

Total synthesis of sulfolipid-1†

Danny Geerdink‡§ and Adriaan J. Minnaard*§

Cite this: *Chem. Commun.*, 2014, 50, 2286Received 21st October 2013,
Accepted 4th January 2014

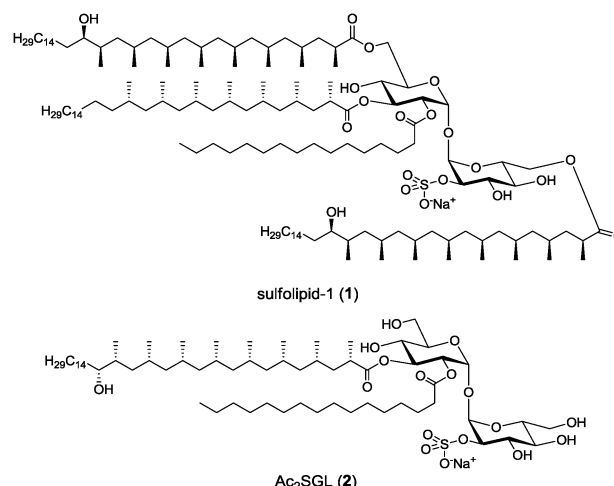
DOI: 10.1039/c3cc48087a

www.rsc.org/chemcomm

Sulfolipid-1, a tetra-acylated sulfotrehalose from *Mycobacterium tuberculosis*, was isolated over 40 years ago. Being a main component of the mycomembrane of *M. tuberculosis*, its biosynthesis and function have been studied in depth, but the chemical synthesis of sulfolipid-1 has not been reported. The synthesis presented here is based on iterative catalytic asymmetric conjugate additions of methylmagnesium bromide for the preparation of the phthioceranic and hydroxyphthioceranic acid side chains, a double regioselective reductive ring-opening and a five-fold deprotection in the final step.

Mycobacterium tuberculosis (*M. tuberculosis*), isolated by Koch 130 years ago, is at present still the cause of the most widespread bacterial infectious disease.¹ Much of the molecular research on *M. tuberculosis*, considerably stimulated by the sequencing of the bacterial genome, has focused on the composition and biosynthesis of its unusual waxy cell envelope.² The outer membrane of the cell envelope, the mycomembrane, is composed of long-tailed (glyco) lipids and, as a consequence, this hydrophobic layer functions as a very efficient barrier for antibiotics.³

A number of these mycomembrane glycolipids are known to modulate the immune response of the host, and several specific glycolipids act as ligands for the CD1 immune system.⁴ One of the most prominent and most complex glycolipids present is sulfolipid-1 (**1**), a 2,3,6,6'-tetraacyl- α,α -trehalose 2'-sulfate, decorated with phthioceranic, hydroxyphthioceranic, and palmitic acid residues (Fig. 1). Remarkably, **1** is only present in pathogenic mycobacteria but its function remains largely unknown. Sulfolipid-1 was isolated, and its structure elucidated using scrupulous degradation studies, by Goren more than 40 years ago.⁵ Recently, Gilleron *et al.* showed, using mass

Fig. 1 SL-1 and Ac₂SGL.

spectrometry and 2D-NMR spectroscopy, that the composition, abundance, and position of the fatty acids on the trehalose core of **1** varies per strain.⁶ The role of **1** in the pathogenicity of *M. tuberculosis* has not been demonstrated and in addition could be species specific, a phenomenon not uncommon in mycobacterial infections.⁷ Studies with knockout mutants, unable to produce the negatively charged **1**, indicate that **1** has a role in host-pathogen interactions by mediating between a cationic human antimicrobial peptide and the bacterium.⁸

In a series of studies, the group of Bertozzi has unraveled the biosynthesis of **1**, that starts with the sulfation of trehalose.⁹ In addition, its membrane transport is a topic of interest.⁹ A comprehensive view on the biosynthesis and role of trehalose-containing lipids in mycobacteria is expected to originate from a bioorthogonal chemistry approach.¹⁰ As far as synthetic precursors and model compounds have been involved in these studies, the multimethyl-branched phthioceranic acid residues were replaced by mono- or disubstituted analogues for synthetic reasons.¹¹

Also the hydroxy group in hydroxyphthioceranic acid was omitted; in addition its stereochemistry was unknown.

Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, NL-9747 AG Groningen, The Netherlands. E-mail: a.j.minnaard@rug.nl

† Electronic supplementary information (ESI) available: Experimental procedures, spectral data, and a HRMS-spectrum of synthetic **1**. See DOI: 10.1039/c3cc48087a

‡ Current address: Department of Organic Chemistry, University of Vienna, Währinger Straße 38, Vienna, Austria.

§ Author contributions: The manuscript was written through contributions of both authors. Both authors have given approval to the final version of the manuscript.

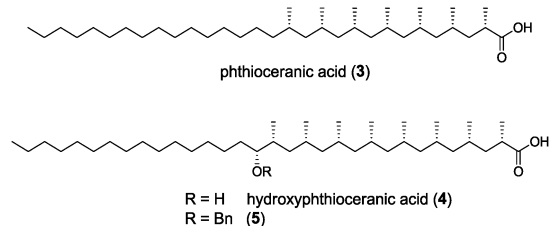
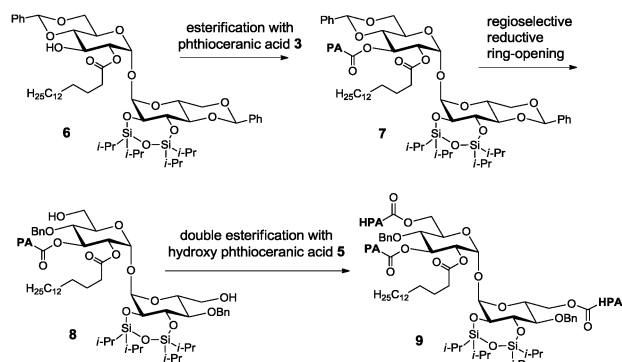


Fig. 2 Phthioceranic and hydroxyphthioceranic acid.

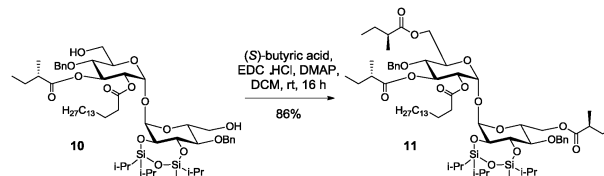
Recently, we reported the first total synthesis of Ac₂SulfoGlycoLipid (Ac₂SGL, **2**), a biosynthetic precursor of **1**.^{12,13} Ac₂SGL is a potent antigen in *M. tuberculosis*-infected humans, specifically recognized by antigen presenting cells expressing CD1b. This points to the potential use of (analogues of) Ac₂SGL in a TB vaccine.¹⁴ In these studies the stereochemistry of the hydroxy group in hydroxyphthioceranic acid was also established. Although still challenging, with the experience gained in the synthesis of Ac₂SGL, a total synthesis of **1** has come within reach. This would potentially assist in the research on *M. tuberculosis* and in addition serve as an illustration of the power of asymmetric catalysis in the synthesis of complex deoxypropionates.¹⁵

Key to the successful synthesis of **1** is the regioselective esterification and sulfation of trehalose, with minimal loss of the precious phthioceranic acids **3** and **4** (Fig. 2). Commencing with intermediate **6**,¹⁶ prepared from trehalose in three steps, esterification with **3** (Scheme 1), followed by regioselective reductive ring-opening of the benzylidene acetals, should produce **8** with the 6- and 6'-OH liberated. Subsequent esterification of both 6-positions with **5** should provide **9**, which upon desilylation, regioselective sulfation and overall deprotection would afford **1**.

