Organic LETTERS

Letter

pubs.acs.org/OrgLett

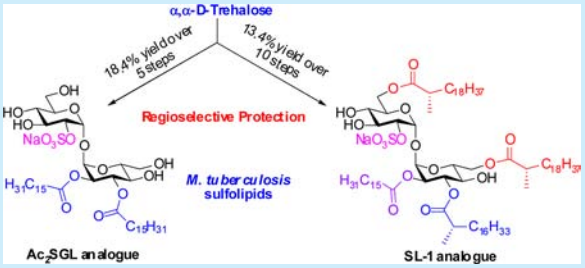
Expeditious Synthesis of *Mycobacterium tuberculosis* Sulfolipids SL-1 and Ac_2SGL Analogues

Vikram A. Sarpe and Suvarn S. Kulkarni*

Department of Chemistry, Indian Institute of Technology Bombay, Powai, Mumbai 400076, India

Supporting Information

ABSTRACT: *M. tuberculosis* sulfoglycolipids SL-1 and Ac_2SGL are highly immunogenic and potential vaccine candidates. A short and efficient methodology is reported for the synthesis of SL-1 and Ac_2SGL analogues via regioselective functionalization of α,α-D-trehalose employing a highly regioselective late stage sulfation, as a key step. The SL-1 analogues **3a** and **4** were obtained in 10 and 9 steps in 13.4% and 23.9% overall yields, respectively. The Ac_2SGL analogue **5** was synthesized in 5 steps in 18.4% yield.



Mycobacterium tuberculosis, the causative agent of tuberculosis, is one of the deadliest pathogens of global importance.[1] Despite decades of intensive research in antibiotics and vaccine development, we are still far from a reliable treatment for tuberculosis. Mycobacterial infections are progressively being recognized as a major public health risk due to coinfection with HIV and the emergence of multidrug resistant strains. Today, approximately 2 billion people, about one-third of the world's population, are estimated to be infected with *M. tuberculosis*. In 2012 alone, nearly 8.6 million people contracted tuberculosis and 1.3 million died from complications of the disease.[2] Thus, novel antibiotics and vaccines are urgently required to control the spread of this lethal disease.

A distinguishing feature of mycobacteria is that it displays a vast array of complex lipids, oligosaccharides, and unique glycolipids on its outer envelope.[3] Particularly, many virulent strains of *M. tuberculosis* contain large quantities of certain glycolipids.[4] Many of these glycolipids are involved in host immunomodulation. Goren, in 1972, elucidated the structures of some of the lipids and established that they possess a common α,α-D-trehalose core.[5] The most remarkable lipid of these is a sulfated glycolipid, termed sulfolipid-1 (SL-1) **1** (Figure 1), which is thought to mediate specific host−pathogen interactions during infection and is a potential virulence factor. Its abundant and preferential expression in virulent strains of *M. tuberculosis* and its location on the outer envelope are suggestive of its putative vital role in the virulence mechanism. Furthermore, it has been shown to be immunogenic in human patients and has utility as a serodiagnostic marker.[6]

Structurally, SL-1 (**1**) consists of a trehalose-2′-*O*-sulfate disaccharide equipped with four fatty acid groups esterified at various positions: two hydroxyphthioceranoyl substituents at the O6 and O6′ positions, a palmitoyl or stearoyl group at the O2 position, and a phthioceranoyl group at O3. In 2004, Gilleron et al. characterized a structurally related diacyl trehalose sulfolipid called Ac_2SGL **2**, specifically found in *M. tuberculosis*, and identified it as a potential vaccine candidate for tuberculosis.[7]

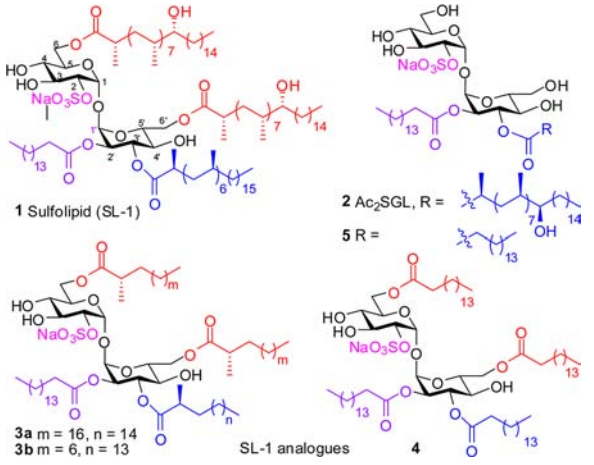


Figure 1. Mycobacterial sulfoglycolipids and their analogues.

