner, I.A. Wilson, J.D. Altman, D.B. Moody, *J. Exp. Med.*; 2011, **208**, 1741.

52 E.P. Grant, E.M. Beckman, S.M. Behar, M. Degano, D. Frederique, G.S. Besra, I.A. Wilson, S.A. Porcelli, S.T. Furlong, M.B. Brenner, *J. Immunol.*, 2002, **168**, 3933.