In the synthesis of Ac₂SGL, the preparation of **3** and **5** had already been combined, thereby minimizing the total number of reactions and purification steps.¹² To study the reductive ring-opening of the benzylidene acetals in **7**, a less precious substrate carrying (*S*)-methylbutyric acid was prepared.¹⁷ Whereas a combination of borane and CoCl₂, contrary to literature reports, gave no conversion,¹⁸ its combination with Cu(OTf)₂¹⁹ and TMSOTf²⁰ afforded **10**, albeit in low yield. The main side product in these reactions was the fully deprotected 4,6-4',6'-tetraol.²¹ Increasing the amount of BH₃·THF to 12 eq. in combination with TMSOTf gave the product in a satisfactory 59% yield. Also the regioselectivity affording the free 6- and 6'-OH was excellent using this approach. A successive double



Scheme 1 Regioselective esterification towards SL-1.

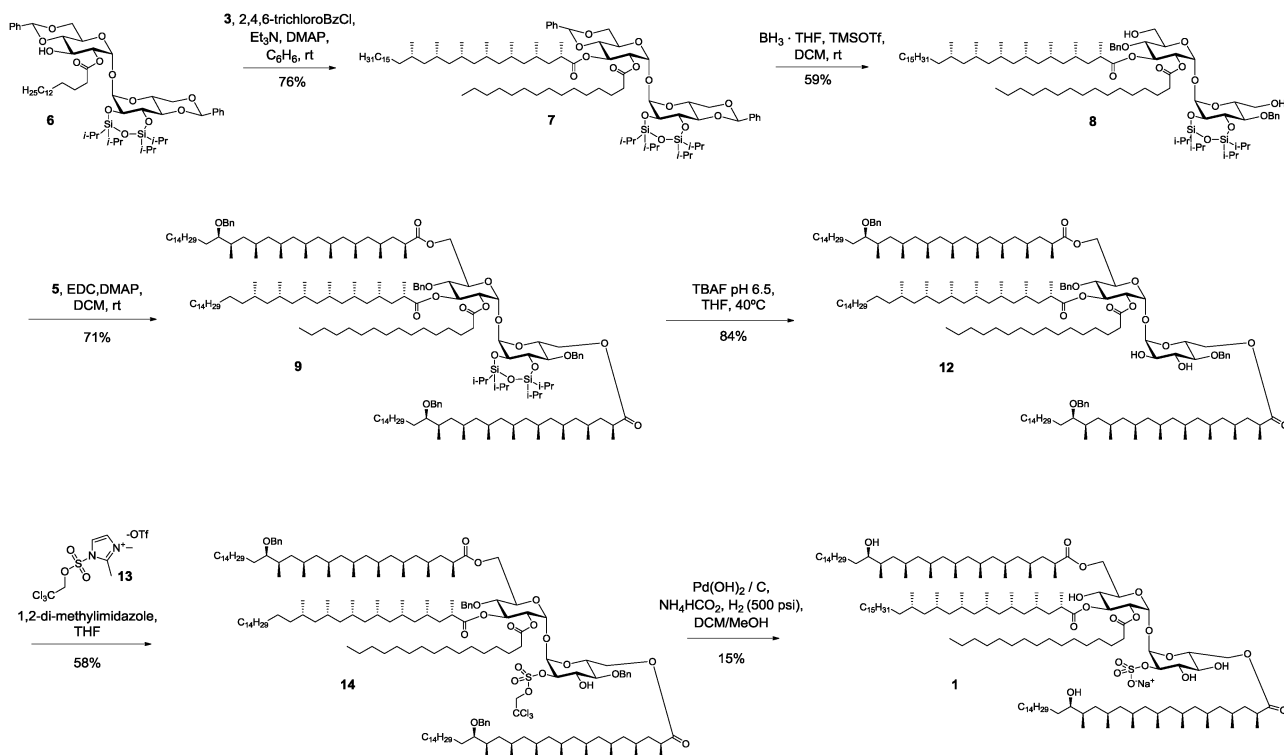


Scheme 2 Double EDC-promoted esterification.

esterification of **10** with (*S*)-methylbutyric acid under Yamaguchi conditions, however, gave mainly a disappointing mixture of 6- and 6'-monoacylated products. The desired diacylated **11** could, on the other hand, be obtained in very good yield using an EDC-promoted esterification (Scheme 2). Straightforward removal of the bis(diisopropylsilyl) ether with buffered TBAF, followed by regioselective introduction of a 2,2,2-trichloroethyl-protected sulfate at the 2-OH,²² and removal of all protecting groups under hydrogenolysis conditions, led to the corresponding analogue of **1** (see ESI,[†] compounds **15–17**).

