Total synthesis of enantiopure lipids
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Chapter 4

Total synthesis of sulfolipid-1

Sulfolipid-1 is a tetra-acylated sulfoglycolipid, discovered and characterized over 40 years ago. Although many reports about its biological function have appeared, no total synthesis of this elusive molecule has been reported. In this chapter, the molecules and knowledge from earlier research on the synthesis of Ac₂SGL are combined, and applied to the first synthesis of sulfolipid-1. Crucial to the completion of the synthesis are a regioselective reductive opening of a benzylidene acetal and a final fivefold deprotection.
Chapter 4

4.1 Introduction

Much of the research into *Mycobacterium tuberculosis* has focused on its unusual, waxy cell envelope, especially after its complete genome was decoded.\(^1\) The outer membrane of the cell envelope is decorated with long-tailed (glyco)lipids and, as a consequence, this very hydrophobic layer functions as an extremely efficient barrier against antibiotics, rendering TB treatment lengthy.\(^2\) Hence, to target the genes responsible for the biosynthesis of these lipids seems a logical approach.\(^3\) Over the past years we have also contributed to the study of the *M. tuberculosis* cell envelope. Our focus has been on the synthesis of a variety of these (glyco)lipids to confirm or elucidate their structure and function.\(^4\) To the host, many of these (glyco)lipids are thought to function as virulence factors. Most notably, we have recently reported the first total synthesis of ac\(_2\)sulfoglycolipid (Ac\(_2\)SGL, 3.1, Figure 4.1).\(^5\)\(^6\) 3.1 is able to function as a potent antigen in *M. tuberculosis*-infected humans by binding to CD1b restricted ab T cells, which points to a potential use of (analogues of) 3.1 in a TB vaccine.\(^7\) A closely related compound to 3.1, present as the most abundant sulfatide in the *M. tuberculosis* outer membrane is sulfolipid-1 (SL-1, 4.1). This 2,3,6,6'-tetraacyl-\(\alpha,\alpha\)-trehalose 2'-sulfate was already isolated, and its structure elucidated using scrupulous degradation studies, by Goren over 40 years.

![Ac\(_2\)SGL (3.1)](image1)

![sulfolipid-1 (4.1)](image2)

*Figure 4.1* Ac\(_2\)SGL (3.1) and sulfolipid-1 (4.1)
Total synthesis of sulfolipid-1

ago from the H37Rv strain. Recently, Gilleron has proven, using mass analysis and 2D-NMR techniques, that the composition, abundance and position of the fatty acids acylated to the trehalose core of 4.1 varies per strain. The function of 4.1, however, remains speculative and there has been reasonable doubt whether it influences *M. tuberculosis* pathogenesis *in vivo*. In recent work by Bertozzi *et al.*, the pathogenicity of 4.1 is claimed to be species-specific, a phenomenon not uncommon to mycobacterial infections. In addition, studies with knockout mutants, unable to produce negatively charged 4.1, indicate that it has a role in host-pathogen interactions by mediating between a cationic human antimicrobial peptide and *M. tuberculosis*. With the recent unraveling of the complete biosynthesis of 4.1 it is clear that, even after four decades, the exact function of this elusive molecule still remains to be discovered. A total synthesis of sulfolipid-1 and perhaps its desulfated analogue, making the pure compounds available for further studies, would therefore be of great interest.

### 4.2 Biosynthesis of sulfolipid-1

The identification of all enzymes responsible for the biosynthesis of sulfolipid-1 has been the subject of numerous studies as sulfolipid-1 has been positively correlated with the virulence of *M. tuberculosis*. To disrupt the biosynthesis of sulfolipid-1 by targeting one (or multiple) of the enzymes responsible for its biosynthesis was consequently thought to reduce *M. tuberculosis* virulence.

The biosynthesis of sulfolipid-1 is initiated with the sulfation of trehalose (4.2) to form trehalose 2'-sulfate (T2S)(Scheme 4.1). Regioselective acylation of the 2-position with a palmitoyl or stearoyl moiety on T2S is then accomplished using the acyltransferase polyketide synthase(PKS2)-associated protein PapA2. The resulting 2-palmitoyl-T2S is subsequently acylated at 3-OH catalyzed by PapA1, introducing the phthioceranic or hydroxyphthioceranic acid sidechain. It is believed that PapA1 catalyzes the transfer of the acyl chain from the acyl carrier protein (ACP) to the 2-palmitoyl-T2S, since PKS2 lacks a thioesterase. This mechanism has precedent in the biosynthesis of PDIM A, where PapA5 ensures the transfer of a mycocerosyl group. The sequential order of 2-OH/3-OHacylation was established by preparing the ΔpapA2 and ΔpapA1 mutant strains. Monitoring formation of sulfolipid-1 indicated that both enzymes are essential.

Whereas the ΔpapA2
mutant afforded no 2-palmitoyl-T2S, the ΔpapA1 mutant led to the formation of 2-palmitoyl-T2S, but lacked the formation of the 3-acyl intermediate. Importantly, it was noticed that both mutants do not affect *M. tuberculosis* virulence.[15]

Over the last few years, the final steps of sulfolipid-1 biosynthesis have been elucidated. Analysis of lipid biosynthesis in *M. tuberculosis* knockout strains revealed that Chp1,[13] a membrane-anchored acyltransferase, and MmpL8,[10b] a lipid transporter, are essential for the formation of sulfolipid-1. Sap, a transmembrane transport protein, was recognized to be required for the transport of sulfolipid-1 across the cell membrane.[13] In addition, also MmpL8 is crucial for the transport, indicating a double role for this enzyme. Sap and MmpL8 also jointly showed to transport Ac₂SGL to the cell surface in a ΔChp1 mutant. Given that in wild-type strains Ac₂SGL resides in the more internal layers, this observation was rather surprising and indicates some structural tolerance in the lipid transfer.

The approach Chp1 uses to acylate the 6-positions of 4.4 is rather unusual. The
enzyme catalyzes the transesterification of hydroxyphthioceranic acid from two donor
\( \text{Ac}_2\text{SGL} \) molecules to create a single sulfolipid-1 molecule. The acceptor molecule can
either be \( \text{Ac}_2\text{SGL} \) acylated with either a phthioceranic or hydroxyphthioceranic acid moiety,
affording two different sulfolipid-1 structures, something already noticed by Goren\[^{[17]}\] and
confirmed recently.\[^{[9]}\] Although sulfolipid-1 production is not crucial for \( \text{M. tuberculosis} \)
virulence, recent work by Bertozzi et al. has shown that, its formation can be inhibited by
blocking Chp1. Addition of THL, a lipase inhibitor, reveals a dose-dependent decrease of
sulfolipid-1. The formation of \( \text{Ac}_2\text{SGL} \) is not altered, leading to its accumulation.\[^{[13]}\]

Whether the unraveling of the enzymes responsible for the biosynthesis of sulfolipid-1
will lead to new insights in how to address the tuberculosis problem is still unclear.
However, these new insights do give an opening to new targets to disrupt the synthesis of
important constituents of the cell envelope of \( \text{M. tuberculosis} \).

4.3 Reported synthesis of sulfolipid-1

The only attempt to date to chemically synthesize 4.1 has also been made by the
Bertozzi group.\[^{[18]}\] After an extensive synthesis, employing a wide variety of orthogonal
protecting groups and a novel intramolecular aglycon delivery (IAD) to form the 1,2-\( \text{cis} \)-
glycoside, they were able to obtain an analogue of 4.1. Nevertheless, the 1,3-polymethyl
fatty acids phthioceranic (PA, 2.2) and hydroxyphthioceranic acid (HPA, 2.1), needed to

\[ \text{Scheme 4.2 Retrosynthesis towards a sulfolipid-1 analogue. DMB: 3,4-dimethoxybenzyl, PCB: para-} \\
\text{chlorobenzyl, PIB: para-iodobenzyl, PMB: para-methoxybenzyl} \]
access native sulfolipid-1, were never prepared by them. Instead, chiral α-methylated 20-carbon-long fatty acids were prepared to mimic the characteristics of phthioceranic and hydroxyphthioceranic acid.