Ac_2SGL **2** is also comprised of a trehalose 2′-*O*-sulfate core, but is esterified with either a palmitic or stearic acid at the 2-position and a hydroxyphthioceranic acid at the 3-position. Due to their immunological potential, both SL-1 and Ac_2SGL have received immense attention from synthetic chemists over the past few years.[8] In 2008, Bertozzi's group reported the first synthesis of a model compound mimicking SL-1 **3a**[9] from D-glucose employing an intramolecular aglycon delivery (IAD) reaction as a key step to form the dissymmetrically substituted trehalose core. Recently, Beau et al. reported a synthesis of SL-1 analogue **3b** as well as its simplified analogue **4** starting from α,α-D-trehalose via a tandem regioselective protection approach.[10] Minnaard et al.[11] accomplished the first total synthesis of SL-1 in its native form. Toward the synthesis of Ac_2SGL, in 2008, Prandi et al.[12] reported a regioselective strategy for the synthesis of various lipid

Received: September 22, 2014
Published: October 16, 2014

dx.doi.org/10.1021/ol5027987 | *Org. Lett.* 2014, 16, 5732−5735

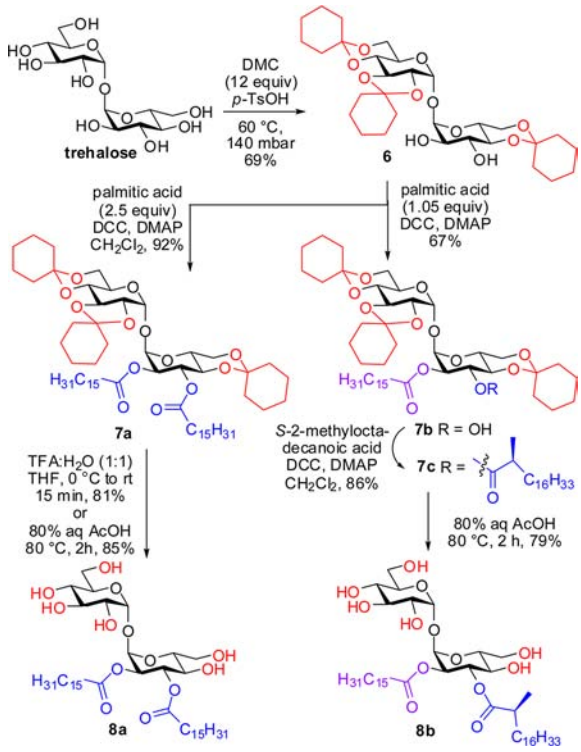
chain analogs of Ac_2SGL including analogue **5**. Along similar lines, total synthesis of Ac_2SGL **2** was reported by Minnaard et al. in 2013.[13] Very recently, the Beau and Prandi groups synthesized various analogues differing in the lipid chains at O3, employing the strategy originally established for SL-1 analogues.[14] In continuation of our studies directed toward the synthesis of trehalose glycoconjugates,[15] herein, we report expeditious syntheses of SL-1 and Ac_2SGL analogues **3a**, **4**, and **5** via regioselective functionalization of α,α-D-trehalose, employing a late stage regioselective 2′-*O*-sulfation as a key step.

In order to obtain the target sulfoglycolipids in good amount, it is necessary to develop efficient strategies for the regioselective protection of trehalose, using minimal protecting groups which can be also removed swiftly in a single deprotection step without affecting the acyl chains and the sulfate group. With this in mind, we decided to explore the tricyclohexylidene acetal derivative of trehalose which offers the opportunity for a quick desymmetrization of trehalose, and which provides access to a regioselectively differentiated 2,3-diol in a single step. Wallace and Minnikin in 1993 reported the preparation of 2,3;4,6;4′,6′-tricyclohexylidene-α,α-D-trehalose **6** from trehalose,[16a] which was further used for the synthesis of 2,3-diacyltrehaloses via sequential acylations with two different acids and subsequent removal of the cyclohexylidene acetal.[16] This short strategy gives ready access to 2,3-diacyltrehaloses, with the only caveat being the moderate yields of the tricyclohexylidene protection (40−45%) and deprotection steps (30−45%).