In order to synthesize **1**, **6** was efficiently acylated with **3** at the 3-position, by Yamaguchi esterification, to afford **7** (Scheme 3). Applying the optimized conditions for regioselective reductive ring-opening with TMSOTf and BH₃·THF, **8** was obtained in 59% yield, similar to the model substrate. EDC-promoted double esterification of **8** using the previously prepared benzyl ether-protected **5** initially proved rather sluggish. Addition of an excess of EDC (8 eq.) and DMAP (8 eq.) was required to obtain complete conversion of the starting material. This diminished reactivity of long-chain fatty acids has been reported before.²³ Despite the formation of 10% of the tri-acyl product, tetra-acyl **9** was obtained in 71% yield. Deprotection of the bis(diisopropylsilyl) ether under buffered conditions proved to be facile affording the 2',3'-diol **12** in 84% yield. Subsequent introduction of the 2,2,2-trichloroethyl protected sulfate at the 2'-OH using **13** turned out to be not completely regioselective.²² In the synthesis of Ac₂SGL, only introduction of the TCE-sulfate at the 2'-OH and minor amounts of difunctionalization was observed. In the current case, also trace amounts of, assumed, 3'-OH sulfated products were obtained. This can be subscribed to the diminished rigidity of the substrate as a consequence of the previously effected benzylidene ring-opening. Nevertheless, pure **14** was isolated in 58% yield. To circumvent the purification of highly polar and charged intermediates, we envisioned, as our final step, the complete removal of all protecting groups using hydrogenolysis. Previously, the fivefold deprotection of our model system had shown to be straightforward. With a combination of ammonium formate, Pd(OH)₂/C and hydrogen at atmospheric pressure, the benzyl groups on both the 4- and 4'-OH, the benzyl group in the side chain and the TCE group were removed.¹² In **14**, however, the benzyl groups on the 4- and 4'-OH were not removed under these conditions. While 15 bar of hydrogen pressure did not lead to full deprotection, prolonged hydrogenolysis at 30 bar hydrogen finally led to **1** in a low, but delivering 15% yield. The low yield is a result of the multiple attempts to remove all protecting groups causing desulfation of the variety of intermediates and the product. Indeed, also a small amount of the desulfated product was isolated.¹⁶ An explanation given earlier,^{23b} to account for the reluctant hydrogenolysis, is most likely the steric hindrance caused by the long-tailed lipids that prevents the





Scheme 3 End game in the synthesis of sulfolipid-1.

approach of the heterogeneous Pd-catalyst. Although natural **1** is isolated as a mixture of homologues, the ^1H -NMR spectrum of synthetic **1** is similar to the one reported for natural **1** (Fig. S1, ESI †). Sulfolipid-1 turned out unstable in CDCl_3 solution (most probably desulfation takes place), 5a accounting for the minor differences. High resolution mass analysis (ESI) confirmed the formation of the desired product.

In summary, forty years after its first isolation as a main component of the mycomembrane of *Mycobacterium tuberculosis*, sulfolipid-1 has been synthesized and its structure confirmed. The availability of synthetic **1** and its precursors will accommodate research on its biosynthesis and function. Key steps in the synthesis are the preparation of hydroxyphthioceranic acid, the regioselective reductive ring-opening of the benzylidene acetals and a final fivefold deprotection.

We thank Dr M. Gilleron for kindly providing a ^1H -NMR spectrum of natural, purified SL-1. Financial support from the Netherlands Organization for Scientific Research (NWO-CW) is gratefully acknowledged.

