Chemical Science

RSC Publishing

EDGE ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Chem. Sci.*, 2013, **4**, 709

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,[be] Germain Puzo,[cd] Anna K. H. Hirsch,[a] Martine Gilleron,[cd] Gennaro de Libero[be] and Adriaan J. Minnaard*[a]

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 C17 hydroxyl 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.

Received 28th September 2012
Accepted 1st November 2012

DOI: 10.1039/c2sc21620e

www.rsc.org/chemicalscience

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**) (Fig. 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

[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/c2sc21620e

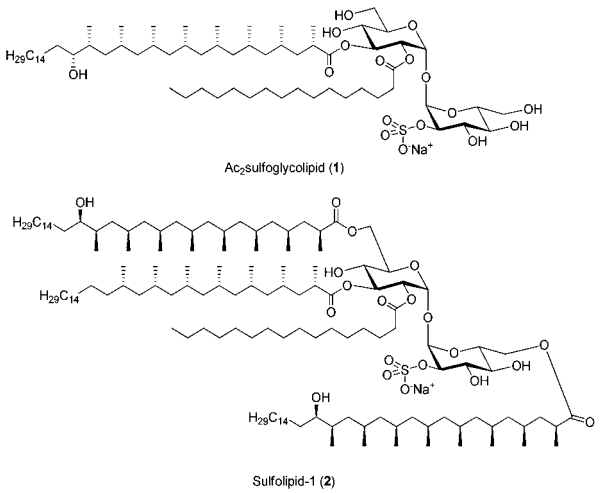


Fig. 1 Structures of sulfoglycolipids Ac_2SGL (**1**) and SL-1 (**2**) as found in the cell wall of *M. tuberculosis*.

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 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 centers, 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^{-1}) 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**) (Fig. 2) is unprecedented in synthesis and the presence of a hydroxyl 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.

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** (Fig. 2), 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 (4): 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 $CuBr \cdot SMe_2$ and **L1**, provided **6** in 76% yield and 98% *ee*. As the stereochemistry of 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,

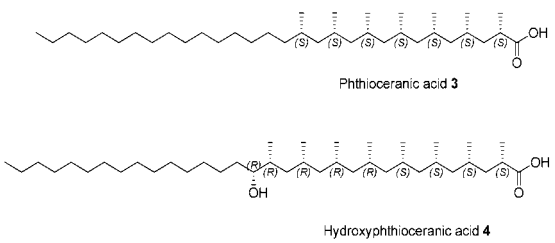
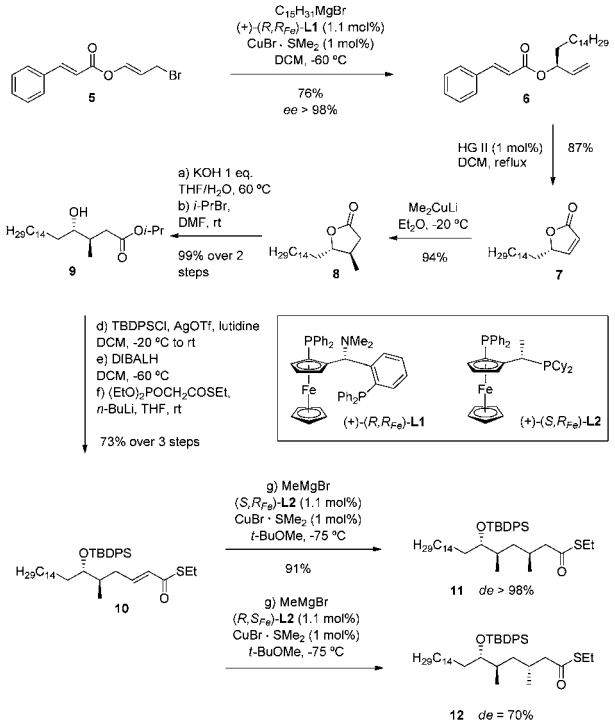


Fig. 2 Phthioceranic (**3**) and hydroxyphthioceranic (**4**) acid and the absolute configuration of their stereocenters.



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

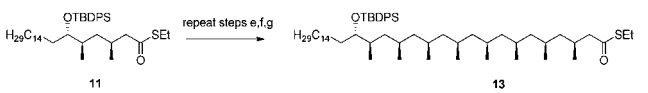
1,4-addition with the Gilman reagent Me_2CuLi gave *anti*-**8** as a single diastereomer in 94% yield.

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 isopropyl 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% (Fig. 1, ESI†).