Retrosynthetically, Bertozzi et al. chose to commence their synthesis with two glucose monomers bearing a total of five orthogonal protecting groups (Scheme 4.2). Coupling of these, using an IAD, would afford the desired α,α-linked trehalose. Regioselective deprotection and subsequent introduction of the acyl groups and the sulfate ester at the corresponding positions would lead to their target molecule.

Preparation of the monomers, installing a variety of protecting groups afforded 4.5 and 4.6 (Scheme 4.3). The 3,4-dimethoxybenzyl moiety on 4.5 was chosen based upon earlier results where the p-methoxybenzyl group gave incomplete oxidation with DDQ and resulted in lower yields. The IAD was initiated with the oxidation of the 3,4-dimethoxybenzyl on 4.5 and trapping of the quinoid with the disarmed glucose monomer, to form mixed acetal 4.7. Activation of the sulfide with MeOTf in the presence of 2,6-di-tert-butyl-4-methyl pyridine, followed by intramolecular attack, gave the desired α,α-dimerized trehalose 4.8 as the sole stereoisomer in 60% yield, along with a molecule of veratraldehyde.

A set of dimethoxybenzyl (DMB) ethers was introduced on both 6-positions by protecting the free 2-OH of 4.8, regioselective ring-opening of the benzylidene acetals

![Scheme 4.3 Intramolecular aglycon delivery](image)
Scheme 4.4 Functionalization towards a sulfolipid-1 analogue

towards the free 6-OH and 6'-OH using DIBALH\cite{19} and a successive etherification with DMB-Cl and NaH. Although DIBALH gave concomitant reduction of the aryl iodide, this deiodinated side product could be separated at a later stage. The formation of 4.9 was considered to be key to the success of the planned route. Amination of the \( p \)-iodobenzyl using Buchwald’s conditions followed by removal with ZnCl\(_2\) gave the free hydroxyl at the 2-position (Scheme 4.4).\cite{20} A second amination of the adjacent \( para \)-chlorobenzyl using a the same conditions but with a different ligand was followed by the introduction of the palmitoyl group at the free 2-OH. Deprotection of the amine, again with ZnCl\(_2\), afforded the free hydroxyl at the 3-position, allowing the introduction of the first chiral fatty acid with an EDC-promoted esterification. Selective cleavage of the DMB groups than gave 4.13. The last part of the synthesis was completed starting with a double EDC-promoted esterification.
using the chiral model lipids (Scheme 4.5). A remarkably high excess of EDC (14 eq.) and DMAP (8 eq.) was needed to achieve good conversion. Cleavage of the PMB group of 4.14 using DDQ afforded the free 3-OH, which was sulfated using pyridiniumSO$_3$. Finally, hydrogenolysis using Pd/C and 500 psi of hydrogen pressure was needed to cleave all benzyl ethers, affording sulfolipid-1 analogue 4.16.

Overall, Bertozzi et al. developed an elegant synthesis of a sulfolipid-1 analogue which, in combination with the synthesis of phthioceranic acid and hydroxyphthioceranic acid, could lead to the synthesis of native sulfolipid-1. In addition, this compound and intermediates have been used to study the biosynthesis of sulfolipid-1.$^{[11, 13, 15]}$

**4.4 Strategy**

In recent years, our group has reported the synthesis of phthioceranic acid using a highly efficient iterative catalytic asymmetric protocol.$^{[4c, 21]}$ Combining the route developed by Bertozzi et al.$^{[18]}$, and our synthesis of phthioceranic and hydroxyphthioceranic acid
Total synthesis of sulfolipid-1

Scheme 4.6 Proposed steps towards SL-1. PA: phthioceranic acid; HPA: hydroxyphthioceranic acid

(Chapter 2), initially seemed obvious to obtain native 4.1. However, we realized that together with the preparation of phthioceranic and hydroxyphthioceranic acid, this would represent a lengthy route with over 70 steps. To minimize the synthetic effort towards 4.1, we sought for a more efficient route, ideally using our experience from the synthesis of Ac$_2$SGL.$^5$ Next to being the biosynthetic precursor of 4.1,$^{13}$ we envisioned that our reported chemical synthesis of Ac$_2$SGL could also function as the guideline for the synthesis of sulfolipid-1. Thus, starting with a Yamaguchi esterification using known intermediate 3.4 (Scheme 4.6),$^{22}$ followed by a regioselective reductive ring-opening of the benzylidene acetals, should produce 4.18 with the free 6- and 6’-OH (Scheme 4.1). Subsequent esterification of both 6-positions gives then 4.19, an intermediate very similar to the one encountered in the synthesis of 3.1. Further functionalization towards 4.1 consequently seemed feasible.

4.5 Synthesis

To study our hypothesis we prepared the less precious substrate 4.20 via our previously developed route and tested a variety of reductive ring-opening reactions of benzylidene acetals as reported in literature (Table 4.1),$^{19b}$ Whereas a combination of
Table 4.1 Optimization of the regioselective reductive ring-opening

<table>
<thead>
<tr>
<th>entry</th>
<th>solvent</th>
<th>Lewis acid (eq)</th>
<th>eq. BH$_3$•THF</th>
<th>yield$^b$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>THF</td>
<td>CoCl$_2$ (5)</td>
<td>6</td>
<td>0$^c$</td>
</tr>
<tr>
<td>2</td>
<td>DCM</td>
<td>Cu(OTf)$_2$ (0.25)</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>DCM</td>
<td>TMSOTf (0.3)</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>DCM</td>
<td>TMSOTf (0.3)</td>
<td>12</td>
<td>59</td>
</tr>
</tbody>
</table>

$^a$ Reactions were performed at 0.018 mmol scale.  $^b$ Yield of the isolated product.  $^c$ No conversion

borane and CoCl$_2$ remarkably gave no conversion (entry 1),$^{[23]}$ Cu(OTf)$_2$$^{[24]}$ and TMSOTf$^{[25]}$ afforded 4.21, albeit in low yield (entry 2 and 3). The main isolated side product in these reactions was the fully deprotected 4,6-4′,6′-tetraol, probably caused by hydrolysis.$^{[26]}$ Increasing the amount of BH$_3$•THF to 12 equivalents in combination with TMSOTf gave the product in a more satisfactory 59% yield. Whether this higher yield is the result of beneficial kinetics for the desired reaction or the quenching of H$_2$O, is not clear. The regioselectivity towards the free 6- and 6′-OH is excellent using the BH$_3$•THF/TMSOTf combination. We never observed any significant side-product other than the fully deprotected tetraol.

