Chapter 7

Toward the synthesis of a sulfo-glycopeptidolipid from *Mycobacterium avium*

Although less known to the general public than *Mycobacterium tuberculosis*, *Mycobacterium avium* represents a severe threat to many people as well, but mainly targets immuno-compromised individuals. In this chapter, we present our efforts toward the synthesis of a sulfo-glycopeptidolipid, present in the outer cell wall of this pathogen. Although its exact function is not yet known, similar molecules are known for their importance in host-pathogen-signaling interactions. The synthesis of chemically pure sulfo-glycopeptidolipid might aid the understanding of the pathogenicity of *Mycobacterium avium*.

The work in this chapter was carried out in collaboration with Wendy van Bruggen, as part of her M.Sc. research project.
7.1 Introduction

*Mycobacterium avium* complex (MAC) is a slow-growing mycobacterial infection which affects mainly immuno-compromised individuals and incidentally elderly people. It consists of 28 serotypes of the species *M. avium* and *M. intracellulare* which, because of their similar characteristics, are together defined as *Mycobacterium avium-intracellulare* (MAI).[1] Acquisition of MAC occurs by inhalation of aerosols or ingestion *via* very common environmental sources, for example, soil, water and even pasteurized milk.[2] Once acquired, it quickly spreads over the body into the blood, bone marrow and tissue. Often reported symptoms are a persistent cough, fever, night sweats and weight loss. Although less prevalent than infections with *M. tuberculosis*, the causative agent of most tuberculosis infections, MAC is one of the most common non-tuberculous mycobacteria in clinical specimen, and is related to the CD4 (cluster of differentiation 4) count. If the amount of these white blood cells, essential for the human immune system, is lower than 50/μL, the risk of infection with MAC increases considerably. For this reason, 20 – 30% of the people with AIDS acquire an infection with MAC. Treatment against MAC infections requires compliance; a combination of two or three drugs has to be taken over a period of at least twelve months. Single-drug treatment has shown to facilitate drug resistance.[3]

As all mycobacteria, MAC has an unusual, lipid-rich cell envelope, made up of soluble lipids, proteins, carbohydrates and a core, consisting of arabinogalactan, peptidoglycan and mycolic acids with very long alkyl chains. The high (glyco)lipid content ensures a strong hydrophobic barrier against antibiotics, providing a rationale for the prolonged treatment against mycobacterial infections. For this reason, the breakdown of the cell wall and the inhibition of its biosynthesis has been part of many investigations.[4] In addition, the MAC outer cell surface, which has the most direct interaction with our immune system, is covered by glycopeptidolipids (GPLs). Of these, several have been found to function as antigens, and their structures are known to be species-specific. The basic structure of all GPLs consists of a β-hydroxy or –methoxy fatty acid, amidated to a tripeptide composed of the D-amino acids phenylalanine, *allo*-threonine and alanine, which is, in turn, terminated by L-alaninol. The hydroxyl group of threonine and alaninol is glycosidated to carbohydrates with varying length.[5]
Toward the total synthesis of a sulfo-glycopeptidolipid from M. avium

One of the GPLs found in clinical strains of M. avium, was identified as a sulfo-GPL (S-GPL, Figure 7.1).\[6\] Next to the basic GPL core, 7.1 carries an α-linked L-rhamnopyranosyl residue, methylated at 4-OH, on the alaninol residue. The allo-threonine is α-glycosidated to 6-deoxy talopyranose, which bears a sulfate moiety at 4-OH. Although metabolites with a sulfate group are rare in prokaryotes, M. avium is known to possess multiple sulfotransferases.\[7\]

Interestingly, S-GPL 7.1 was found to be overproduced in an AIDS patient with a drug resistant strain of M. avium who was treated with ethambutol.\[6\] Although the exact function of 7.1 is not yet known, speculations have been made regarding its involvement in drug resistance. A chemical synthesis of 7.1 could confirm its chemical structure and shed light on its precise function. For these reasons, we embarked on its first total synthesis, with the purpose to supply biologists with chemically pure material.

Figure 7.1 Sulfo-glycopeptidolipid

Figure 7.2 Glycotetrapeptide from M. fortuitum as synthetized by Gurjar
7.2 Reported syntheses of glycopeptidolipids

To date, no chemical synthesis of any S-GPL has been reported. However, two syntheses prepare GPLs that largely resemble 7.1. In 1993, the first synthesis of a glycopeptidolipid core was reported by Gurjar and Saha (Figure 7.2).7 Although their work does not reflect the exact natural product, isolated from *M. fortuitum*, which has a long β-methoxy fatty acid attached, it is the first complete synthesis of the glycotetrapeptide and was used as a guideline for subsequent studies. Starting from tetra-acetyl L-rhamnopyranoside (Scheme 7.1), the trichloroacetimidate was introduced *via* a selective cleavage of the acetyl group at the anomeric position, followed by treating the resulting alcohol with trichloroacetonitrile in the presence of base. Glycosidation with Cbz-protected L-alaninol, employing catalytic BF$_3$•OEt$_2$, led to the α-configured anomer.8 Deacetylation,
followed by protection of the cis 2,3-diol as its acetonide, allowed the regioselective methylation of 4-OH with NaH and MeI. The acetonide was then cleaved and regioselective methylation of 3-OH, using stannane chemistry, led to the glycosyl acceptor. Condensation of the acceptor with an, earlier reported, rhamnopyranosyl-trichloroacetimidate glycosyl donor afforded the α,α-linked disaccharide. Removal of the acetyl and Cbz protecting groups allowed the connection of Cbz-protected D-alanine, giving rise to the eastern part of the molecule.

The synthesis of the western part started from a known rhamnopyranosyl intermediate (Scheme 7.2), which, after cleavage of the acetonide, was regioselectively methylated via the 2,3-dibutylstannyl acetal. Acidic hydrolysis of the methyl glycoside was followed by acetylation. Regioselective deprotection at C-1 of the diacetyl, allowed the introduction of the trichloroacetimidate, affording the glycosyl donor of the western part. Glycosylation with a previously prepared dipeptide, employing Lewis-acid catalysis, afforded the pure α-anomer as determined by 13C-decoupled NMR spectroscopy. Cleavage of the benzyl group finally allowed the DCC-promoted amidation of the western and eastern part, affording the desired glycotetrapeptide.