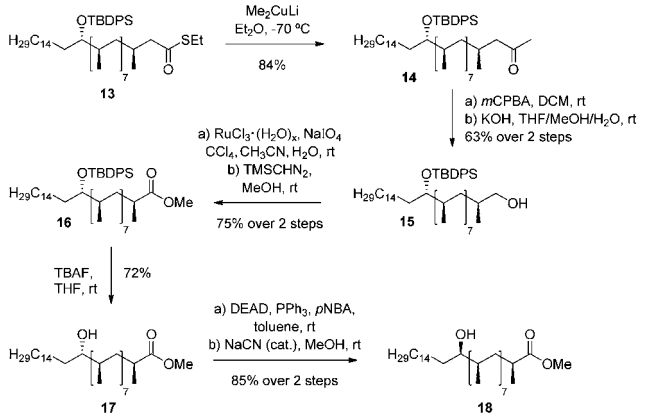
The preparation of **11** set the stage for the introduction of all subsequent methyl substituents applying an iterative protocol following the sequence of DIBALH reduction–HWE olefination–asymmetric conjugate addition.[28,29] Repetition of steps e, f and g (Schemes 1 and 2) led to thioester **13** with 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 ESI†).

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 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 ($[\alpha] = +16.0$ for **17** and $[\alpha] = +16.4$ for **18**, both in $CHCl_3$), and in turn close to that reported in the literature for a mixture of homologues ($[\alpha] = +23$).[34] An extract of cell-wall lipids containing SL-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 (Fig. 2, ESI†). The esterified natural sample was compared to synthetic **17** and **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 (Fig. 3, ESI†). Additional and conclusive evidence was



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



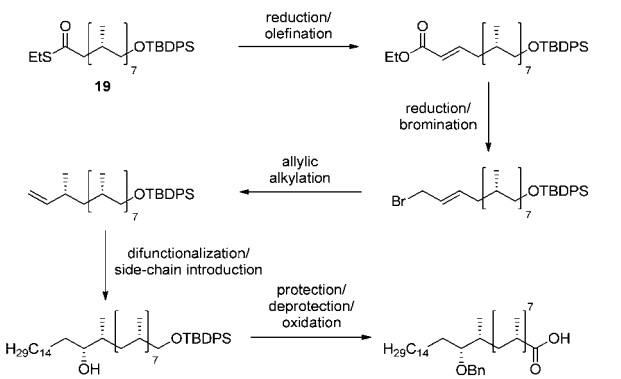
Scheme 3 Synthesis of C17 *syn*- and *anti*-hydroxyphthioceranic acid methyl esters.

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 (Fig. 4, ESI†). 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 SL-1 (Fig. 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



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

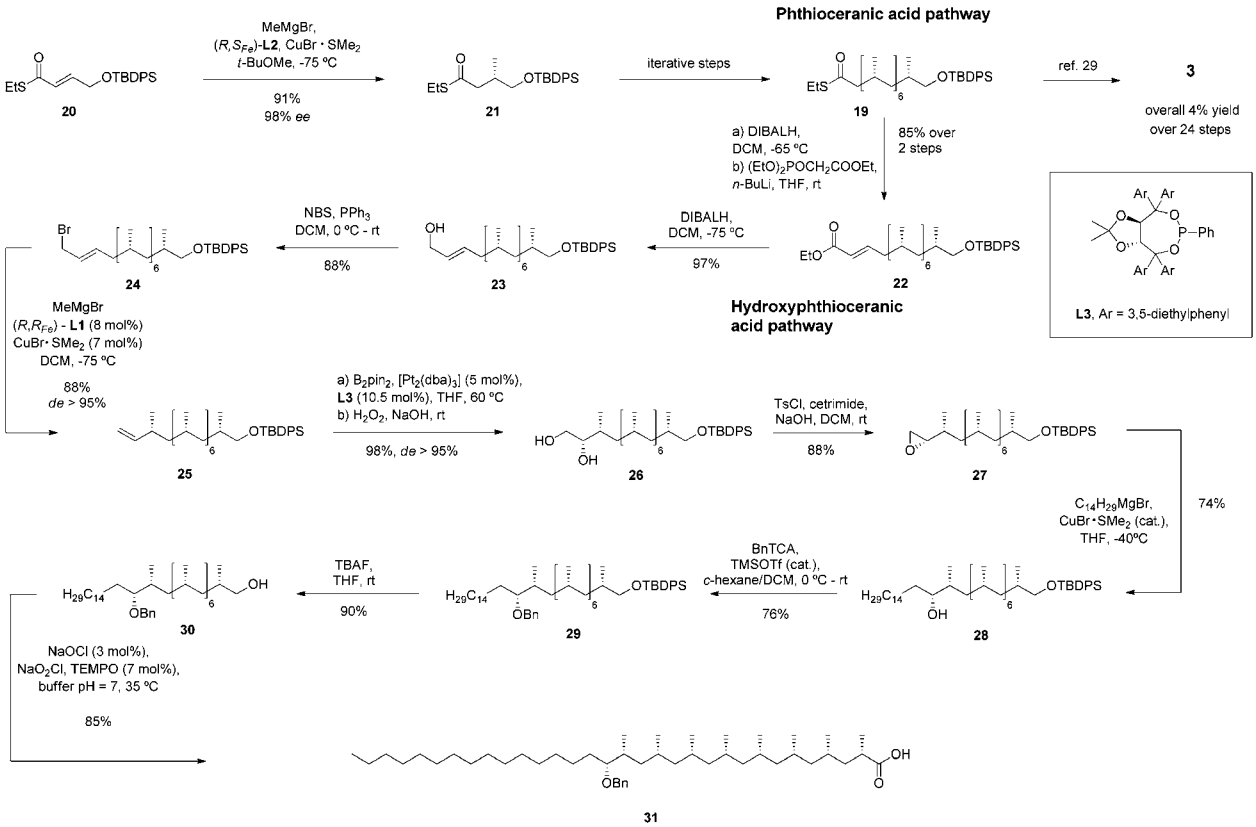
terminus. Even though the asymmetric allylic alkylation is a reasonably well explored reaction, we were not certain of its viability in the presence of such an extended array of chiral centers nearby. Moreover, enantio- or diastereoselective difunctionalization of monosubstituted aliphatic terminal olefins 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 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%. Fig. 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 was 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.