A successive double esterification of 4.21 with (S)-butyric acid using Yamaguchi conditions disappointingly gave a mixture of mainly 6- and 6′-monoacylated products. The diacylated 4.22 could, on the other hand, be obtained in very good yield using an EDC-promoted esterification (Scheme 4.7). Following our previously developed strategy, i.e. removal of the bis(diisopropylsilyl) ether, regioselective introduction of a protected sulfate, and removal of all protecting group under hydrogenolysis conditions, we were pleased to obtain an analogue of native SL-1, pure and in yields similar to those described for the synthesis of Ac$_2$SGL.$^\dagger$

$^\dagger$ See the experimental procedures for detailed information
In order to synthesize native sulfolipid-1, we commenced our synthesis from 3.4, an intermediate we used in the synthesis towards Ac₂SGL (Scheme 4.8). By means of a Yamaguchi esterification, previously prepared phthioceranic acid (2.2, Chapter 2) was efficiently acylated at the 3-position of 3.4 affording 4.17. Using the optimized conditions for regioselective reductive ring-opening with TMSOTf and BH₃•THF, 4.18 was obtained in 56% yield, similar to that obtained for our model substrate. EDC-promoted double esterification of 4.18 using the previously prepared benzyl-ether-protected 2.18, proved somewhat sluggish initially. Addition of a fairly large amount of EDC (8 eq) and DMAP (6 eq) was needed to obtain complete conversion of the starting material. This diminished reactivity has been observed earlier in the aforementioned synthesis of Leigh and Bertozzi. [27] Despite of isolating 10% of monoacylated product, tetra-acylated 4.19 was obtained in 71% yield. Deprotection of the bis(diisopropysilyl) ether under buffered conditions proved to be facile affording the 2',3'-diol 4.23 in 84% yield. Regioselective introduction of the 2,2,2-trichloroethyl protected sulfate at the 2'-OH proved less discriminating than anticipated. Towards the synthesis of 3.1 we had only observed incorporation of the sulfate at the 2'-OH and minor amounts of difunctionalization. In this case, additional trace amounts of, what is assumed to be, selective 3'-OH functionalization were obtained, which could be due to the diminished rigidity of the molecule as a consequence of the previous benzylidene ring-opening. Nevertheless, pure 4.24 was isolated in 58% yield. To circumvent the purification of highly polar intermediates we envisioned as our final step, as in the synthesis of 3.1, the complete removal of all protecting groups using hydrogenolysis.
Scheme 4.8 Final step in the synthesis of sulfolipid-1
Although the fivefold deprotection of our model system proved straightforward using ammonium formate, Pd(OH)$_2$/C and atmospheric hydrogen pressure (see experimental procedures), the benzyl groups at both the 4- and 4'-OH of 4.24 could not be removed under these conditions. While even 250 psi of hydrogen pressure showed to be insufficient, an increase to 500 psi finally led to 4.1 in a low, but delivering 15% yield. A possible explanation mentioned before, accounting for the high hydrogen pressure needed, could be the difficulty of the heterogeneous Pd catalyst to reach the reaction center due to the nearby long-tailed lipids.\cite{27b} The low yield can be explained by our multiple attempts to remove all protecting groups causing degradation of the highly labile sulfate moiety. Indeed, mass analysis of the less polar side product (4.25) indicates the loss of the sulfate.

![Figure 4.2](image)

**Figure 4.2** $^1$H-NMR spectra of natural (up) and synthetic (bottom) 4.1

Although natural 4.1 is isolated as a mixture of homologues, comparison of the $^1$H-NMR spectra of natural and synthetic 4.1, shows a very strong similarity between both. Although minor differences can be observed, the instability of 4.1 in solution, is most probably the reason for this.

### 4.6 Conclusion

In summary, we have developed the first total synthesis of 4.1. The developed route is
much more efficient than a previously reported route for an analogue of 4.1. The synthesis was completed using the previously prepared 1,3-methyl-branched fatty acids PA and HPA, over a total of 46 steps. Key steps in this synthesis are the preparation of hydroxyphthioceranic acid (Chapter 2), the regioselective reductive ring-opening of the benzyldiene acetals and the final fivefold deprotection. In addition, an analogue of 4.1 has been prepared. For future preparations of 4.1, the reductive ring-opening using BH$_3$•THF and TMSOTf deserves additional investigation. In our hands, the yield of this reaction proved to be not very reproducible. Sulfolipid-1 proved to be unstable under a range of conditions and care has to be taken in the final steps of its preparation, its purification and storage. The easily cleaved sulfate moiety is considered to be responsible for this behavior.$^{[28]}$

To conclude, after 40 years, synthetic sulfolipid-1 is now available to study its precise role in $M$. tuberculosis infection. Moreover, the function of the sulfate moiety can be studied as desulfated 4.1 was isolated as a side-product.

### 4.7 Experimental section

For general experimental information: see Chapter 2.

(2$R$,4a$R$,6$R$,7$R$,8$S$,8a$R$)-8-(((S)-2-methylbutanoyl)oxy)-2-phenyl-6-(((5a$R$,6$R$,7a$R$,10$R$,11a$R$,11b$S$)-2,2,4,4-tetraisopropyl-10-phenylhexahydro-[1,3]dioxino[4',5':5,6]pyrano[3,4-f][1,3,5,2,4]trioxadisilepin-6-yl)oxy)hexahydropyrano[3,2-d][1,3]dioxin-7-yl palmitate (4.20): To (S)-butyric acid (1.2 eq, 24 mg, 0.24 mmol) in benzene (3 mL) was added Et$_3$N (2.2 eq, 44 mg, 0.44 mmol) and 2,4,6-trichlorobenzoyl chloride (1.2 eq, 58 mg, 0.24 mmol). The mixture was stirred for 1 h and subsequently 3.4 (1 eq, dissolved in 1 mL benzene) and DMAP (1.3 eq, 32 mg, 0.26 mmol) were added, upon which the mixture turned into a white suspension. After 48 h the suspension was quenched with a sat. aq. solution of NaHCO$_3$ (4 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried, filtered and all volatiles were evaporated. Purification using column chromatography (pentane/EtOAc, 20:1) afforded 4.20 as a colorless oil with traces of an inseparable impurity (161 mg, 74%).
**Total synthesis of sulfolipid-1**

**1H NMR** (400 MHz, CDCl$_3$) $\delta$ 7.53 – 7.39 (m, 4H), 7.39 – 7.30 (m, 6H), 5.69 (t, $J$ = 9.8, 1H), 5.54 (s, 1H), 5.50 (s, 1H), 5.40 (d, $J$ = 3.8, 1H), 5.15 (d, $J$ = 4.1, 1H), 5.04 (dd, $J$ = 9.9, 3.9, 1H), 4.34 (td, $J$ = 10.0, 4.9, 1H), 4.27 – 4.19 (m, 2H), 4.15 (dd, $J$ = 10.1, 4.7, 1H), 3.93 (dd, $J$ = 8.4, 4.2, 1H), 3.85 (td, $J$ = 10.0, 4.7, 1H), 3.78 – 3.66 (m, 3H), 3.53 (t, $J$ = 9.3, 1H), 2.41 – 2.25 (m, 3H), 1.72 – 1.53 (m, 3H), 1.47 – 1.38 (m, 1H), 1.34 – 1.00 (m, 55H), 0.90 (t, $J$ = 6.8, 3H), 0.86 (t, $J$ = 7.4, 3H); **HRMS**-(ESI+) calculated for C$_{59}$H$_{95}$O$_{14}$Si$_{2}$ [M + H]$^+$ 1083.6260 Da, found 1083.6251 Da.

(2R,3R,4S,5R,6R)-5-(benzylxoy)-2-(((5aR,6R,8R,9aS)-9-(benzylxoy)-8-(hydroxymethyl)-2,2,4,4-tetraisopropyltetrahydro-5aH-pyrano[3,4-f][1,3,5,2,4]trioxadisilepin-6-yl)xox)-6-(hydroxymethyl)-4-((S)-2-methylbutanoyl)xox)tetrahydro-2H-pyran-3-yl palmitate (4.21): To a solution of 4.20 (20 mg, 0.018 mmol, stripped three times with toluene) in DCM (1 mL) under argon atmosphere, was added BH$_3$•THF complex (12 eq, 0.221 mL, 1 M solution in THF) and after 5 min, freshly distilled (N$_2$-atmosphere) TMSOTf (0.3 eq, 0.8 $\mu$L). After stirring for 16 h, the reaction was quenched with Et$_3$N (0.1 mL) and MeOH (2 mL). The mixture was concentrated and the crude material was purified using column chromatography (pentane/EtOAc, 2:1) affording 4.21 as a colorless oil (11.3 mg, 59% yield).