We envisioned that such an appropriately functionalized 2,3-diacyltrehalose could be further acylated at the 6,6′-positions by using the unique reactivity of TMS-protecting groups,[15a,17] to obtain the desired 2,3,6,6′-tetra-*O*-ester, which in turn could be expected to undergo regioselective *O*-sulfation at the 2′-*O*-position, owing to the greater acidity of the C2′-OH proton as compared to the C3′-OH, C4′-OH, and C4-OH. Such a route would allow a rapid access to SL-1 analogues and could also be adapted for the synthesis of Ac_2SGL analogues. Although the strategy looked promising, it would involve a highly challenging regioselective sulfation at a late stage.

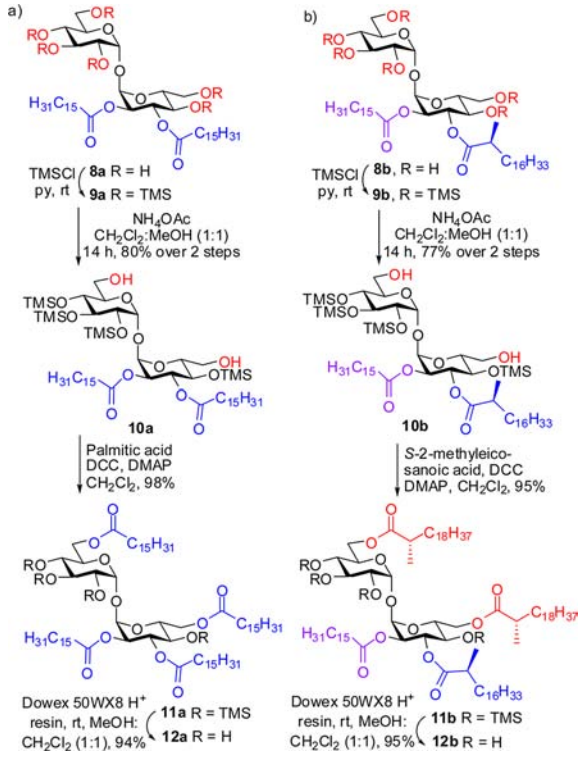
We began our synthesis with optimization of the tricyclohexylidene protection and deprotection reaction conditions (Scheme 1). The one-step preparation of **6** from α,α-D-trehalose was performed using an increased amount of 1,1-dimethoxycyclohexane (DMC, 12 equiv instead of 8 equiv) and by adding the reagent in two portions. The reaction was held at 60 °C and 140 mbar pressure on a rotary evaporator for 5 h, and the pH of the reaction was carefully maintained in the range of 1−2 by addition of *p*-TsOH with each portion of the reagent, to obtain better yields of **6** (69%). Diol **6** upon treatment with palmitic acid (2.5 equiv) and DCC furnished the di-*O*-palmitoyl derivative **7a** (92%), whereas a regioselective 2-*O*-palmitoylation (**7b**, 67%) followed by acylation at the 3-*O*-position with *S*-2-methyloctadecanoic acid[18] using DCC-mediated coupling reactions afforded **7c** (86%). Hydrolysis of the cyclohexylidene acetals in diacyltrehalose derivatives has been reported using either 10% aq HCl−THF (2:1) at 20 °C (31%)[16a] or 75% aq AcOH at 85 °C for 16 h (44%).[16b] Slight modifications to the conditions, i.e. heating **7a** or **7b** in 80% aq AcOH at 80 °C for 2 h, furnished **8a** (85%) and **8b** (79%), respectively, in much higher yields. Alternatively, addition of aq TFA to a solution of **7a** in THF at 0 °C and stirring at rt for 15 min followed by immediate evaporation of the solvents on a rotary evaporator (at 35−37 °C) furnished **8a** (81%).