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 (Fig. 6, ESI†). 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**



Scheme 5 Synthesis of phthioceranic and hydroxyphthioceranic acid.

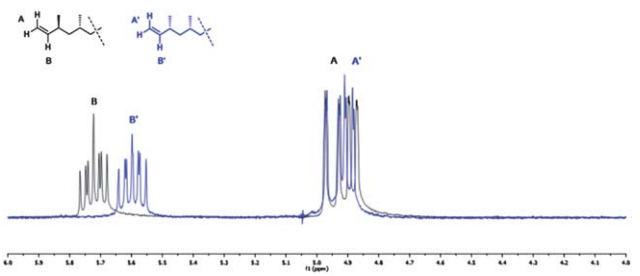
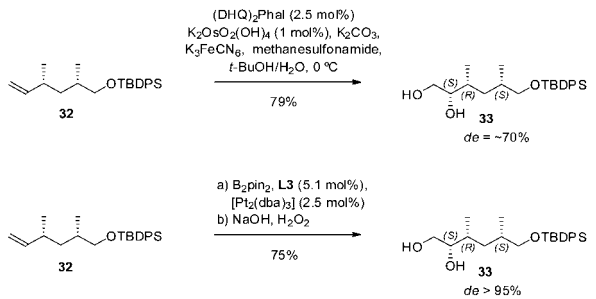


Fig. 3 Highly diastereoselective allylic alkylation affording *syn* and *anti* products.