**1H NMR** (400 MHz, CDCl$_3$) $\delta$ 7.38 – 7.22 (m, 10H), 5.64 (t, $J$ = 9.7, 1H), 5.27 (d, $J$ = 3.7, 1H), 5.04 (d, $J$ = 3.9, 1H), 4.97 (d, $J$ = 11.2, 1H), 4.87 (dd, $J$ = 10.2, 3.7, 1H), 4.70 – 4.57 (m, 3H), 4.26 (t, $J$ = 8.7, 1H), 4.16 (d, $J$ = 10.0, 1H), 3.82 – 3.70 (m, 4H), 3.69 – 3.57 (m, 3H), 3.45 (t, $J$ = 8.9, 1H), 2.29 – 2.22 (m, 3H), 1.75 – 1.34 (m, 4H), 1.28 – 0.94 (m, 55H), 0.88 (t, $J$ = 7.0, 3H), 0.86 (t, $J$ = 7.5, 3H); **13C NMR** (101 MHz, CDCl$_3$) $\delta$ 175.05, 173.34, 138.18, 137.98, 128.35, 128.28, 128.19, 128.14, 127.78, 127.47, 127.16, 127.13, 94.01, 91.45, 77.65, 77.47, 75.49, 75.19, 74.58, 74.07, 71.35, 71.10, 71.05, 70.88, 61.70, 61.13, 41.05, 33.93, 31.90, 29.68, 29.66, 29.64, 29.60, 29.55, 29.45, 29.34, 29.22, 29.12, 26.59, 24.50, 22.67, 17.49, 17.44, 17.34, 17.29, 17.26, 17.23, 17.15, 17.13, 17.11, 16.21, 14.09, 12.81, 12.68, 12.22, 12.00, 11.59; **HRMS**-(ESI+) calculated for C$_{59}$H$_{88}$O$_{14}$Si$_{2}$Na [M + Na]$^+$ 1109.6387 Da, found 1109.6380 Da.
(2R,3R,4S,5R,6R)-5-(benzylxy)-2-(((5aR,6R,8R,9R,9aS)-9-(benzylxy)-2,2,4,4-tetraisopropyl-8-(((S)-2-methylbutanoyl)oxy)methyl)tetrahydro-5h-pyran-3(4H,3,5,2,4)-tioxadisilepin)-5-(benzyloxy)-2-(((S)-2-methylbutanoyl)oxy)methyl)tetrahydro-2H-pyran-3-yl palmitate (4.22): To a solution of diol 4.21 (128 mg, 0.12 mmol) in DCM (4 mL) was added EDC•HCl (2.2 eq., 49.6 mg, 0.26 mmol), DMAP (4.1 eq, 59 mg, 0.48 mmol) and (S)-butyric acid (2.15 eq, 27.6 μL, 0.25 mmol) at rt. The mixture was stirred for 16 h and was then diluted with 10 mL EtOAc. The organic layer was washed with sat. aq. NaHCO₃ (10 mL), brine (10 mL) and dried over MgSO₄. After filtration, all volatiles were evaporated and the crude product was purified using flash column chromatography (silica, pentane/EtOAc 20:1) to afford 4.22 as a colorless oil (127 mg, 86% yield).

1H NMR (400 MHz, CDCl₃) δ 7.40 – 7.17 (m, 10H), 5.66 (t, J = 9.4, 1H), 5.21 (d, J = 3.6, 1H), 5.08 (d, J = 3.8, 1H), 4.99 (AB-6, J = 11.0, 1H), 4.96 (dd, J = 10.2, 3.7, 1H), 4.63 (AB-6', J = 11.1, 1H), 4.57 (AB-6, J = 11.0, 1H), 4.51 (AB-6', J = 11.1, 1H), 4.37 – 4.14 (m, 6H), 3.87 – 3.81 (m, 1H), 3.79 (dd, J = 9.0, 3.8, 1H), 3.65 (t, J = 9.5, 1H), 3.36 (dd, J = 10.1, 8.5, 1H), 2.48 – 2.35 (m, 2H), 2.32 – 2.21 (m, 3H), 1.77 – 1.34 (m, 6H), 1.32 – 1.01 (m, 63H), 0.99 – 0.83 (m, 12H); 13C NMR (101 MHz, CDCl₃) δ 176.36, 176.29, 174.91, 172.85, 137.95, 137.58, 128.31, 128.20, 127.90, 127.72, 127.54, 127.04, 93.22, 90.82, 78.41, 77.46, 76.35, 75.32, 74.48, 74.16, 71.53, 70.54, 69.01, 68.64, 62.77, 62.20, 41.03, 40.95, 40.79, 33.98, 31.91, 29.68, 29.67, 29.65, 29.64, 29.58, 29.42, 29.34, 29.19, 29.12, 26.76, 26.56, 24.54, 22.67, 17.49, 17.43, 17.38, 17.29, 17.18, 17.14, 17.12, 16.51, 16.45, 16.13, 14.10, 12.83, 12.74, 12.32, 12.05, 11.58, 11.57, 11.52; HRMS-(ESI+) calculated for C₆₉H₁₁₆O₁₆Si₂ [M + H]+ 1255.7724 Da, found 1255.7718 Da.

(2R,3R,4S,5R,6R)-5-(benzylxy)-2-(((2R,3R,4S,5R,6R)-5-(benzylxy)-3,4-dihydroxy-6-(((S)-2-methylbutanoyl)oxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4-(((S)-2-methylbutanoyl)oxy)-6-(((S)-2-methylbutanoyl)oxy)methyl)tetrahydro-2H-pyran-3-yl palmitate: To the starting material (30 mg, 0.024 mmol) in THF (0.5 mL) was added TBAF (40 eq, 0.96 mL, 1 M solution in THF, acidified to pH 6.5 with TFA). The mixture was heated to 40 °C for 24 h, and diluted with EtOAc (2 mL) afterwards.
The organic layer was washed with water (2 mL) and then dried over MgSO₄. After filtration, the solvent was evaporated and the product was purified using flash column chromatography (pentane/EtOAc 3:2) to afford the title compound (22 mg, 91% yield) as a colorless oil.

\(^1\)H NMR (400 MHz, CDCl₃) δ 7.38 – 7.20 (m, 10H), 5.62 (t, J = 9.6 1H), 5.24 (d, J = 3.8, 1H), 5.11 (d, J = 3.7, 1H), 4.97 (dd, J = 10.3, 3.8, 1H), 4.82 (AB-6, J = 11.3, 1H), 4.66 (AB-6, J = 11.3, 1H), 4.62 (AB-6, J = 10.8, 1H), 4.52 (AB-6, J = 10.8, 1H), 4.38 (d, J = 10.2, 1H), 4.26 – 4.16 (m, 4H), 4.01 (t, J = 9.2, 1H), 3.81 (dt, J = 9.7, 3.7, 1H), 3.65 (t, J = 9.5, 1H), 3.61 – 3.54 (m, 1H), 3.37 (t, J = 9.7, 1H), 2.49 – 2.35 (m, 2H), 2.34 – 2.21 (m, 3H), 1.77 – 1.37 (m, 8H), 1.28 – 1.19 (m, 24H), 1.17 (d, J = 7.0, 3H), 1.13 (d, J = 7.0, 3H), 1.10 (d, J = 7.0, 3H), 0.95 – 0.84 (m, 12H); \(^{13}\)C NMR (101 MHz, CDCl₃) δ 176.30, 176.23, 175.50, 172.73, 137.94, 137.04, 128.53, 128.50, 128.07, 127.96, 127.86, 127.84, 127.79, 93.53, 91.36, 77.69, 76.29, 74.77, 74.66, 74.41, 71.95, 71.37, 70.30, 69.63, 69.14, 62.50, 62.13, 41.01, 40.97, 40.88, 33.93, 31.92, 29.69, 29.68, 29.65, 29.60, 29.45, 29.35, 29.24, 29.16, 26.77, 26.72, 26.59, 24.60, 22.68, 16.57, 16.46, 16.11, 14.11, 11.62, 11.57; HRMS-(ESI+) calculated for C₅₇H₈₈O₁₅Na [M + Na]⁺ 1035.6021 Da, found 1035.6024 Da.