Scheme 7.2 Final steps in the preparation of the glycotetrapeptide
Although the synthesis of Gurjar is straightforward, many steps lack an experimental procedure and isolated yield. For this reason, the synthesis of a complete glycopeptidolipid from \textit{M. avium} (Figure 7.3), as reported by Heidelberg and Martin, was considered as a better starting point for our investigations.\textsuperscript{[12]}

\textbf{Figure 7.3 Structure of the glycopeptidolipid from \textit{M. avium} as synthesized by Martin et al.}  

The structure of this glycopeptidolipid has the standard tetrapeptide core as all GPLs. In addition, the N-terminal position is acylated with a long \(\beta\)-hydroxy fatty acid, the L-alaninol is connected to a 3,4-dimethoxy \(\alpha\)-L-rhamnopyranosyl residue and the D-\textit{allo}-threonine is linked to a 6-deoxy \(\alpha\)-L-talopyranose, which in turn is glycosylated at C-2 with a trisaccharide. Although the synthesis of the trisaccharide part is interesting, it is not relevant to our synthesis and will therefore not be discussed.

For the synthesis of the eastern part of the molecule, the authors started with the formation of an \(\alpha\)-benzyl ortho ester by means of a previously reported procedure (Scheme 7.3).\textsuperscript{[13]} A one-pot acetyl to methyl transfer, followed by hydrogenolysis of the benzyl ether, allowed the introduction of a trichloroacetimidate on the anomeric position.

\textbf{Scheme 7.3 Synthesis of the Eastern part of the tetrapeptide}
Glycosidation with the alaninol-alanine dipeptide, afforded the desired intermediate as its α-anomer in 72% yield. The synthetic route was shortened by two steps, as the 3,4-di-OMe O-4-pentenyl ortho ester proved to be directly applicable as glycosyl donor, giving rise to the α-anomer in 81% yield. Cleavage of the acetyl and the Cbz group, led to the formation of the eastern part of the glycotetrapeptide.

The synthesis of the western part of the glycopeptide commenced from α-L-rhamnopyranoside, which was treated with 4-penten-1-ol under acidic conditions, followed by protection of the cis-2,3-diol as its acetonide. Subsequent oxidation of 4-OH was performed using PCC, taking into account the presence of an olefin (Scheme 7.4). Reduction of the resulting ketone with NaBH₄, afforded the L-talopyranose, of which 4-OH was protected as its benzyl ether. The acetonide was then cleaved, and a benzyl ether at 3-OH was introduced via the stannylidene acetal. Although the regioselectively was nearly 1:1, the 3-OBn and 2-OBn product could be separated using column chromatography.

**Scheme 7.4 Synthesis of the western part of the glycotetrapeptide**

Introduction of a chloroacetyl group at the 2-OH, allowed an α-selective glycosidation with the allo-threonine-phenylalanine glycosyl acceptor, promoted by NIS and TMSOTf. With respect to the formation of the dipeptide, D-allo-threonine was prepared making use of an earlier reported asymmetric synthesis (not shown) and attached to Troc-protected D-phenylalanine by a standard peptide coupling protocol. Selective removal of the chloroacetyl group is a prerequisite, since in later stages of the synthesis the trisaccharide had to be introduced at this position.
Chapter 7

Scheme 7.5 Preparation of the $\beta$-hydroxy fatty acid chloride

The fatty acid synthesis started with an enantioselective hydrogenation of a tetracosanoyl $\beta$-keto ester, using a ruthenium-BINAP catalyst (Scheme 7.5).\(^{[17]}\) Although these hydrogenations usually afford the product with high stereoselectivity, it is remarkable that the authors did not address this issue. Saponification of the methyl ester afforded the fatty acid, however, this compound proved to be very poorly soluble in subsequent coupling reactions. Therefore, the $\beta$-hydroxyl group was protected as its benzyl ether via the procedure of Nishizawa.\(^{[18]}\) Unfortunately, saponification of this intermediate was low-yielding (41%), most probably due to concomitant elimination of the benzyloxy group. As solubility remained poor afterwards, the carboxylic acid was transformed into its corresponding acid chloride. In order to prevent hydrolysis, this was only done prior to the successive coupling.

Scheme 7.6 Completion of the glycopeptidolipid synthesis
The ultimate coupling of the building blocks started with the glycosidation of the trisaccharide to the talose moiety. Cleavage of the Troc group with activated zinc at the N-terminal position, allowed the connection of the long β-hydroxy fatty acid chloride (Scheme 7.6). Chemoselective deprotection of the benzyl ester could be achieved by means of hydrogenolysis under slightly basic conditions. The liberated acid was coupled to the eastern part of the glycotetrapeptide, using an EDCI-mediated coupling. Finally, the cleavage of all benzyl ethers via hydrogenolysis gave rise to the desired GPL.

7.3 Strategy

Equipped with the knowledge from the reported chemical syntheses of Martin[12] and Gurjar,[8] we decided to disconnect 7.1 in an A, B and C part, keeping in mind the solubility problems encountered by Heidelberg and Martin (Scheme 7.7). In addition, we reasoned that, toward completion of the synthesis, the purification of any highly polar intermediates should be kept to a minimum. To this end, protecting groups that lead to easily removable side products after their cleavage were considered most attractive.

Scheme 7.7 Building blocks for the synthesis of S-GPL
7.4 Synthesis
7.4.1 Building block A

The synthesis of 7.1 commenced with the enantioselective preparation of the β-hydroxy fatty acid. Our first approach started from pentadecanolide (7.2) which was ring-opened using a Claisen condensation to afford β-ketoester 7.3 in a low 24% yield (Scheme 7.8). As we initially attempted to anyway prepare the 30-carbon alkyl chain, the primary alcohol afforded a handle for subsequent chain elongation. However, first 7.3 was subjected to an asymmetric hydrogenation with a Ru-based catalyst which had proven to give good results with similar substrates.\(^{[19]}\) Although the yield of 7.4 was somewhat disappointing, derivatization of a small sample to the primary TBDPS-protected alcohol indicated an ee of 95%. In order to elongate the alkyl chain, the primary alcohol of 7.4 was converted to a tosylate. However, addition of an excess of hexadecylmagnesium bromide to 7.5 did not afford any desired product. Therefore, we decided to first convert the secondary hydroxyl group into its benzyl ether. Whereas basic conditions mostly gave elimination of the hydroxyl group, acidic conditions afforded 7.6, although in very low yield. Because of the low yields in this approach and the multistep synthesis to introduce the long 30 carbon alkyl chain, we decided to abandon the synthesis towards building block A via this approach.