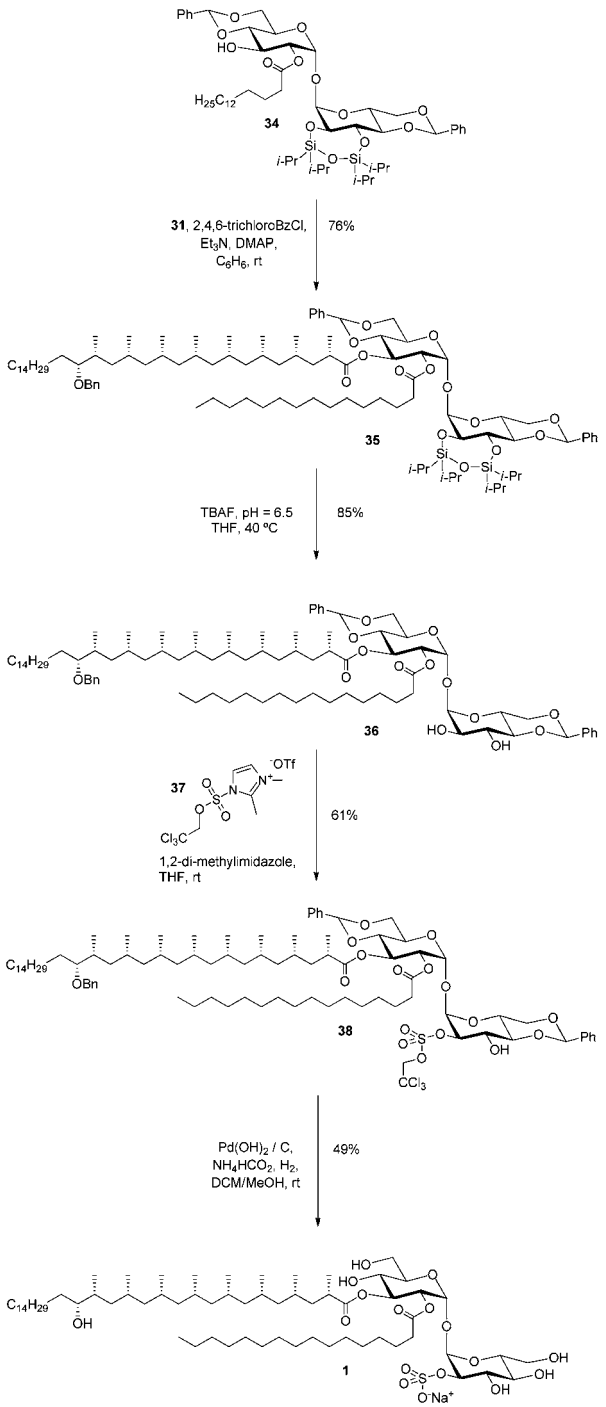
Scheme 6 Dihydroxylation of terminal olefins using procedures by Sharpless and Morken.

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 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 (Fig. 5, ESI†). 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 Ac_2SGL: combining 4 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



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

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 (Fig. 4). In these very sensitive assays, the activity of sulfoglycolipid **1** was remarkably comparable to that of purified 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]

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

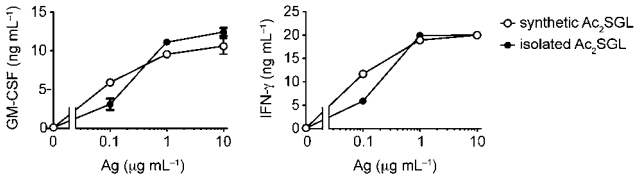


Fig. 4 Activity profiles comparing the cytokine release of synthetic and natural Ac_2SGL (**1**).

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 (Fig. 5 and Fig. 7, ESI†). 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 fewer 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 Fig. 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]

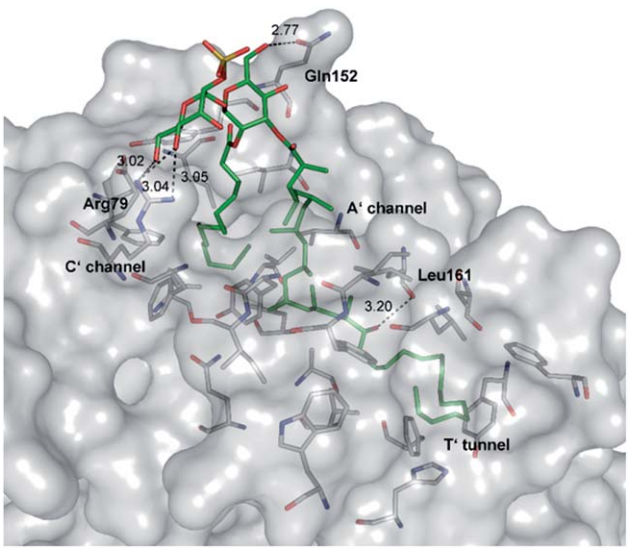


Fig. 5 MOLOC-generated molecular model of **1** in the hydrophobic binding pocket of human CD1b (PDB code: 3T8X).[45] Color code: protein skeleton: C: gray; inhibitor skeleton: C: green; O: red; S: yellow. Hydrogen bonds are represented as dashed lines. Distances between heavy atoms are given in Å. Image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

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 an 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 of 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

1 A. Koul, E. Arnoult, N. Lounis, J. Guillemont and K. Andries, *Nature*, 2011, **469**, 483.
2 www.who.int/topics/tuberculosis/en/.
3 C. E. Barry and J. S. Blanchard, *Curr. Opin. Chem. Biol.*, 2010, **14**, 456.
4 M. J. Brennan and J. Thole, *Tuberculosis*, 2012, **92**, S6.
5 M. Daffé and J.-M. Reyrat, *The Mycobacterial Cell Envelope*, ASM Press, Washington, DC, 2008.
6 D. E. Minnikin, L. Kremer, L. G. Dover and G. S. Besra, *Chem. Biol.*, 2002, **9**, 545.