\((2R,3R,4S,5R,6R)-5-(benzylxylo)-2-(((2R,3R,4S,5S,6R)-5-(benzyloxy)-4-hydroxy-6-(((S)-2-methylbutanoyl)oxy)methyl)-3-(((2,2,2-trichloroethoxy)sulfonyl)oxy)tetrahydro-2H-pyan-2-yl)oxy)-4-(((S)-2-methylbutanoyl)oxy)-6-(((S)-2-methylbutanoyl)oxy)methyl)tetrahydro-2H-pyan-3-yl palmitate: To the diol (32 mg, 0.032 mmol) in DCM (1 mL) was added imidazolium salt 3.7 (2 eq, 29 mg, 0.063 mmol, prepared according to a previously reported procedure).

The mixture was cooled to 0 ºC and 1,2-dimethylimidazole (2.5 eq, 7.6 mg, 0.079 mmol) was added as a solution in DCM (0.5 mL) over 4 h. The reaction was allowed to reach rt after which it was stirred for 72 h. The mixture was diluted with DCM (3 mL) and the organic layer was washed with brine. The organic layer was dried over MgSO₄, filtered and all volatiles were evaporated. The product was purified using flash chromatography (pentane/EtOAC 1:10) to afford the title compound as a colorless oil (19 mg, 49% yield).

\(^1\)H NMR (400 MHz, CDCl₃) δ 7.42 – 7.20 (m, 10H), 5.60 (t, J = 9.4, 1H), 5.40 (d, J = 3.7, 1H), 5.21 (d, J = 3.8, 1H), 5.00 (dd, J = 10.3, 3.8, 1H), 4.87, 4.76 (AB-CH2CCl3, J = 10.8,
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2H), 4.71 (d, J = 4.6, 2H), 4.59, 4.54 (AB system, J = 10.8, 2H), 4.50 (dd, J = 10.0, 3.7, 1H), 4.37 (ddd, J = 16.0, 12.6, 2.8, 2H), 4.30 – 4.20 (m, 3H), 4.17 (dt, J = 10.1, 2.7, 1H), 3.95 – 3.89 (m, 1H), 3.69 (t, J = 9.7, 1H), 3.45 (t, J = 7.2, 1H), 2.52 – 2.36 (m, 3H), 2.35 – 2.20 (m, 2H), 1.80 – 1.35 (m, 8H), 1.27 – 1.15 (m, 24H), 1.17 (d, J = 7.0, 3H), 1.09 (d, J = 7.0, 3H), 0.96 – 0.81 (m, 12H); 13C NMR (101 MHz, CDCl3) δ 176.20 (2 x C), 175.42, 172.57, 137.25, 137.13, 128.82, 128.44, 128.42, 127.94, 127.91, 127.71, 92.66, 92.45, 92.00, 81.02, 79.90, 78.41, 75.89, 75.27, 74.35, 71.05, 70.67, 70.20, 69.54, 69.27, 61.82, 40.93, 40.90, 40.87, 33.91, 31.91, 29.69, 29.67, 29.65, 29.60, 29.44, 29.35, 29.25, 29.18, 26.79, 26.56, 24.58, 22.68, 16.54, 16.51, 16.06, 14.12, 11.63, 11.60; HRMS-(ESI+) calculated for C91H146Cl3O17S [M + H]+ 1647.9346 Da, found 1647.9355 Da.

sodium (2R,3R,4S,5S,6R)-4,5-dihydroxy-2-(((2R,3R,4S,5R,6R)-5-hydroxy-4-((S)-2-methylbutanoyloxy)-6-(((S)-2-methylbutanoyloxy)methyl)-3-(palmitoyloxy)tetrahydro-2H-pyran-2-yl)oxy)-6-(((S)-2-methylbutanoyloxy)methyl)tetrahydro-2H-pyran-3-yl sulfate: To the TCE protected sulfate (16 mg, 0.013 mmol) was added DCM (0.5 mL) and MeOH (1 mL). Ammonium formate (20 eq, 16.5 mg, 0.26 mmol) was added and after everything had dissolved, Pd(OH)2 (1 eq, 9 mg, 20% weight on carbon) was added. The mixture was placed under a H2 atmosphere (1 bar, balloon) using three vacuum/N2 cycles followed by four vacuum/H2 cycles. After 16 h, TLC showed complete conversion of the starting material and the mixture was filtered over Celite and concentrated. 1H NMR spectroscopy showed complete removal of the TCE group but incomplete deprotection of the benzyl ethers. The crude product was redissolved in DCM (0.5 mL) and MeOH (1 mL). Pd(OH)2 (1.2 eq, 11 mg) was added and the reaction was placed under a H2 atmosphere (1 bar, balloon) and left for 48 h. After this period, TLC indicated the appearance of one major product, which was purified using column chromatography (8% MeOH in DCM). The ammonium salt was flushed over a DOWEX Na+ ion-exchange column (DCM/MeOH 9:1) to give the title compound as a viscous colorless oil (6.0 mg, 49%).

1H NMR (400 MHz, CDCl3/MeOD 9:1) δ 5.33 (d, J = 4.0, 1H), 5.31 (t, J = 9.7, 1H), 5.13 (d, J = 3.4, 1H), 4.86 (dd, J = 10.3, 3.5, 1H), 4.31 (m, 2H) 4.25 – 4.11 (m, 4H), 3.83 (t, J = 9.3, 1H), 3.76 – 3.69 (m, 1H), 3.49 (t, J = 9.8, 1H), 3.32 (t, J = 9.6, 1H), 2.41 – 2.25 (m, 3H), 2.23 – 2.15 (m, 2H), 1.66 – 1.31 (m, 8H), 1.24 – 1.13 (m, 24H), 1.07 (d, J = 7.0, 3H), 1.06
Total synthesis of sulfolipid-1

(\(d, J = 7.0, 3H\)), 1.04 (d, \(J = 7.0, 3H\)) 0.87 – 0.76 (m, 12H); HRMS-(ESI-) calculated for C\(_{43}H_{75}O_{18}SNa\) [M + Na]\(^+\) 957.4470 Da, found 957.4468

\(2S,4S,6S,8S,10S,12S,14S\)-(2\(R\),4\(a\)R,6\(R\),7\(R\),8\(S\),8\(a\)R)-7-(palmitoyloxy)-2-phenyl-6

\(((5aR,6\(R\),7\(a\)R,10\(R\),11\(a\)R,11\(b\)S)-2,2,4,4-tetraisopropyl-10-phenylhexahydro

[1,3]dioxino[4',5':5,6]pyrano[3,4-\(f\)][1,3,5,2,4]trioxadisilepin-6-yl)oxy)hexahydropyrano[3,2-d][1,3]dioxin-8-yl 2,4,6,8,10,12,14-heptamethyltriacontanoate (4.17)

To carboxylic acid 2.2 (1.05 eq, 40 mg, 0.073 mmol) in benzene (1 mL) was added Et\(_3\)N (2.2 eq, 15.4 mg, 0.152 mmol) and 2,4,6-trichlorobenzoyl chloride (1.1 eq, 5.2 mg, 0.021 mmol). The mixture was stirred for 1 h, and 3.4 (1 eq, dissolved in 0.4 mL benzene) and DMAP (1.1 eq, 9.3 mg, 0.076 mmol) were added, upon which the mixture turned into a white suspension. After 48 h, the suspension was quenched with sat. aq. NaHCO\(_3\) (4 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried, filtered and all volatiles were evaporated. Purification using column chromatography (pentane/EtOAc 20:1) afforded 4.17 as a colorless oil (81 mg, 76%).