\[
\begin{align*}
7.2 & \xrightarrow{\text{LDA, THF, } -40 \degree \text{C}} 7.3 \\
7.4 & \xrightarrow{\text{Et}_2\text{N, TsCl, DMAP, DCM}} 7.5 \\
7.6 & \xrightarrow{\text{C}_{16}H_{33}\text{MgBr, THF}} 7.7 \\
7.3 & \xrightarrow{(\text{R})[\text{RuCl} \ (\text{T-BINAP})]_2 \ \ (\mu-\text{Cl})_3[\text{NH}_2\text{Me}_2]} \xrightarrow{\text{H}_2 \ (20 \text{ bar}), \ MeOH, 60 \degree \text{C}} 7.4 \\
7.5 & \xrightarrow{\text{TMSOTf, BnTCA, c-hexane/DCM, rt}} 7.6 \\
7.7 & \xrightarrow{\text{OBr, DCM, rt}}
\end{align*}
\]

Scheme 7.8 First approach to building block A
In order to facilitate the synthesis of building block A and ultimately 7.1, it was decided to substitute the 30-carbon side chain for a shorter 22-carbon alkyl chain, accessible in a single step approach using commercially available starting materials. Thus, methyl acetoacetate (7.8) was elongated with 1-bromodocosane using a previously reported approach, which afforded 7.9 in good yield (Scheme 7.9). Asymmetric hydrogenation of 7.9 using the same catalyst as previously employed afforded 7.10 in a satisfying 91% yield. HPLC-ELSD analysis of 7.10 showed the presence of only a single enantiomer.

Scheme 7.9 Preparation of building block A

The hydroxyl group was protected as its benzyl ether, relying on a procedure developed by Nishizawa. First, the TMS-ether was formed, which upon treatment with benzaldehyde and TMSOTf under reducing conditions afforded 7.11 in good yield. For the preparation of the carboxylic acid, Heidelberg and Martin relied on a standard saponification with aqueous NaOH. However, the reported yield was only 41%, most likely due to concommitant elimination. To obtain carboxylic acid 7.12 in higher yield, an alternative strategy was investigated. The method reported by Bartlett initially proved successful, affording full conversion after 4 h. In this reaction, the mercaptide attacks the methyl ester in an SN2 fashion, liberating the carboxylate salt. Aqueous acidic work-up afforded the crude product. Unfortunately, the majority of the material was lost during column chromatography. Due to the small scale of the reaction only 7% of 7.12 could be recovered. Although low, we believe that, on the basis of the full conversion and a promising crude 1H-NMR spectrum, the reaction has good potential and leaves room for improved yields in future experiments on larger scale.
7.4.2 Building block B

Due to a lack of time, the synthesis of building block B has only been partly completed. Because of this, the results will be discussed and a possible outline to complete the synthesis will be given.

![Scheme 7.10](Proposed preparation of the peptide part of building block B)

Our strategy for the synthesis of building block B started with the epimerization of readily available L-threonine to, considerably more expensive, D-allo-threonine following a procedure developed by Shair et al. (Scheme 7.10).\[^{22}\] To this end, L-threonine (7.13) was transformed to its methyl ester and the amine was Boc-protected to afford 7.14 in quantitative yield over two steps. Treatment of 7.14 with pivaldehyde under acidic conditions afforded oxazolidine 7.15 in 57% yield, and was the final executed step. Epimerization of 7.15 can be performed by deprotonation with LDA, followed by a reverse quench to afford D-allo-threonine 7.16. Ring-opening under acidic conditions, followed by removal of the Boc protecting group and coupling with N-protected D-phenylalanine should allow the formation of the glycosyl acceptor 7.20.

For the glycosyl donor, a procedure that relies on the epimerization of 4-OH of L-rhamnose (7.21) to afford the corresponding talopyranoside was envisioned.\[^{15}\] Moreover, a sulfate has to be introduced at this position. As sulfates are rather labile under a variety of reaction conditions, the use of a protected sulfate allows for its introduction at any point during the synthesis and prevents its degradation throughout successive steps.
Toward the total synthesis of a sulfo-glycopeptidolipid from *M. avium*

**Scheme 7.11 Proposed route towards the glycosyl donor of building block B**

An example of such a sulfate-protecting group is the 2,2,2-trichloroethyl (TCE) group. As mentioned in the synthesis of Ac₂SGL (Chapter 3) and sulfolipid-1 (Chapter 4), this protected sulfate can be efficiently introduced by its easily prepared imidazolium salt 3.7.[23]

Starting from *L*-rhamnose (7.21), we realized that the introduction of a pentenyl group at the anomeric position should allow for the efficient preparation of an appropriate glycosyl donor (Scheme 7.11). Subsequent acetalization of 2-OH and 3-OH leaves 4-OH unprotected, which, in turn, can be epimerized using a Swern oxidation,[24] followed by a stereoselective reduction using NaBH₄ to afford 7.23.[15] Introduction of the TCE-sulfate, followed by replacing the acetal for acetyl moieties gives rise to the envisioned glycosyl donor 7.25.

**Scheme 7.12 Final steps towards building block B**

Activation of 7.25 with NIS/TMSOTf in the presence of 7.20 should allow the formation of 7.26 (Scheme 7.12). Subsequent saponification of the methyl ester, and concommitant
removal of the acetyl groups with aqueous base should afford block B (7.27).

### 7.4.3 Building block C

The synthesis of building block C was initiated with the Boc-protection of L-alanine (7.28), followed by the reduction of carboxylic acid 7.29, affording protected L-alaninol glycosyl acceptor 7.30 (Scheme 7.13). Although the yield of both steps is lower than anticipated, this is most likely due to problems during the work-up.

**Scheme 7.13 Synthesis of the glycosyl acceptor of building block C**

For the preparation of the glycosyl donor, we sought to prepare glycosyl donors with different activating groups, in order to study the influence on the yield. For this reason, a strategy was developed which allowed for the late-stage functionalization with any activating group. Starting from L-rhamnose (7.21), the anomeric hydroxyl group was protected as its allyl ether under acidic conditions, followed by the acetalization of the cis-oriented 2- and 3-OH, affording 7.32 in good yield over two steps (Scheme 7.14). After methylation of 4-OH, the acetonide was cleaved using acid, and the now liberated 2- and

**Scheme 7.14 General approach towards different glycosyl donors of building block C**
3-OH were acetylated. In order to obtain a versatile building block, which could be functionalized with different activating groups, the allyl group of 7.34 was cleaved using Pd(PPh$_3$)$_4$ and polymethylhydrosiloxane which gave 7.35 in moderate yield.[25]

To install the activating groups, standard procedures were followed. Treatment of 7.35 with trichloroacetonitrile and NaH afforded 7.36 in 46% yield (Scheme 7.15). Upon activation and addition of the glycosyl acceptor 7.30, a 2 : 1 mixture of the desired 7.37 and an inseparable side product was obtained in 70% yield. $^1$H-NMR spectroscopy provided no evidence for the structure of the side product. However, the chemical shift in its $^{13}$C-NMR spectrum for the (second) anomic proton showed a signal at 97.8 ppm, most likely indicating an additional α-anomer.