Scheme 1. Synthesis of Diacyltrehaloses 8a and 8b



All attempts to directly acylate O6 and O6′ in hexaols **8a** and **8b** failed. So, the free hydroxyl groups in **8a** and **8b** were protected as TMS ethers using TMSCl and pyridine;[17] this afforded compounds **9a** and **9b**, respectively (Scheme 2). Regioselective deprotection of the primary TMS groups of the hexa-TMS derivatives **9a**/**9b** also turned out to be a difficult task.

Scheme 2. Synthesis of Tetra-*O*-esters 12a and 12b

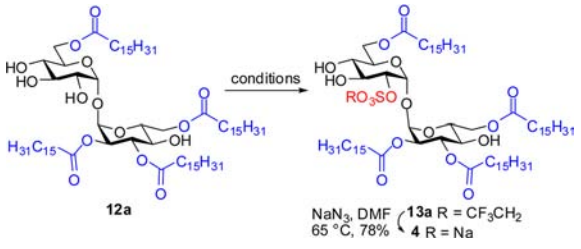


dx.doi.org/10.1021/ol5027987 | Org. Lett. 2014, 16, 5732−5735

The most commonly used method, K_2CO_3−MeOH,[15a,17] when applied to compound **9a**, offered a poor yield (20%) of the desired 6,6′-diol **10a**. Acidic conditions (AcOH−MeOH/Acetone)[19] also turned out to be nonselective. When compound **9a** was subjected to TMS deprotection using 2 equiv of NH_4OAc in MeOH/CH_2Cl_2 (1:1) at rt,[20] very little conversion was observed even after 12 h. The best results were obtained by using 10 equiv of NH_4OAc in MeOH/CH_2Cl_2 (1:1) for 14 h at rt to afford **10a** in 80% yields. These conditions worked equally well for **9b**, furnishing **10b** (77%). Diols **10a** and **10b** were then subjected to DCC-mediated 6,6′-diacylations with palmitic acid and the known *S*-2-methyl-eicosanoic acid[18] to obtain the tetra-*O*-ester derivatives **11a** (98%) and **11b** (95%). The remaining TMS groups were removed by acid promoted hydrolysis using Dowex 50WX8 in MeOH/CH_2Cl_2 to obtain **12a** (94%) and **12b** (95%).