\(^1\text{H NMR}\) (400 MHz, CDCl\(_3\)) \(\delta\) 7.46 (dd, \(J = 6.6, 3.0, 2H\)), 7.40 (dd, \(J = 6.7, 3.0, 2H\)), 7.36 – 7.31 (m, 6H), 5.67 (t, \(J = 9.8, 1H\)), 5.54 (s, 1H), 5.47 (s, 1H), 5.39 (d, \(J = 3.8, 1H\)), 5.13 (d, \(J = 4.1, 1H\)), 5.03 (dd, \(J = 10.0, 3.8, 1H\)), 4.32 (td, \(J = 9.9, 5.0, 1H\)), 4.27 – 4.18 (m, 2H), 4.17 – 4.11 (m, 1H), 3.91 (dd, \(J = 8.5, 4.1, 1H\)), 3.82 (td, \(J = 10.0, 4.6, 1H\)), 3.78 – 3.61 (m, 3H), 3.52 (t, \(J = 9.2, 1H\)), 2.63 – 2.52 (m, 1H), 2.41 – 2.24 (m, 2H), 1.83 – 1.72 (m, 1H), 1.65 – 1.45 (m, 8H), 1.37 – 1.03 (m, 93H), 0.92 – 0.75 (m, 27H); \(^{13}\text{C NMR}\) (101 MHz, CDCl\(_3\)) \(\delta\) 175.22, 173.15, 137.60, 137.06, 128.80, 128.56, 127.98, 127.93, 126.11, 125.90, 101.42, 101.09, 94.44, 91.92, 81.10, 79.43, 75.24, 73.40, 70.88, 68.73 68.71, 68.38, 62.82, 62.51, 45.63, 45.42, 45.04, 44.95, 39.83, 37.55, 36.44, 33.90, 31.93, 30.06, 29.99, 29.76, 29.70, 29.68, 29.66, 29.56, 29.48, 29.38, 29.36, 29.15, 29.11, 27.82, 27.48, 27.40, 27.30, 27.02, 26.88, 26.55, 24.61, 22.69, 21.26, 21.13, 21.05, 20.66, 20.60, 20.56, 18.52, 17.45, 17.40, 17.32, 17.21, 17.15, 17.12, 17.00, 14.12, 12.89, 12.65, 12.29, 11.70; HRMS-(ESI+) calculated for C\(_{91}H_{159}O_{14}Si_2\) [M + H]\(^+\) 1532.1268 Da, found 1532.1263 Da.
\[(2S,4S,6S,8S,10S,12S,14S)\-(2R,3R,4S,5R,6R)\-3\-(benzyloxy)\-6\-(((5aR,6R,8R,9R,9aS)\-9\-(benzyloxy)\-8\-(hydroxymethyl)\-2,2,4,4\-tetraisopropyltetrahydro-5aH-pyran[3,4-f][1,3,5,2,4]trioxadisilepin\-6\-yl)oxy)\-2\-(hydroxymethyl)\-5\-(palmitoyloxy)tetrahydro-2H-pyran-4\-yl 2,4,6,8,10,12,14\-heptamethyltriacontanoate (4.18)\]

To a solution of 4.17 (48 mg, 0.031 mmol, stripped three times with toluene) in DCM (1 mL) under argon atmosphere, was added BH\(_3\)\-THF complex (12 eq, 0.38 mL, 1 M solution in THF) and after 5 min, freshly distilled TMSOTf (0.3 eq, 3.40 μL). After stirring for 16 h, the reaction was quenched with Et\(_3\)N (0.1 mL) and MeOH (2 mL). The mixture was concentrated and the crude material was purified using column chromatography (pentane/EtOAc, 3:1) affording 4.18 as a colorless oil (28 mg, 59%).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 7.41 – 7.17 (m, 10H), 5.64 (t, \(J = 9.6\), 1H), 5.28 (d, \(J = 3.6\), 1H), 5.04 (d, \(J = 3.8\), 1H), 4.97 (AB-6, \(J = 11.2\), 1H), 4.86 (dd, \(J = 12.1\), 5.6, 1H), 4.67 (AB-6', \(J = 11.4\), 1H), 4.66 (AB-6, \(J = 11.2\), 1H), 4.61 (AB-6', \(J = 11.6\), 1H), 4.27 (t, \(J = 8.8\), 1H), 4.15 (d, \(J = 10.0\), 1H), 3.82 – 3.69 (m, 4H), 3.69 – 3.56 (m, 3H), 3.45 (t, \(J = 9.0\), 1H), 2.48 (q, \(J = 14.5\), 6.9, 1H), 2.24 (td, \(J = 7.1\), 1.8, 2H), 1.79 – 1.65 (m, 2H), 1.63 – 1.46 (m, 8H), 1.34 – 1.12 (m, 78H), 1.11 – 0.95 (m, 14H), 0.91 – 0.76 (m, 27H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) δ 175.33, 173.24, 138.17, 137.95, 130.26, 128.38, 128.20, 128.17, 128.10, 127.82, 127.47, 127.10, 93.92, 91.28, 77.58, 77.42, 77.20, 75.60, 75.21, 74.57, 73.99, 71.29, 71.09, 70.78, 61.70, 61.23, 45.46, 45.39, 45.33, 45.02, 44.80, 41.58, 41.35, 40.48, 37.91, 37.88, 37.05, 36.43, 33.91, 32.12, 31.92, 30.06, 29.99, 29.76, 29.70, 29.67, 29.65, 29.52, 29.37, 29.36, 29.28, 29.20, 27.82, 27.48, 27.45, 27.41, 27.29, 27.17, 26.87, 24.91, 24.48, 22.69, 21.31, 21.20, 21.14, 21.01, 20.79, 20.60, 18.47, 17.77, 17.50, 17.45, 17.36, 17.26, 17.17, 17.14, 14.12, 14.05, 12.82, 12.70, 12.24, 12.02; HRMS -(ESI+) calculated for C\(_{91}H_{162}O_{14}Si_2Na [M + Na]^+ 1558.1401 Da, found 1558.1395 Da.
Total synthesis of sulfolipid-1

\[(2S,4S,6S,8S,10R,12R,14R,16R,17R)-\{(5aR,8R,9R,9aS)-9-(benzyloxy)-6-((2R,3R,4S,5R,6R)-5-(benzyloxy)-6-(((2S,4S,6S,8S,10R,12R,14R,16R,17R)-17-(benzyloxy)-2,4,6,8,10,12,14,16-octamethyltetradecanoyloxy)methyl)-4-((2S,4S,6S,8S,10S,12S,14S)-2,4,6,8,10,12,14-heptamethyltetradecanoyloxy)-3-(palmitoyloxy)tetrahydro-2H-pyran-2-yl)oxy)-2,2,4,4-tetraisopropyltetrahydro-5aH-pyran][3,4-f][1,3,5,2,4]trioxadisilepin-8-yl)methyl 17-(benzyloxy)-2,4,6,8,10,12,14,16-octamethyltetradecanoylate (4.19) \]

To a solution of diol 4.18 (27 mg, 0.018 mmol) in DCM (1 mL) was added EDC•HCl (2.1 eq, 7.1 mg, 0.037 mmol), DMAP (4.1 eq, 8.8 mg, 0.072 mmol) and acid 2.18 (2.1 eq, 25.8 mg, 0.037 mmol) at rt. The mixture was stirred for 16 h, after which TLC showed incomplete conversion. Additional EDC•HCl (6 eq) and DMAP (4 eq) were added and stirring was continued for 6 h. The reaction was diluted with EtOAc (5 mL), and the organic layer was washed with a sat. aq. NaHCO₃ solution (10 mL), brine (10 mL) and dried over MgSO₄. After filtration, all volatiles were evaporated and the crude product was purified using flash column chromatography (silica, pentane/EtOAc 40:1) to afford 4.19 as a colorless oil (36 mg, 71% yield).