A signal at 123.8 ppm in the HSQC spectrum without any coupling, most probably suggests the formation of the orthoester 7.38. Although the orthoester creates an additional chiral center, nucleophilic attack from the back-side by the glycosyl acceptor is not likely due to a strong difference in steric hindrance. Time did not permit further investigation of the side product, but treatment of the mixture with TfOH in DCM is known to force the equilibrium towards 7.37.[26] To prevent orthoester formation, alternative participating groups, like benzoyl or pivaloyl, can be considered.[27]
For the preparation of the 4-pentenyl-activated glycosyl donor, 7.35 was treated at 95 °C with 4-penten-1-ol under acidic conditions. Although the 4-pentenyl group was installed, both acetyl groups were also cleaved in the process. The 2,3-diol was therefore again treated with Ac₂O and pyridine, affording 7.39 in 46% yield over two steps. Glycosylation of 7.39 with 7.30 upon activation with NIS and TMSOTf showed a promising crude ¹H-NMR spectrum, the 4-pentenyl disappeared and all protecting groups remained intact. However, the crude product proved to be unstable as its storage for a week unfortunately left no remaining product. No further attempts to prepare building block C were made due to time constraints.

If the aforementioned selectivity and stability problems are solved, completion of building block C (7.43) can most probably be achieved by (simultaneous) cleavage of the Boc and acetyl protecting groups of 7.40, followed by coupling of amine 7.41 with protected D-alanine (7.42) (Scheme 7.16).

7.4.4 Connecting the building blocks: final steps towards S-GPL

To assemble the building blocks towards S-GPL, two strategies can be employed. One is to first connect building block A to B, and subsequently attach building block C, followed by a final deprotection step. However, due to our choice of protecting groups in the early stages of the synthesis, the connection of 7.43 and 7.27, followed by the attachment of 7.12 minimizes the protection and deprotection steps towards S-GPL. Thus, deprotection of 7.43 allows for the EDCI-promoted coupling with 7.27 (Scheme 7.17). A subsequent deprotection of the phenylalanine sets the stage for the coupling of the glycopeptide with the fatty acid 7.12. For the formation of the amide, the acid should be transformed to its acid chloride (7.13) as this has shown to increase solubility, and reactivity, under the reaction conditions. As a final step, removal of the remaining protecting groups has to be
performed. Since the 2,2,2-trichloroethyl and the benzyl ether of 7.46 are labile under hydrogenolysis conditions, this will hopefully afford S-GPL (7.1).

\[ \text{Scheme 7.17 Towards S-GPL} \]

7.5 Conclusions

Although the synthesis of S-GPL has not been completed, much has been learned from the steps performed until now. Whether the envisioned strategy will deliver the desired product has to be investigated. In particular the connection of the building blocks could be troublesome. However, as the order of assembly is to a certain extent modular, several approaches lead to 7.1.

As sulfate groups are known to be important signaling groups in important physiological functions, the exact function of 7.1 is very interesting.\[^{12}\] The availability of synthetic material will help to elucidate this function.
7.6 Experimental section

For general experimental information: see Chapter 2.

**methyl 3-oxohexacosanoate (7.9):** A suspension of NaH (0.68 g, 16.9 mmol, 60% suspension in mineral oil) in THF (50 mL) was cooled in an ice bath, and a solution of methyl acetoacetate (7.8) (1.8 g, 15.4 mmol) in THF (5 mL) was added slowly and left stirring for 1 h. Then n-BuLi (10.6 mL, 16.9 mmol, 1.6 M solution in hexane) was added slowly at -5°C in a ice-salt bath, affording an orange mixture. The mixture was stirred for 1 h while allowing it to warm to rt and was subsequently cooled in an ice bath. Then a solution of 1-bromodocosane (3.0 g, 7.7 mmol) in THF (10 mL) was added slowly. After stirring the mixture at rt for 22 h, the reaction was quenched by adding aq. saturated NH₄Cl. The resulting slurry was extracted 3 times with EtOAc, and the combined organic layers were washed with water and brine, dried over MgSO₄, filtered and concentrated. Column chromatography (5% EtOAc in pentane) provided 7.9 (2.4 g, 73%) as a colorless solid. Analytical data were in accordance to those reported.[20]

**(R)-methyl 3-hydroxyhexacosanoate (7.10):** Racemic reaction: To a suspension of 7.9 (100 mg, 0.235 mmol) in MeOH (6.5 mL), was added NaBH₄ (14.3 mg, 0.377 mmol), and the mixture was allowed to stir for 2 h. When TLC (10% EtOAc in pentane) showed poor conversion, THF (2.5 mL) was added to try and dissolve the remaining precipitate, and the mixture was stirred overnight. As the precipitate was still present and TLC showed no further improvement of the conversion, another equivalent of NaBH₄ was added, and the mixture was heated to 60 °C, affording a solution. After 5 h, TLC showed full conversion of the starting material. The reaction was quenched by adding 1 M HCl while cooling on ice, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried with MgSO₄, filtered, concentrated, and purified by column chromatography (10% EtOAc in pentane) to afford racemic 7.10 (82 mg, 82%) as a white solid.

*Enantoiselective reaction:* To a suspension of β-ketoester 7.9 (500 mg, 1.18 mmol) in MeOH (10 mL) was added [(R,R)-RuCl(T-BINAP)]₂(μ-Cl)₃[NH₂Me₂] (21 mg, 0.012 mmol). The resulting yellow mixture was stirred in an autoclave under H₂ pressure (20 bar) at 60 °C for 21 h. The solvent was then evaporated and the residue was purified by column chromatography (10% EtOAc in pentane) to give (R)-7.10 (455 mg, 91%, >99% ee). Note:
The absolute stereochemistry was not determined, however the use of the catalyst with the 
(R)-configuration, suggests the formation of the product with (R)-stereochemistry. [19]

1H NMR (400 MHz, CDCl3) δ 4.00 (bs, 1H), 3.71 (s, 3H), 2.82 (s, 1H), 2.46 (ddd, 2H), 1.68 – 1.36 (m, 2H), 1.25 (s, 42H), 0.88 (t, 3H); 13C NMR (50 MHz, CDCl3) δ 173.2, 68.01, 51.70, 41.06, 36.51, 31.91, 29.68, 29.57, 29.34, 25.46, 22.67, 14.10; HRMS-(ESI+) for C27H54O3Na [M + Na]+ calculated 449.3971, found 449.3965; Chiral HPLC(ELSD) analysis, Chiralpak OD-H 95:5, n-heptane/2-propanol, flow 0.5 ml/min, retention times (min): 7.9 (major) and 8.9 minutes (minor).