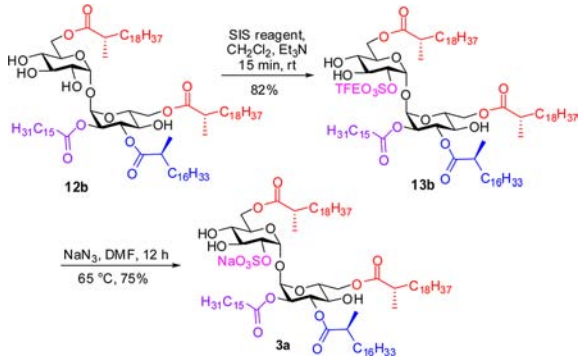
As anticipated, regioselective 2′-*O*-sulfation of the tetraol **12a** proved a challenging task. Initial attempts at 2′-*O*-sulfation with SO_3·Pyridine and SO_3·Et_3N (DMF/Pyridine)[12] met with little success (<10% conversion). Use of catalyst dimethyltin dichloride and SO_3·Me_3N for the transformation improved the yield and selectivity of the reaction to afford compound **4** (45%), mixed with a minor regioisomer which could not be separated from the product even after repeated column chromatographic purifications. Toward this end, we turned our attention to the protected sulfuryl imidazolium salts (SIS), which were used earlier as mild and regioselective sulfating agents for carbohydrate and noncarbohydrate substrates.[21] Trichloroethyloxysulfuryl imidazolium triflate salt[21a] has been used in the synthesis of trehalose based glycolipids SL-1 and Ac_2SGL.[11,13] Although this is a good method for regioselective sulfation, yields for removal of the trichloroethyl protecting group using catalytic hydrogenation conditions were not satisfactory in the case of trehalose glycolipids. In comparison, the other and less explored variant of the SIS salt, trifluoroethyloxysulfuryl imidazolium triflate[21b] was shown to be equally selective on 2,3-diols and the trifluoroethyl group (TFE) could be deprotected by heating with NaN_3. This reagent combination has not previously been explored on a trehalose scaffold. Owing to the mild conditions this requires for deprotection, we decided to try out TFE SIS as a sulfation agent for our studies. The results of the various reaction conditions, reagents, and yields are summarized in Table 1. As shown in entry 1, when *O*-sulfation was carried out using the TFE SIS reagent (4 equiv) and 1,2-dimethylimidazole as a base at 0 °C, with stirring at rt for 3 days, we obtained the 2′-*O*-sulfated product **13a** in a low yield of 33%, along with recovery of starting material **12a** (47%). Increasing the equivalents of the SIS reagent up to 8 equiv did not change the outcome of the reaction (entry 2). Use of DMAP in place of 1,2-DMI also proved futile, and **13a** was obtained in 36% yield (SM recovered, 40%, entry 3). Gratifyingly, the best yields for sulfation of **12a** were obtained when Et_3N was employed as a base (entry 4). When Et_3N was added into the solution of **12a** and the SIS reagent (4 equiv) in CH_2Cl_2 at rt, the reaction was complete in 20 min affording the 2′-*O*-sulfated product **13a** (77%) in a highly regioselective manner. The regioselectivity of sulfation was confirmed by observing the downfield movement of the C2′ proton in the ^1H NMR spectrum recorded in $CDCl_3$ (δ 4.46, dd, J = 9.5, 3.5 Hz) and by using COSY analysis (see Supporting Information (SI)). Deprotection of the TFE-protecting group proceeded smoothly with NaN_3 to furnish the 2′-*O*-sulfated tetra-*O*-palmitoyl analogue of sulfolipid **4** in 78% yield. The identity of the product was established with the help of 2D NMR spectroscopy and by comparing with literature data.[10] In a similar manner (Scheme 3), the sequence of *O*-sulfation and TFE group deprotection was carried out on **12b** with similar efficiency to obtain sulfolipid analogue **3a** (75%), via intermediate **13b** (82%). The NMR data of **3a** matched well with the data reported by Bertozzi et al.[9] The structure of **3a** was also independently confirmed by 2D NMR analysis (see SI).

Table 1. Regioselective Sulfation of 12a



entry	reagents	t (°C)	time	yield (%)
1	SIS (4 equiv), 1,2-DMI	0 to rt	3 d	33
2	SIS (8 equiv), 1,2-DMI	0 to rt	3 d	30
3	SIS (4 equiv), DMAP	0 to rt	3 d	36
4	SIS (4 equiv), Et_3N	rt	20 min	77