7 M. Jackson, G. Stadthagen and B. Gicquel, *Tuberculosis*, 2007, **87**, 78.
8 M. B. Goren, *Biochim. Biophys. Acta, Lipids Lipid Metab.*, 1970, **210**, 116.
9 M. B. Goren, *Biochim. Biophys. Acta, Lipids Lipid Metab.*, 1970, **210**, 127.
10 The synthesis of a sulfolipid-I analogue was reported earlier: C. D. Leigh and C. R. Bertozzi, *J. Org. Chem.*, 2008, **73**, 1008.
11 J. P. Brozna, M. Horan, J. M. Rademacher, K. M. Pabst and M. J. Pabst, *Infect. Immun.*, 1991, **59**, 2542.
12 M. B. Goren, O. Brokl and W. B. Schaeffe, *Infect. Immun.*, 1974, **9**, 142.
13 Y. Okamoto, Y. Fujita, T. Naka, M. Hirai, I. Tomiyasu and I. Yano, *Microb. Pathog.*, 2006, **40**, 245.
14 L. Zhang, M. B. Goren, T. J. Holzer and B. R. Andersen, *Infect. Immun.*, 1988, **56**, 2876.
15 M. Kato and M. B. Goren, *Infect. Immun.*, 1974, **10**, 733.
16 O. Marjanovic, A. T. Iavarone and L. W. Riley, *J. Microbiol.*, 2011, **49**, 441.
17 S. E. Converse, J. D. Mougous, M. D. Leavell, J. A. Leary, C. R. Bertozzi and 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 and 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 and M. Jackson, *Infect. 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 and 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 and G. De Libero, *J. Exp. Med.*, 2004, **199**, 649.
23 D. C. Barral and 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 and 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 and G. Puzo, *Angew. Chem., Int. Ed.*, 2008, **47**, 9734.
26 R. P. van Summeren, D. B. Moody, B. L. Feringa and A. J. Minnaard, *J. Am. Chem. Soc.*, 2006, **128**, 4546.
27 R. D. Mazery, M. Pullez, F. Lopez, S. R. Harutyunyan, A. J. Minnaard and B. L. Feringa, *J. Am. Chem. Soc.*, 2005, **127**, 9966.
28 B. ter Horst, B. L. Feringa and A. J. Minnaard, *Chem. Commun.*, 2007, 489.
29 B. ter Horst, B. L. Feringa and A. J. Minnaard, *Org. Lett.*, 2007, **9**, 3013.
30 B. ter Horst, B. L. Feringa and A. J. Minnaard, *Chem. Commun.*, 2010, **46**, 2535.
31 K. Geurts, S. P. Fletcher and 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: B. ter Horst, PhD thesis, University of Groningen, 2010, ISBN 978-90-367-4085-2.
33 R. J. Anderson, C. A. Henrick and L. D. Rosenblum, *J. Am. Chem. Soc.*, 1974, **96**, 3654.
34 M. B. Goren, O. Brokl, B. C. Das and E. Lederer, *Biochemistry*, 1971, **10**, 72.
35 F. Lopez, A. W. van Zijl, A. J. Minnaard and B. L. Feringa, *Chem. Commun.*, 2006, 409.
36 A. B. Zaitsev and H. Adolfsson, *Synthesis*, 2006, **11**, 1725.
37 A. Francais, O. Bedel and A. Haudrechy, *Tetrahedron*, 2008, **64**, 2495.
38 A. V. R. Madduri and A. J. Minnaard, *Chem.–Eur. J.*, 2010, **16**, 11726.
39 A. B. Smith, III, T. Bosanac and 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 and E. Lee, *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 ESI.†
42 L. T. Kliman, S. N. Mlynarski and 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 and P. J. Reider, *J. Org. Chem.*, 1999, **64**, 2564.
44 L. J. Ingram, A. Desoky, A. M. Ali and S. D. Taylor, *J. Org. Chem.*, 2009, **74**, 6479.
45 A. Y. Desoky and 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 and G. De Libero, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 17755.
47 P. R. Gerber and 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 and E. Y. Jones, *J. Immunol.*, 2004, **172**, 2382.
49 *The PyMOL Molecular Graphics System, Version 1.3*, Schrödinger, LLC.
50 D. C. Young and 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 and 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 and M. B. Brenner, *J. Immunol.*, 2002, **168**, 3933.