(R)-methyl 3-(benzyloxy)hexacosanoate (7.11): To a solution of 7.10 in anhydrous DCM (1.2 mL), was added Et3N (44 μL, 0.32 mmol) followed by TMSCl (35 μL, 0.28 mmol). The solution was stirred at rt under N2 atmosphere until TLC (5% EtOAc in pentane) showed full conversion after 3.5 h. After cooling in an ice bath, the mixture was diluted with DCM and washed with ice-cold aq. NaHCO3 and water. The organic layer was dried over MgSO4, filtered and concentrated to give the O-TMS derivative of 7.10 (45 mg, 67%).

1H NMR (400 MHz, CDCl3) δ 4.21 – 3.96 (m, 1H), 3.67 (s, 3H), 2.43 (d, J = 6.1 Hz, 2H), 1.52 – 1.35 (m, 2H), 1.30 – 1.18 (m, 42H), 0.88 (t, J = 6.5 Hz, 3H), 0.10 (s, 9H).

The O-TMS derivative and freshly distilled benzaldehyde (11 μL, 0.11 mmol) were dissolved in anhydrous DCM (1 mL) and the solution was cooled in an ice bath. TMSOTf (2 μL, 0.011 mmol) was added and the mixture was stirred on ice for 1 h after which triethylsilane (15 μL, 0.095 mmol) was added. The reaction mixture was stirred at rt overnight, diluted with EtOAc and washed with aqueous saturated NaHCO3 and water. The organic solution was dried over MgSO4, concentrated and purified by automated column chromatography (EtOAc : n-pentane). The benzylated compound 7.11 was obtained as an off-white solid (28 mg, 60%).

1H NMR (400 MHz, CDCl3) δ 7.43 – 7.06 (m, 5H), 4.49 (s, 2H), 3.81 (m, 1H), 3.62 (s, 3H), 2.63 – 2.31 (ddd, J = 20.4, 15.0, 6.3 Hz, 2H), 1.40-1.63 (m, 2H), 1.36 – 1.02 (m, 42H), 0.82 (t, J = 6.7 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 171.26, 137.49, 137.23, 127.37, 127.28, 126.75, 126.73, 126.60, 126.52, 75.67, 75.04, 71.08, 70.50, 50.58, 38.75, 33.36, 30.91, 28.69, 28.64, 28.60, 28.57, 28.55, 28.35, 24.13, 21.68, 13.11; HRMS-(ESI+) for C34H61O3 [M + H]+ calculated 517.4621 Da, measured 517.4615 Da.
(R)-3-(benzyloxy)hexacosanoic acid (7.12): Propane-1-thiol (0.5 mL, 5.52 mol) was added to a suspension of LiH (0.15 g, 18.87 mmol) in dry, oxygen-free HMPA (5 mL) under a N₂ atmosphere. After stirring at rt for 1.5 h, the suspension was filtered without contact to air. The resulting viscous liquid (4.5 mL) was stirred with compound 7.11 (90 mg, 0.174 mmol) at rt under a N₂ atmosphere for 3 h before it was transferred to aq. HCl (1 m, 5 mL). The aq. layer was extracted with EtOAc which was then extracted with NaOH aq (2 m). The aqueous layer was washed with EtOAc and acidified to pH 2 to precipitate the product. No precipitate was formed, so the organic layers were combined and concentrated. The crude product was flushed through a short silica column (5% EtOAc in pentane). However, no product was detected by TLC analysis (40% EtOAc in pentane). All fractions were combined and again concentrated. A second attempt was made by automated column chromatography which afforded only little of pure 7.12 (5.8 mg, 7%) was obtained as a white solid.

¹H NMR (400 MHz, CDCl₃) δ 7.43 – 6.78 (m, 5H), 4.51 (s, 2H), 3.85 – 3.68 (m, 1H), 2.62 – 2.44 (m, 2H), 1.42-1.50 (m, 2H), 1.40 – 0.97 (m, 43H), 0.82 (t, J = 6.5 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 175.9, 137.78, 128.33, 127.76, 127.70, 113.75, 75.64, 71.44, 39.2, 33.91, 31.82, 29.60, 29.55, 29.48, 29.44, 29.26, 24.98, 22.59, 14.02; HRMS-(ESI⁺) for C₃₃H₅₉O₃Na [M + Na⁺] calculated 525.4284 Da, measured 525.4278 Da.

(S)-2-((tert-butoxycarbonyl)amino)propanoic acid (7.29): L-alanine (1.0 g, 11.2 mmol), KOH (0.7 g, 12.4 mmol) and di-tert-butyl-dicarbonate (2.7 g, 12.4 mmol) were stirred in a mixture of water (45 mL) and THF (4.5 mL) at 50 °C for 3 h. The mixture was stirred at rt overnight after which it was acidified with HCl aq (1 m) to pH 5. The aqueous layer was extracted with EtOAc and DCM, after which the combined organic layers were dried over MgSO₄, filtered and concentrated. Only 119 mg of 7.29 was obtained, so the aqueous layer was acidified to pH 3 and extraction with DCM was repeated to afford another 595 mg of product 7.29. The combined fractions afforded 714 mg (34%) of 7.29. Analytical data were in accordance with those reported.