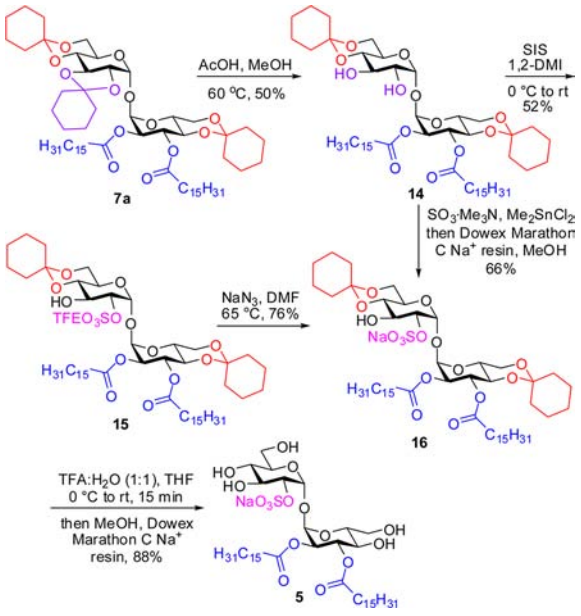
Scheme 3. Synthesis of SL-1 Analogue 3a



Ac_2SGL **2** is an intermediate metabolite in the synthesis of SL-1 in *M. tuberculosis*. We envisioned that it would be possible to synthesize the Ac_2SGL analogue **5**, if the five-membered 1,2-*trans* cyclohexylidene acetal at the 2,3-position in **7a** was hydrolyzed selectively (Scheme 4). Accordingly, we attempted selective hydrolysis of the 2,3-cyclohexylidene acetal in **7a** using pyridinium *p*-toluene sulfonate (PPTS) in methanol at rt. Under the conditions, we obtained the desired 2′,3′-diol **14** in a low yield of 35%. Alternatively, treatment of **7a** with AcOH (4 mL/g) in MeOH (25 mL/g) at 60 °C for 1 h afforded **14** in 50% isolated yield along with recovered starting material **7a** (18%). When sulfation of **14** was carried using the above optimized conditions, i.e. using the SIS reagent (4 equiv) and employing triethylamine as a base, we found that the reaction was very sluggish and offered poor yields (15%) and selectivity. When the same reaction was carried out under earlier reported conditions,[21b] viz. SIS reagent and employing 1,2-DMI as a base, we observed a dramatic change in the reaction output, obtaining the 2′-*O*-sulfated product **14** in 52% yield along with the minor 3′-*O*-sulfated product. The reasons behind the reversed efficiency of sulfation of tetraols **12a**/**12b** and diol **14** are not clear. Perhaps, this could be attributed to the difference in the amphiphilicity and conformational flexibility of the two

dx.doi.org/10.1021/ol5027987 | Org. Lett. 2014, 16, 5732−5735

Scheme 4. Synthesis of Ac_2SGL Analogue 5



substrates. The sulfate protecting TFE group was removed under NaN_3 heating conditions to furnish **16** (76%). Alternatively, sulfation of **14** with SO_3·Me_3N (1.5 equiv) using Me_2SnCl_2 (0.25 equiv) as a catalyst delivered **16** in a single step in 66% yield (**14** recovered, 15%). The cyclohexylidene acetals were removed by hydrolysis with aq TFA to afford the Ac_2SGL analogue **5** in good yield (88%). The NMR data of **5** corroborated well with the reported data[12] revealing its identity which was further independently confirmed by 2D NMR analysis (see SI).

In conclusion, we have established a short and efficient route for the synthesis of trehalose-based mycobacterial glycolipid SL-1 via tricyclohexylidene acetal protection, exploiting a unique reactivity difference of the TMS protecting groups at the primary and secondary positions and regioselective 2′-*O*-sulfation. We applied this strategy for the synthesis of two analogues of SL-1, compounds **3a** (10 steps, 13.4% overall) and **4** (9 steps, 23.9% overall). We also report the synthesis of Ac_2SGL analogue **5** in 5 steps with 18.4% overall yields. Glycolipids **3a**, **4**, and **5** are immunogenic compounds. These structurally well-defined and chemically homogeneous compounds, which are also free from biological contaminants, can now be potentially utilized as serodiagnostic markers and for vaccine development. This straightforward methodology can also be used for the rapid synthesis of various lipid chain analogues of sulfolipids and related metabolites of *M. tuberculosis*.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and copies of ^1H and ^{13}C NMR spectra for all compounds and ^1H–^1H COSY spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: suvarn@chem.iitb.ac.in.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Department of Science and Technology (Grant No. SR/S1/OC-40/2009) and Board of Research in Nuclear Sciences (Grant No. 2013/37C/51/BRNS) for financial support. V.A.S. thanks CSIR-New Delhi for a fellowship.