\(^1\)H NMR (400 MHz, CDCl₃) δ 7.37 – 7.17 (m, 20H), 5.65 (t, J = 9.6, 1H), 5.23 (d, J = 3.6, 1H), 5.06 (d, J = 3.7, 1H), 4.99 (d, J = 10.8, 1H), 4.93 (dd, J = 10.2, 3.6, 1H), 4.63 (d, J = 11.1, 1H), 4.54 (d, J = 10.9, 1H), 4.51 (s, 4H), 4.50 (d, J = 11.0, 1H), 4.33 – 4.24 (m, 5H), 4.15 (dd, J = 11.5, 1.6, 1H), 3.83 – 3.74 (m, 2H), 3.65 (t, J = 9.5, 1H), 3.37 (dd, J = 10.0, 8.5, 1H), 3.26 – 3.19 (m, 2H), 2.66 – 2.57 (m, 2H), 2.48 (q, J = 14.7, 6.9, 1H), 2.22 (t, J = 7.8, 1H), 1.85 – 1.76 (m, 4H), 1.75 – 1.66 (m, 2H), 1.65 – 1.38 (m, 24H), 1.35 – 1.12 (m, 153H), 1.11 – 0.94 (m, 18H), 0.93 – 0.73 (m, 81H); \(^{13}\)C NMR (101 MHz, CDCl₃) δ 176.70, 176.63, 175.20, 172.74, 139.35, 138.00, 137.57, 128.30, 128.19, 127.85, 127.70, 127.57, 127.51, 127.23, 126.97, 93.15, 90.62, 82.84, 78.38, 76.37, 75.38, 74.47, 74.05, 71.81,
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71.42, 70.64, 69.07, 68.59, 62.61, 62.22, 52.62, 45.48, 45.38, 45.34, 45.27, 45.22, 45.18, 45.02, 44.92, 44.89, 44.75, 40.68, 40.62, 40.54, 40.41, 38.73, 37.24, 37.12, 37.03, 36.44, 33.97, 31.93, 30.76, 30.07, 29.99, 29.89, 29.76, 29.70, 29.68, 29.66, 29.52, 29.38, 29.36, 29.26, 29.20, 28.12, 28.10, 26.88, 26.20, 24.56, 22.69, 21.58, 21.48, 21.41, 21.31, 21.19, 21.17, 21.14, 20.96, 20.82, 20.81, 20.71, 20.64, 20.60, 18.47, 18.38, 17.75, 17.50, 17.45, 17.41, 17.30, 17.23, 17.17, 15.69, 14.12, 12.84, 12.77, 12.33, 12.06; HRMS-(APPI+) calculated for C\textsubscript{185}H\textsubscript{330}O\textsubscript{18}Si\textsubscript{2}Na [M + Na]\textsuperscript{+} 2921.4405 Da, found 2921.4186 Da.

\((2S,4S,6S,8S,10R,12R,14R,16R,17R)\)\(-(2R,3R,4S,5R,6R)\)\-3-(benzyloxy)-6-\((2R,3R,4R,5S,6R)\)\-5-(benzyloxy)-6-\((2S,4S,6S,8S,10R,12R,14R,16R,17R)\)\-17-(benzyloxy)-2,4,6,8,10,12,14,16-octamethyltriacontanoyloxy)methyl)-3,4-dihydroxytetrahydro-2H-pyran-2-yl]oxy)\-4-\((2S,4S,6S,8S,10S,12S,14S)\)\-2,4,6,8,10,12,14-heptamethyltriacontanoyloxy)-5-(palmitoyloxy)tetrahydro-2H-pyran-2-yl]methyl 17-(benzyloxy)-2,4,6,8,10,12,14,16-octamethyltriacontanoate (4.23)

To compound 4.19 (32 mg, 0.011 mmol) in THF (0.5 mL), was added TBAF (40 eq, 0.44 mL, 1 M solution in THF, acidified to pH = 6.5 with TFA). The mixture was heated to 40 °C for 24 h and then EtOAc (2 mL) was added. The organic layer was washed with water (2 mL) and subsequently dried over MgSO\textsubscript{4}. After filtration, the solvent was evaporated, and the product was purified using flash column chromatography (pentane/EtOAc 5:1) to afford 4.23 (25 mg, 84% yield) as a colorless oil.

\(^1\text{H NMR}\) (400 MHz, CDCl\textsubscript{3}) \(\delta\) 7.36 – 7.20 (m, 20H), 5.61 (t, \(J = 9.7, 1\)H), 5.25 (d, \(J = 3.3, 1\)H), 5.10 (d, \(J = 3.4, 1\)H), 4.96 (dd, \(J = 10.2, 3.5, 1\)H), 4.82 (d, \(J = 11.2, 1\)H), 4.70 – 4.59 (m, 2H), 4.53 (d, \(J = 11.0, 1\)H), 4.51 (s, 4H), 4.36 (d, \(J = 11.5, 1\)H), 4.25 – 4.17 (m, 4H), 4.01 (t, \(J = 8.9, 1\)H), 3.82 – 3.76 (m, 1H), 3.66 (t, \(J = 9.5, 1\)H), 3.61 – 3.51 (m, 1H), 3.40 (t,
Total synthesis of sulfolipid-1

$J = 9.1, 1H), 3.26 – 3.18 (m, 2H), 2.69 – 2.55 (m, 3H), 2.51 (q, J = 13.9, 7.0, 1H), 2.33 (d, J = 7.6, 1H), 2.23 (t, J = 7.8, 2H), 1.89 – 1.71 (m, 4H), 1.64 – 1.36 (m, 24H), 1.35 – 1.12 (m, 130H), 1.12 – 0.96 (m, 14H), 0.95 – 0.74 (m, 81H); $\textsuperscript{13}C$ NMR (101 MHz, CDCl$_3$) $\delta$ 176.65, 176.58, 175.80, 172.59, 139.34, 137.95, 137.05, 128.53, 128.47, 128.19, 128.01, 127.95, 127.82, 127.66, 127.57, 127.24, 93.51, 91.28, 82.84, 77.72, 77.20, 76.37, 74.84, 74.42, 71.98, 71.81, 71.30, 70.36, 69.58, 69.00, 62.44, 62.20, 45.39, 45.31, 45.26, 45.22, 45.17, 45.02, 44.89, 44.70, 40.74, 40.63, 40.54, 37.26, 37.16, 37.03, 36.44, 33.94, 32.88, 31.92, 31.90, 30.76, 30.07, 29.99, 29.89, 29.76, 29.70, 29.68, 29.66, 29.56, 29.51, 29.42, 29.36, 29.33, 29.30, 29.24, 28.10, 27.88, 27.80, 27.66, 27.54, 27.47, 27.46, 27.37, 27.36, 27.21, 27.15, 27.12, 26.88, 26.55, 26.20, 25.86, 24.63, 22.69, 21.58, 21.49, 21.42, 21.32, 21.25, 21.19, 21.15, 21.13, 20.85, 20.82, 20.80, 20.71, 20.64, 20.60, 18.44, 18.35, 17.67, 17.07, 17.05, 15.69, 14.12, 14.10, 13.05, 13.03; HRMS-(ESI+) calculated for C$_{173}$H$_{304}$O$_{17}$Na$_2$ [M + 2Na]$^+$ 2700.2719 Da, found 2700.2637 Da.