(S)-tert-butyl 1-hydroxypropan-2-ylcarbamate (7.30): A solution of compound 7.29 (275 mg, 1.45 mmol) in THF (7.3 mL), was slowly added over 1 h to a solution of BH₃-THF (1 m, 2.9 mL, 2.9 mmol) while cooling in an ice bath. The mixture was stirred at this temperature for another 2.5 h, after which it was quenched with a 10% solution of AcOH in MeOH. The solvents were evaporated and the crude
product was dissolved in EtOAc and washed with HCl\textsubscript{aq} (1 M), water and NaHCO\textsubscript{3(aq)} (1 M). The organic layer was dried over MgSO\textsubscript{4}, filtered and concentrated. Purification by automated column chromatography (EtOAc : pentane, 0 – 100% EtOAc in 20 min) afforded 7.30 (165 mg, 65%) as a white solid. Analytical data were in accordance with those reported.\cite{29}

(2\text{R},3\text{R},4\text{R},5\text{R},6\text{S})-2-(allyloxy)-6-methyltetrahydro-2H-pyran-3,4,5-triol (7.31): A solution of L-rhamnose (5.0 g, 30.5 mmol) in allyl alcohol (60 mL) and concentrated H\textsubscript{2}SO\textsubscript{4} (0.5 mL) was stirred at 100 °C for 2 h. After TLC (10% MeOH in DCM) showed full conversion, K\textsubscript{2}CO\textsubscript{3} was added to neutralize the solution. All volatiles were evaporated and the crude product was purified by column chromatography (10% MeOH in DCM). 7.31 was obtained (5.26 g, 85%) as a colorless oil. The \textsuperscript{1}H-NMR spectrum showed ~23% of a contamination at 1.34 ppm (d, \(J = 6.1\) Hz), this side product was not separated from the product before the succeeding reaction. All analytical data were in accordance to those reported.\cite{26}

(3\text{aR},4\text{R},6\text{S},7\text{S},7\text{aR})-4-(allyloxy)-2,2,6-trimethyltetrahydro-3aH-[1,3]dioxolo[4,5-c]pyran-7-ol (7.32)\cite{25,26}. To a solution of 7.31 (2.0 g, 9.8 mmol) in acetone (5 mL) was added 2,2-dimethoxypropane (4.6 mL, 38 mmol) and p-toluenesulfonic acid (40 mg, 0.23 mmol), and the reaction was stirred at rt for 2.5 h. Although TLC (10% MeOH in DCM) showed incomplete conversion, the reaction was quenched by neutralization with NaHCO\textsubscript{3}. The aqueous layer was extracted three times with EtOAc (3 x 10 mL) and the combined organic layers were dried over MgSO\textsubscript{4}, filtered and concentrated. Purification by automated column chromatography (MTBE : heptane) afforded compound 7.32 (1.37 g, 57%) as a colorless oil.

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta 5.93 – 5.86\) (m, 1H), 5.29 (dd, \(J = 17.2, 1.6\) Hz, 1H), 5.21 (dd, \(J = 10.4, 1.2\) Hz, 1H), 5.00 (s, 1H), 4.21 – 4.14 (m, 2H), 4.09 (dd, \(J = 12.8, 5.7\) Hz, 1H), 4.00 (ddt, \(J = 12.8, 6.3, 1.2\) Hz, 1H), 3.67 (dq, \(J = 9.2, 6.3\) Hz, 1H), 3.38 (ddd, \(J = 9.2, 7.2, 4.4\) Hz, 1H), 2.65 (d, \(J = 4.4\) Hz, 1H), 1.51 (s, 3H), 1.34 (s, 3H), 1.28 (d, \(J = 6.3\) Hz, 3H);

\textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}) \(\delta 133.56, 117.74, 109.40, 96.15, 78.48, 75.81, 74.39, 67.92, 65.79, 27.93, 26.09, 17.38;\) HRMS-(ESI\textsuperscript{+}) for C\textsubscript{12}H\textsubscript{26}O\textsubscript{5}Na [M + Na]\textsuperscript{+} calculated 267.1208 Da, measured 267.1203 Da.
To a solution of 7.32 (3.6 g, 14.7 mmol) in THF (30 mL), was added NaH (1.2 g, 31 mmol, 60% dispersed in mineral oil). The mixture was stirred at rt for 30 min and subsequently cooled in an ice bath. Iodomethane (1.9 mL, 31 mmol) was added and the mixture was stirred in an ice bath, allowing it to warm to rt overnight. Excess hydride was destroyed by slow addition of MeOH, and the solvents were evaporated. CHCl₃ was added, and the organic layer was washed with brine (2x) and water. The organic layer was dried over MgSO₄, filtered and concentrated. Column chromatography (EtOAc : n-heptane) afforded 7.33 (3.6 g, 95%) as a colorless oil.

**1H NMR** (400 MHz, CDCl₃) δ 5.91 – 5.83 (m, 1H), 5.27 (ddd, J = 17.2, 3.1, 1.5 Hz, 1H), 5.17 (dd, J = 10.4, 1.4 Hz, 1H), 4.97 (s, 1H), 4.18 – 4.09 (m, 3H), 3.96 (ddt, J = 12.8, 6.2, 1.2 Hz, 1H), 3.59 (dq, J = 9.9, 6.3 Hz, 1H), 3.50 (s, 3H), 3.00 – 2.89 (m, 1H), 1.52 (s, 3H), 1.33 (s, 3H), 1.25 (d, J = 6.3 Hz, 3H); **13C NMR** (101 MHz, CDCl₃) δ 133.62, 117.63, 109.02, 96.06, 83.56, 78.24, 76.00, 67.79, 64.62, 59.38, 27.98, 26.24, 17.64; **HRMS-(ESI+)** for C₁₁H₂₂O₅Na [M + Na]⁺ calculated 281.1365 Da, measured 281.1359 Da.

To a solution of 7.32 (2.5 g, 9.77 mmol) in MeOH (40 mL), was added aq. solution of H₂SO₄ (1 m, 3.8 mL) and the mixture was stirred at 70 °C for 4 h. After the solution was cooled down to rt, NaHCO₃ (s) was added, and most of the MeOH was subsequently removed under reduced pressure. The resulting aqueous layer was extracted with EtOAc (2x). The combined organic layers were washed with water, dried over MgSO₄, filtered and concentrated. Automated column chromatography (EtOAc : n-heptane) afforded 7.33a (1.3 g, 61%) as a colorless oil.

**1H NMR** (400 MHz, CDCl₃) δ 5.92 – 5.79 (m, 1H), 5.25 (d, J = 17.2 Hz, 1H), 5.16 (dd, J = 10.4, 0.5 Hz, 1H), 4.77 (s, 1H), 4.19 – 4.04 (m, 2H), 3.99 – 3.89 (m, 2H), 3.84 (d, J = 8.9 Hz, 1H), 3.69 – 3.59 (m, 1H), 3.54 (d, J = 2.2 Hz, 3H), 3.12 – 3.01 (m, 3H), 1.29 (dd, J = 6.3, 2.0 Hz, 3H); **13C NMR** (101 MHz, CDCl₃) δ 133.72, 117.25, 98.67, 83.10, 71.14, 71.05, 67.80, 67.31, 60.59, 17.81; **HRMS-(ESI+)** for C₁₀H₁₈O₇Na [M + Na]⁺ calculated 241.1052 Da, measured 241.1046 Da.