References

- (a) http://www.nobelprize.org/nobel_prizes/medicine/laureates/1905/koch-bio.html; (b) A. Zumla, M. Ravighione, R. Hafner and C. F. von Reyn, *N. Engl. J. Med.*, 2013, **368**, 745–755.
- S. T. Cole, *et al.*, *Nature*, 1998, **393**, 537.
- M. Daffé and J.-M. Reyat, *The mycobacterial cell envelope*, ASM Press, Washington, DC, 2008, p. 395.
- G. Bricard and S. A. Porcelli, *Cell. Mol. Life Sci.*, 2007, **64**, 1824–1840.
- (a) M. B. Goren, *Biochim. Biophys. Acta*, 1970, **210**, 116; (b) M. B. Goren, *Biochim. Biophys. Acta*, 1970, **210**, 127; (c) M. B. Goren, O. Brokl, B. C. Das and E. Lederer, *Biochemistry*, 1971, **10**, 72.
- E. Layre, D. Cala-De Paepe, G. Larrouy-Maumus, J. Vaubourgeix, S. Mundayoor, B. Lindner, G. Puzo and M. Gilleron, *J. Lipid Res.*, 2011, **52**, 1098–1110.
- A. Apt and I. Kramnik, *Tuberculosis*, 2009, **89**, 195–198.
- S. A. Gilmore, M. W. Schelle, C. M. Holsclaw, C. D. Leigh, M. Jain, J. S. Cox, J. A. Leary and C. R. Bertozzi, *ACS Chem. Biol.*, 2012, **7**, 863–870.
- J. C. Seeliger, C. M. Holsclaw, M. W. Schelle, Z. Botyanszki, S. A. Gilmore, S. E. Tully, M. Niederweis, B. F. Cravatt, J. A. Leary and C. R. Bertozzi, *J. Biol. Chem.*, 2012, **287**, 7990–8000.
- B. M. Swarts, C. M. Holsclaw, J. C. Jewett, M. Alber, D. M. Fox, M. S. Siegrist, J. A. Leary, R. Kalscheuer and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2012, **134**, 16123–16126.
- (a) C. D. Leigh and C. R. Bertozzi, *J. Org. Chem.*, 2008, **73**, 1008–1017; (b) A. Lemétais, Y. Bourdreux, P. Lesot, J. Farjon and J.-M. Beau, *J. Org. Chem.*, 2013, **78**, 7648–7657.
- D. Geerdink, B. ter Horst, M. Lepore, L. Mori, G. Puzo, A. K. H. Hirsch, M. Gilleron, G. de Libero and A. J. Minnaard, *Chem. Sci.*, 2013, 709–716.
- The depicted structure of **1** corresponds to the most abundant homologue.
- 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**, 649–659.
- (a) B. ter Horst, B. L. Feringa and A. J. Minnaard, *Org. Lett.*, 2007, **9**, 3013–3015; (b) M. C. Pischl, C. F. Weise, M. A. Mueller, A. Pfaltz and C. Schneider, *Angew. Chem., Int. Ed.*, 2013, **52**, 8968–8972.
- J. Prandi, J. Guiard, A. Collmann, M. Gilleron, L. Mori, G. De Libero and G. Puzo, *Angew. Chem., Int. Ed.*, 2008, **47**, 9734–9738.
- See the ESI † for further details.
- S. Tani, S. Sawadi, M. Kojima, S. Akai and K.-i. Sato, *Tetrahedron Lett.*, 2007, **48**, 3103–3104.
- C. R. Shie, Z. H. Tzeng, S. S. Kulkarni, B. J. Uang, C. Y. Hsu and S. C. Hung, *Angew. Chem., Int. Ed.*, 2005, **44**, 1665–1668.
- K. Daragics and P. Fuegedi, *Tetrahedron Lett.*, 2009, **50**, 2914–2916.
- We suspect that with these small scale reactions, minimal amounts of water have a strong influence on the reaction outcome.
- (a) A. Y. Desoky and S. D. Taylor, *J. Org. Chem.*, 2009, **74**, 9406–9412; (b) L. J. Ingram, A. Desoky, A. M. Ali and S. D. Taylor, *J. Org. Chem.*, 2009, **74**, 6479–6485.
- (a) A. A. Khan, S. H. Chee, R. J. McLaughlin, J. L. Harper, F. Kamena, M. S. M. Timmer and B. L. Stocker, *ChemBioChem*, 2011, **12**, 2572–2576; (b) C. D. Leigh, *PhD thesis*, University of California, Berkeley, Progress Toward the Total Synthesis of *Mycobacterium tuberculosis* Sulfolipid-I, 2006.