■ REFERENCES

(1) Koul, A.; Arnoult, E.; Lounis, N.; Guillemont, J.; Andries, K. *Nature* **2011**, *469*, 483–490.
(2) *WHO Fact Sheets: Tuberculosis*. http://www.who.int/mediacentre/factsheets/fs104/en/.
(3) Goren, M. B. *Bacteriol. Rev.* **1972**, *36*, 33–64.
(4) (a) Middlebrooke, G.; Coleman, C. M.; Schaefer, W. B. *Proc. Natl. Acad. Sci. U.S.A.* **1959**, *45*, 1801–1804. (b) Elbion, A. D.; Pan, Y. T.; Pastuszak, I.; Caroll, D. *Glycobiology* **2003**, *13*, 17R–27R.
(5) (a) Goren, M. B. *Biochim. Biophys. Acta* **1970**, *120*, 116–126. (b) Goren, M. B. *Biochim. Biophys. Acta* **1970**, *120*, 127–138.
(6) (a) Goren, M. B.; D'Arcy Hart, P.; Young, M. R.; Armstrong, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2510–2514. (b) Pabst, M. J.; Gross, J. M.; Brozna, J. P.; Goren, M. B. *J. Immunol.* **1988**, *140*, 634–640.
(7) Gilleron, M.; Stenger, S.; Mazorra, Z.; Wittke, F.; Mariotti, S.; Böhmer, G.; Prandi, J.; Mori, L.; Puzo, G.; De Libero, G. *J. Exp. Med.* **2004**, *199*, 649–659.
(8) (a) Khan, A. A.; Stocker, B. L.; Timmer, M. S. M. *Carbohydr. Res.* **2012**, *356*, 25–36. (b) Chaube, M. A.; Kulkarni, S. S. *Trends Carbohydr. Res.* **2012**, *4*, 1–19. (c) Sarpe, V. A.; Kulkarni, S. S. *Trends Carbohydr. Res.* **2013**, *5*, 8–33. (d) Wu, C.-H.; Wang, C.-C. *Org. Biomol. Chem.* **2014**, *12*, 5558–5562.
(9) Leigh, C. D.; Bertozzi, C. R. *J. Org. Chem.* **2008**, *73*, 1008–1017.
(10) Lemétais, A.; Bourdreux, Y.; Lesot, P.; Farjon, J.; Beau, J.-M. *J. Org. Chem.* **2013**, *78*, 7648–7657.
(11) Geerdink, D.; Minnaard, A. J. *Chem. Commun.* **2014**, *50*, 2286–2288.
(12) Guiard, J.; Collmann, A.; Gilleron, M.; Mori, L.; De Libero, G.; Prandi, J.; Puzo, G. *Angew. Chem., Int. Ed.* **2008**, *47*, 9734–9738.
(13) Geerdink, D.; ter Horst, B.; Lepore, M.; Mori, L.; Puzo, G.; Hirsch, A. K. H.; Gilleron, M.; de Libero, G.; Minnaard, A. J. *Chem. Sci.* **2013**, *4*, 709–716.
(14) Gau, B.; Lemétais, A.; Lepore, M.; Garcia-Alles, L. F.; Bourdreux, Y.; Mori, L.; Gilleron, M.; De Libero, G.; Puzo, G.; Beau, J.-M.; Prandi, J. *ChemBioChem* **2013**, *14*, 2413–2417.
(15) (a) Sarpe, V. A.; Kulkarni, S. S. *J. Org. Chem.* **2011**, *76*, 6866–6870. (b) Sarpe, V. A.; Kulkarni, S. S. *Org. Biomol. Chem.* **2013**, *11*, 6460–6465.
(16) (a) Wallace, P. A.; Minnikin, D. E. *J. Chem. Soc., Chem. Commun.* **1993**, 1292–1293. (b) Wallace, P. A.; Minnikin, D. E.; Ridell, M. *J. Chem. Soc., Chem. Commun.* **1994**, 329–330.
(17) Toubiana, R.; Das, B. C.; Defaye, J.; Mompon, B.; Toubiana, M. J. *Carbohydr. Res.* **1975**, *44*, 308–312.
(18) Besra, G. S.; Minnikin, D. E.; Wheeler, P. R.; Ratledge, C. *Chem. Phys. Lipids* **1993**, *66*, 23–34.
(19) Fernández, C.; Nieto, O.; Rivas, E.; Montenegro, G.; Fontenla, J. A.; Fernández-Mayoralas, A. *Carbohydr. Res.* **2000**, *327*, 353–365.
(20) Cui, Y.; Cheng, Z.; Mao, J.; Yu, Y. *Tetrahedron Lett.* **2013**, *54*, 3831–3833.
(21) (a) Desoky, A. Y.; Taylor, S. D. *J. Org. Chem.* **2009**, *74*, 9406–9412. (b) Desoky, A. Y.; Hendel, J.; Ingram, L. J.; Taylor, S. D. *Tetrahedron* **2011**, *67*, 1281–1287.