To a solution of 7.32a (1.2 g, 5.5 mmol) was dissolved in Ac₂O (5.5 mL) and pyridine (5.5 mL) and stirred at rt for 22 h. The solvent was removed...
under reduced pressure and automated column chromatography (EtOAc : n-heptane) afforded 7.34 (1.7 g, quant) as a viscous colorless oil.

1H NMR (400 MHz, CDCl3) δ 5.83 (ddd, J = 21.9, 10.7, 5.5 Hz, 1H), 5.34 – 5.01 (m, 4H), 4.67 (s, 1H), 4.11 (dd, J = 12.0, 5.5 Hz, 1H), 4.01 – 3.88 (m, 1H), 3.71 (dt, J = 15.7, 6.1 Hz, 1H), 3.43 (s, 3H), 3.16 (td, J = 10.0, 4.8 Hz, 1H), 2.08 (s, 3H), 2.00 (s, 3H), 1.29 (d, J = 6.0 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 169.95, 169.70, 133.35, 117.64, 96.30, 80.23, 71.40, 70.32, 68.01, 67.59, 60.45, 20.90, 20.83, 17.68; HRMS-(ESI+) for C14H22O7Na [M + Na]+ calculated 325.1263 Da, measured 325.1258 Da.

(2R,3R,4R,5S,6S)-2-hydroxy-5-methoxy-6-methyltetrahydro-2H-pyran-3,4-diyl diacetate (7.35): To a stirred solution of 7.34 (200 mg, 0.662 mmol) in THF (6.6 mL) was added polymethylhydrosiloxane (PMHS) (2.5 g, 1.32 mmol), Pd(PPh3)4 (23 mg, 0.02 mmol) and ZnCl2 (16.4 mg, 0.12 mmol).[25] The mixture was stirred at 50 °C overnight, after which a thick syrup had formed on the bottom of the flask, which did not dissolve in EtOAc, Et2O, DCM, MeOH and water. Therefore some syrup remained in the flask. The solvents used to try to dissolve the syrup were combined, and the aq. layer was separated and extracted with EtOAc. The combined organic layers were dried over MgSO4, filtered and concentrated. Automated column chromatography (EtOAc : n-heptane) afforded 7.35 (101 mg, 58%) as a colorless oil.

1H NMR (400 MHz, CDCl3) δ 5.31 – 5.23 (m, 2H), 5.10 (s, 1H), 3.99 (dq, J = 12.4, 6.2 Hz, 1H), 3.48 (s, 3H), 3.22 (t, J = 9.5 Hz, 1H), 2.88 (d, J = 2.8 Hz, 1H), 2.14 (s, 3H), 2.06 (s, 3H), 1.33 (d, J = 6.2 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 170.21, 170.02, 92.04, 80.22, 71.07, 70.86, 70.73, 67.61, 60.43, 20.91, 17.70; HRMS-(ESI+) for C11H18O7Na [M + Na]+ calculated 285.0950 Da, measured 285.0945 Da.

(2S,3R,4R,5S,6S)-5-methoxy-6-methyl-2-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2H-pyrano-3,4-diyl diacetate (7.36): 7.35 (410 mg, 1.56 mmol, stripped three times with toluene) was dissolved in DCM (4 mL), and 2,2,2-trichloroacetonitrile (1.1 g, 7.82 mmol) and NaH (3.75 g, 0.156 mmol) were added. After stirring the mixture for 1 h, silica was added to quench the reaction and the solvent was evaporated. Automated column chromatography (EtOAc : n-heptane) afforded 7.36 (293 mg, 46%) as a colorless viscous oil, which was stored at –20 °C.

1H NMR (400 MHz, CDCl3) δ 8.67 (s, 1H), 6.15 (d, J = 2.0 Hz, 1H), 5.44 (dd, J = 3.4, 2.1 Hz, 1H), 5.30 (dd, J = 9.7, 3.4 Hz, 1H), 3.97 (dq, J = 9.8, 6.1 Hz, 1H), 3.51 (s, 3H), 3.30 (t, J = 9.6 Hz, 1H), 2.17 (s, 3H), 2.06 (s, 3H), 1.38 (d, J = 6.2 Hz, 3H); 13C NMR (101 MHz,
CDCl$_3$ $\delta$ 169.75, 169.73, 160.13, 94.73, 90.71, 79.64, 71.05, 70.52, 68.54, 60.69, 20.89, 20.80, 17.83; HRMS-(ESI+) for C$_{13}$H$_{18}$Cl$_3$NO$_7$Na [M + Na]$^+$ calculated 428.0047 Da, measured 428.0041 Da.

$\text{(2R,3R,4R,5R,6S)-2-((S)-2-(tert-butoxycarbonylamino)proproxy)-5-methoxy-6}$-methyltetrahydro-2H-pyran-3,4-diy diacetate (7.37 + 7.38): To a solution of 7.36 (100 mg, 0.246 mmol) and 7.30 (56 mg, 0.32 mmol) in anhydrous DCM (6 mL), were added freshly activated powdered 4 Å molecular sieves (300 mg) and the mixture was stirred at rt under a N$_2$ atmosphere. After 10 min, the mixture was cooled to $-78 \, ^\circ$C, TMSOTf (13 μL, 0.074 mmol) was added, and stirring was continued for 1 h. TLC analysis (30% EtOAc in n-pentane) showed full conversion of the starting material, and the reaction was quenched by addition of Et$_3$N. The mixture was diluted with DCM and filtered. The filtrate was concentrated and the residue was purified by automated column chromatography (EtOAc : n-heptane) to afford 7.37 (72 mg, 70%) as a white solid. The $^1$H-NMR spectrum shows approx. 30% of another product at 6.67, 5.36, 4.99, 4.52, 3.62, 3.49 and 1.12 ppm. From a signal at 123.8 in the HSQC spectrum without any coupling, formation of the orthoester (7.38) as a side product was concluded.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.46 (bs, 1H), 5.22 (dd, $J = 3.3$, 1.8 Hz, 1H), 5.16 (dd, $J = 9.7$, 3.5 Hz, 1H), 4.64 (d, $J = 1.5$ Hz, 1H), 4.11 (q, $J = 7.2$ Hz, 1H), 3.81 (bs, 1H), 3.71 (dt, $J = 15.6$, 6.3 Hz, 1H), 3.47 (s, 3H), 3.40 (d, $J = 4.2$ Hz, 2H), 3.20 (td, $J = 9.5$, 6.5 Hz, 1H), 2.13 (s, 3H), 2.05 (s, 3H), 1.43 (s, 9H), 1.32 (d, $J = 6.2$ Hz, 3H), 1.16 (d, $J = 6.8$ Hz, 3H); HRMS-(ESI+) for C$_{19}$H$_{34}$NO$_9$ [M + H]$^+$ calculated 420.2234 Da, measured 420.2228 Da.

