Chapter 3
Total Synthesis of Mycolipenic and Mycolipanolic acid

In this chapter the first catalytic enantioselective synthesis of mycolipenic and mycolipanolic acid is described. Iterative copper-catalyzed asymmetric conjugate addition reactions, selective olefination and an enantioselective aldol reaction are key strategic elements in the synthesis of both acids. The optical rotation and other spectroscopic data of the two synthetic acids were compared to the literature values reported for the naturally occurring acids from M. tuberculosis.*

3.1 Introduction

Mycolipenic acid (3), also known as phthienoic acid, and mycolipanolic acid (4) (Figure 2) are two methyl-branched acids found in *Mycobacterium tuberculosis* (*M. tuberculosis*). The two acids are presented in trehalose-based glycolipids called penta-acyltrehalose (PAT) and diacyltrehalose (DAT) (Figure 1). PAT and DAT are examples of cell-wall lipids from *M. tuberculosis* and are known to have biological activity.\(^1,2,3,6\)

Mycolipenic acids, the major acyl substituents found in PAT and some forms of DAT, have been shown to be potent inhibitors of leukocyte migration (immune response), *in vitro*.\(^4,6b\) It has also been shown that they act as B-cell antigens.\(^1,5,11\)

Similar to SL-I (chapter 2), PAT and DAT are exclusively found in virulent strains of *M. tuberculosis*.\(^6b\) Genetically modified *M. tuberculosis* strains that are unable to produce PAT and DAT by gene knockout showed different morphology.\(^7,8,9b\) It was found that the absence of DAT and PAT resulted in defects in capsule attachment causing the mutant to aggregate in liquid broth (cell-cell interaction). This indicates that these lipids play an important role in the build up and stability of the cell wall and are positioned at the outer layer.
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Figure 1: Penta-acyl trehalose PAT (1) and diacyl trehalose DAT (2).

Mycolipenic acid (3) and mycolipanolic acid (4) are both considered to be produced by a polyketide synthase, Pks2 (polyketide synthase 2), member of the same enzyme class involved in the synthesis of phthioceranic (Chapter 2) and hydroxyphthioceranic acid (Chapter 6). This also accounts for the stereochemical relationship of the methyl substituents, which is all-syn and of the S-configuration at the stereogenic centers, identical to phthioceranic acid and hydroxyphthioceranic acid.

In addition, the biosynthetic pathway of mycolipenic and mycolipanolic are closely related, as both are most likely derived from the same precursor. Mycolipenic acid is probably the dehydrated product of mycolipanolic acid. The dehydratase unit of Pks2 can eliminate water from mycolipanolic acid resulting in $\alpha,\beta$-unsaturated mycolipenic acid.
Olefination and enantioselective aldol reactions have been described in Chapter 2. Mycolipenic and mycolipanolic, however, have been synthesized as a racemate by Minnikin et al. in 1992. A racemic total synthesis of mycolipanolic acid was reported by Minnikin in 1996.

Since only pathogenic *M. tuberculosis* contains 3 and 4, isolating these lipids and acids from *M. tuberculosis* is undesirable. Besides the danger of working with pathogenic *M. tuberculosis*, the extremely slow growth of the bacteria (cell division every 22-24 h compared to 20 min for *E. coli*) makes it difficult to obtain sufficient quantities for biological studies. Most of the glycolipids occur as analogues, which are different in the number of acyl chains on trehalose and the number of methyl substituents and the chain length. To study the biological activity of PAT (1) and DAT (2) it is important to obtain pure and fully identified material and therefore a synthetic approach is highly desirable.

For the synthesis of both acids we anticipated the use of iterative enantioselective copper-catalyzed 1,4-addition reactions with MeMgBr, as described in Chapter 2. Mycolipenic and mycolipanolic, however, have additional functionality compared to mycocerosic and phthioceranic acid described in Chapter 2. Mycolipenic acid is an α,β-unsaturated acid with E stereochemistry. Mycolipanolic acid contains an all-syn methyl-hydroxy-methyl relationship. Olefination and enantioselective aldol reactions have

Figure 2: Structures of mycolipenic (3) and mycolipanolic acid (4).

Mycolipenic acid (3) was first isolated and characterized in the fifties by Polgar. The compound was synthesized in 1958 by a lengthy route comprising a kinetic resolution which confirmed the stereochemistry to be all S and the olefin to have the E-geometry. Mycolipenic acid has been synthesized as a racemate by Minnikin et al. in 1992.