Compound 4.24

To 4.23 (24 mg, 9.7 μmol) in DCM (1 mL) was added imidazolium salt 3.7 (2 eq, 8.3 mg, 0.018 mmol, prepared according to a previously reported procedure).[29] The mixture was cooled to 0 °C and 1,2-dimethylimidazole (2.8 eq, 2.4 mg, 0.025 mmol) was added as a solution in DCM (0.5 mL) over 4 h. The reaction was allowed to reach rt, after which it was stirred for 24 h, and more imidazolium (0.5 eq) salt was added. After an additional 48 h the mixture was diluted with DCM (3 mL) and the organic layer was washed with brine (2 mL). The organic layer was dried over MgSO$_4$, filtered and all volatiles were evaporated. The product was purified using flash chromatography (pentane/EtOAc 20:1) to afford 4.24 as a colorless oil (15 mg, 58% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.40 – 7.17 (m, 20H), 5.59 (t, $J = 9.7, 1H), 5.39 (d, J = 3.1,
1H), 5.22 (d, J = 3.3, 1H), 4.98 (dd, J = 10.2, 3.2, 1H), 4.86, 4.75 (AB-CH2CCl3, J = 10.8, 2H), 4.70 (s, 2H), 4.59, 4.54 (AB system, J = 10.7, 2H), 4.52 – 4.48 (m, 5H), 4.42 – 4.20 (m, 5H), 4.17 (d, J = 10.2, 1H), 3.91 (d, J = 7.3, 1H), 3.70 (t, J = 9.6, 1H), 3.47 (t, J = 9.3, 1H), 3.24 – 3.19 (m, 2H), 2.69 – 2.60 (m, 2H), 2.52 – 2.45 (m, 2H), 2.25 – 2.18 (m, 2H), 1.85 – 1.73 (m, 4H), 1.65– 1.35 (m, 24H), 1.08 – 0.97 (m, 14H), 0.93 – 0.74 (m, 81H); 13C NMR (101 MHz, CDCl3) δ 176.54, 176.50, 175.68, 172.43, 139.35, 137.28, 137.17, 128.81, 128.40, 128.19, 127.86, 127.57, 127.24, 92.64, 92.48, 92.02, 82.84, 81.03, 79.91, 78.43, 75.94, 75.30, 74.04, 71.81, 70.99, 70.67, 70.30, 69.35, 69.23, 61.88, 61.80, 45.38, 45.33, 45.30, 45.26, 45.22, 45.17, 45.01, 44.92, 44.83, 44.65, 40.67, 40.62, 40.54, 37.23, 37.18, 36.99, 36.44, 33.93, 32.88, 31.93, 30.76, 30.07, 29.99, 29.95, 29.90, 29.85, 29.82, 29.76, 29.70, 29.68, 29.66, 29.51, 29.36, 29.34, 29.32, 29.27, 28.14, 27.87, 27.80, 27.64, 27.53, 27.47, 27.35, 27.23, 27.12, 26.88, 26.20, 24.62, 22.69, 21.58, 21.48, 21.41, 21.33, 21.27, 21.17, 20.83, 20.81, 20.72, 20.66, 20.61, 18.49, 18.39, 17.58, 15.69, 14.12; HRMS-(ESI-) calculated for C175H305Cl3O20S [M – H]− 2866.1556 Da, found 2866.1323 Da.

**Sulfolipid-1 (4.1)**

To a solution of **4.24** (13 mg, 4.5 μmol) in DCM/MeOH (1:1, 1.2 mL) was added ammonium formate (15 eq, 4.3 mg, 0.07 mmol). After everything had dissolved, Pd(OH)2 (1.2 eq, 3.8 mg, 20% weight on carbon) was added. The mixture was placed under a H2 atmosphere (1 bar, balloon) using three vacuum/N2 cycles followed by four vacuum/H2 cycles. After 16 h, TLC showed complete conversion of the starting material and the mixture was filtered over Celite and concentrated. 1H NMR spectroscopy showed complete removal of the TCE group but incomplete deprotection of the benzyl ethers. The crude product was redissolved in DCM (0.5 mL) and MeOH (1 mL). Pd(OH)2 (1.2 eq, 11 mg) was added and the reaction was placed under a H2 atmosphere (1 bar, balloon) and left for 48 h. After this period, TLC
indicated the formation of multiple products. ¹H NMR still indicated the presence of benzyl ethers. The procedure was repeated, first with 250 psi of hydrogen for 3 h (little change) and then at 500 psi for 3 h in a Parr bomb. After this, ¹H NMR showed complete removal of all protecting groups, however with substantial amounts of side products. The crude product was flushed over a DOWEX Na⁺ ion-exchange column (DCM/MeOH 9:1) and further purified by flash column chromatography (silica, first DCM/MeOH 99:1, then DCM/MeOH 97:3). 4.1 was obtained as a glass-like colorless oil (1.6 mg, 15%).

¹H NMR (400 MHz, CDCl₃/MeOD - 4:1) δ 5.28 (d, J = 3.8, 1H), 5.25 (t, J = 9.7, 1H), 5.07 (d, J = 3.5, 1H), 4.77 (dd, J = 10.2, 3.5, 1H), 4.27, 4.21 (AB system, J = 12.2, 2H), 4.14 – 4.06 (m, 4H), 3.78 (t, J = 9.5, 1H), 3.68 – 3.63 (m, 1H), 3.44 (t, J = 10.1, 1H), 3.33 – 3.27 (m, 2H), 3.24 (t, J = 9.2, 1H), 2.55 – 2.42 (m, 3H), 2.20 – 2.10 (m, 3H), 1.64 – 1.55 (m, 4H), 1.50 – 1.35 (m, 20H), 1.33 – 0.95 (m, 147H), 0.92 – 0.63 (m, 81H); HRMS-(ESI−) calculated for C₁₄₅H₂₇₉O₂₀S [M – Na] 2374.0564 Da, found 2374.0462 Da.

**Compound 4.25**

¹H NMR (400 MHz, CDCl₃) 5.39 (t, J = 9.4, 1H), 5.28 (d, J = 2.6, 1H), 5.12 (d, J = 2.7, 1H), 4.95 (dd, J = 10.0, 2.7, 1H), 4.49 (dd, J = 12.4, 4.0, 1H), 4.40 (dd, J = 12.8, 4.1, 1H), 4.34 – 4.28 (m, 1H), 4.17 – 4.06 (m, 2H), 3.92 (t, J = 8.7, 1H), 3.78 – 3.71 (m, 1H), 3.63 – 3.56 (m, 1H), 3.54 – 3.44 (m, 3H), 3.36 – 3.23 (m, 3H), 2.68 – 2.55 (m, 3H), 2.29 – 2.18 (m, 3H), 1.82 – 1.70 (m, 4H), 1.68 – 1.38 (m, 22H), 1.35 – 0.97 (m, 145H), 0.93 – 0.77 (m, 81H); HRMS-(ESI+) calculated for C₁₄₅H₂₈₁O₁₇ [M + H]⁺ 2296.1152 Da, found 2296.0999 Da.

**4.8 References**


[6] The depicted structure of 4.1 corresponds to the most abundant one in the mixture of homologues.


[26] We suspect that with these small scale reactions, minimal amounts of water have a strong influence on the reaction outcome.