$\text{(2R,3R,4R,5S,6S)-5-methoxy-6-methyl-2-(pent-4-enyloxy)tetrahydro-2H-pyran-3,4-diy}$ diacetate (7.39). A mixture of 7.35 (200 mg, 0.763 mmol) and ρ-toluenesulfonic acid (1.7 mg, 9.9 μmol) was treated with 4-penten-1-ol (1.1 mL, 10.8 mmol) under a N$_2$ atmosphere. After stirring at 95 $^\circ$C for 45 h, TLC (30% EtOAc in pentane) indicated the presence of remaining starting material. Nevertheless, the solvent was evaporated, and the residue purified by automated column chromatography (EtOAc : n-heptane). $^1$H-NMR spectroscopy showed that the fraction with the same $R_f$-value as the starting material (of which 141 mg was isolated) did contain the pentenyl group, but that the acetyl protecting groups were cleaved. (The $^{13}$C-NMR spectrum showed some impurities at $\delta$ 127.46, 126.89, 126.41, 125.82, 32.66, 27.19, 17.94 ppm).

The obtained product (112 mg, 0.455 mmol) was re-acetylated by addition of acetic
anhydride (453 μL, 4.8 mmol) and pyridine (453 μL, 5.6 mmol) and stirred at rt for 19 h. The solvent was evaporated and automated column chromatography (EtOAc : n-heptane) finally afforded compound **7.39** (115 mg, 77%) as a white solid in 46% yield after both steps.

**1H NMR** (400 MHz, CDCl₃) δ 5.78 (ddt, J = 16.9, 10.2, 6.5, 1H), 5.60 – 5.31 (m, 1H), 5.24 – 5.16 (m, 2H), 4.99 (dd, J = 21.2, 13.7, 1H), 4.69 – 4.60 (m, 1H), 3.79 – 3.57 (m, 2H), 3.47 (s, 3H), 3.43 – 3.37 (m, 1H), 3.18 (t, J = 9.6, 1H), 2.35 – 2.21 (m, 1H), 2.12 (s, 3H), 2.04 (s, 3H), 1.73 – 1.59 (m, 2H), 1.32 (d, J = 6.2, 3H); **13C NMR** (101 MHz, CDCl₃) δ 170.08, 169.80, 137.89, 114.96, 97.36, 80.31, 71.46, 70.44, 67.48, 67.18, 60.48, 30.16, 28.46, 20.97, 20.92, 17.74; **HRMS**-(ESI+) for C₁₆H₂₆O₇Na [M + Na]+ calculated 353.1576 Da, measured 353.1571 Da.

**(2S,3R)-methyl 2-(tert-butoxycarbonylamino)-3-hydroxybutanoate (7.14):** A solution of thionyl chloride (3.7 mL, 50.5 mmol) in MeOH (10 mL) was cooled in an ice bath, and L-threonine (7.13) (2.0 g, 17.8 mmol) was added in portions. The mixture was stirred while cooling in an ice bath for 1 h and then at rt for another 47 h. Removal of the solvent under reduced pressure afforded the methyl ester of L-threonine as its HCl salt, as a colorless viscous oil.

**1H NMR** (400 MHz, CDCl₃) δ 5.37 (bs, 1H), 4.33 – 4.18 (m, 1H), 4.10 (dd, J = 14.2, 7.1 Hz, 1H), 3.75 (s, 3H), 2.51 (bs, 1H), 1.43 (s, 9H), 1.23 (d, J = 6.5 Hz, 3H).

Without further purification, Et₃N (3.2 mL, 23 mmol) was added slowly to a suspension of the obtained oil in acetonitrile (30 mL) while cooling in an ice bath under a N₂ atmosphere. Di-tert-butyl dicarbonate (3.95 g, 18.1 mmol) in acetonitrile (35 mL) was added slowly, and the resulting suspension was stirred at rt for 72 h. The solvents were removed under reduced pressure, and the residue was dissolved in EtOAc and washed with saturated aq NaHCO₃, water and brine. The organic solution was dried over MgSO₄, filtered and concentrated. Purification by automated column chromatography (EtOAc : n-heptane) afforded 7.14 (3.5 g, quant) as a white solid. Analytical data were in accordance with those reported.[30]

**(2S,5R)-3-tert-butyl 4-methyl 2-tert-butyl-5-methyloxazolidine-3,4-dicarboxylate (7.15):** To a solution of 7.14 (200 mg, 0.857 mmol) in anhydrous toluene (4.3 mL), was added freshly distilled pivaldehyde (4.3 mL, 39.8 mmol), triethylformate (1.4 mL, 8.66 mmol) and pyridinium ρ-toluenesulfonate (PPTS) (431 mg, 1.72 mmol).[22] **(NOTE:** a mistake was made for the
calculation of the amount of pivaldehyde and triethylformate needed. 4 eq of pivaldehyde and 1.5 eq of triethylformate would have been sufficient according to the literature). This emulsion was stirred at 95 °C until TLC (30% EtOAc in pentane) showed full conversion of the starting material (3.5 h). After evaporation of the solvent, the residue was purified by flash column chromatography (DCM) to afford 7.15 (147 mg, 57%) with a diastereomeric ratio of 9:1 as determined by $^1$H-NMR.

$^1$H NMR (400 MHz, CDCl$_3$) δ 5.16 (s, 1H), 4.10 (dt, $J = 12.2$, 6.0 Hz, 1H), 3.77 (s, 3H), 3.72 (d, $J = 8.6$ Hz, 1H), 1.42 (s, 9H), 1.30 (d, $J = 6.0$ Hz, 3H), 0.92 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 152.52, 96.49, 80.96, 77.54, 66.38, 52.34, 38.03, 28.10, 27.01, 26.06, 25.64, 17.49.

7.7 References
Toward the total synthesis of a sulfo-glycopeptidolipid from M. avium