Mycolipanolic acid (4) was isolated and characterized by degradation studies in 1968 by Polgar. A racemic total synthesis of mycolipanolic acid was reported by Minnikin in 1996.

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been investigated for the total synthesis of mycolipenic and mycolipanolic acid.

### 3.2 Reported synthetic strategies for mycolipenic and mycolipanolic acid

The first synthesis of mycolipenic acid was reported in 1958 by Polgar, starting with enantiopure pure (S)-2-methylpent-4-enoic acid (5) which was reduced in the first step to alcohol (6) with LiAlH₄ (Scheme 1). Tosylation of the alcohol followed by substitution with sodium ethyl methylmalonate resulted in the corresponding diester 7. Saponification of 7 followed by acidic decarboxylation resulted in a mixture of two diastereomers (8) which could be separated by column chromatography.

After several functional group transformation steps, iodide 9 was obtained. Reaction of the latter with methyl 3-oxohexadecanoate, followed by hydrolysis and decarboxylation, led to ketone 10. The ketone was reduced by a Clemmensen reduction and subsequent reduction of the acid yielded alcohol 11. Tosylation of the alcohol and substitution with sodium methyl ethylmalonate followed by hydrolysis and decarboxylation yielded trimethyl substituted acid 12. Bromination of the acid by a Hell-Volhard-Zelinsky reaction followed by dehydrobromination with pyridine resulted in the desired E-isomer of mycolipenic acid (13). The optical rotation matched with the natural product which was considered sufficient evidence for the S-stereochemistry of the methyl substituents and the E-stereochemistry of the double bond.
Scheme 1: First total synthesis of mycolipenic acid.

A second synthesis of mycolipenic acid was published by Minnikin (Scheme 2). In this racemic synthesis the methyl ester of mycolipenic acid is obtained in 12 synthetic steps. The same synthetic
strategy was used in 1996 for the first total synthesis of racemic mycolipanolic acid (Scheme 2).

In both syntheses dimethylcyclohexanol 14 (mixture of isomers) was first oxidized to the corresponding ketone 15, which subsequently underwent a Baeyer-Villiger reaction to seven-membered lactone 16. Ring opening by saponification resulted in hydroxy acid 17. After seven synthetic steps, including the introduction of the C-18 aliphatic chain, alcohol 18 was obtained in 8% yield. Alcohol 18 was oxidized to aldehyde 19 which was used in the synthesis of both mycolipenic and mycolipanolic acid.

In the synthesis of mycolipenic acid, aldehyde 19 was treated with the Wittig reagent Ph₃PCH(CH₃)COOEt and the ethyl ester of mycolipenic acid was obtained as a mixture of diastereomers.

Scheme 2: Racemic total synthesis of mycolipenic acid and mycolipanolic acid.

The synthesis of mycolipanolic acid was completed by an aldol reaction between 19 and the lithium enolate of methyl propionate. The product was isolated as a horrendous mixture of 16 isomers!
3.3 Strategic synthetic analysis

The synthetic analysis starts with the formation of 1,3-dimethyl bifunctional substrate $21$ (Scheme 3). The syn-dimethyl thioester $21$ can be obtained by two consecutive copper-catalyzed asymmetric 1,4-addition reactions as is described in chapter 2. The starting substrate is bifunctional substrate $20$, which can be made in three steps from glycol. After the introduction of both methyl groups, the thioester can be reduced to the corresponding alcohol. Tosylation of the alcohol followed by a Grignard coupling reaction with $C_{15}H_{31}MgBr$ for the introduction of the long aliphatic chain would result in silylether $24$.

Scheme 3: Retrosynthetic analysis of mycolipenic acid (3).
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Cleavage of the silyl-protecting group followed by mild oxidation would result in aldehyde 26, which can be used in an olefination reaction with Wittig reagent 27. This would yield the desired mycolipenic acid 3 after hydrolysis.

For the retrosynthetic analysis of mycolipanolic acid we envisioned that up to aldehyde 26, the synthesis would be identical to the synthesis of mycolipenic acid. An Evans aldol reaction of aldehyde 26 with the boron enolate of oxazolidone 28 allows us to introduce the third methyl substituent and the alcohol functionality (29) in a single step. The chiral auxiliary can be removed in the final step resulting in the desired mycolipanolic acid (4).

Scheme 4: Retrosynthetic analysis of mycolipanolic acid (4).
3.4 Results and discussion

3.4.1 Total synthesis of mycolipenic acid

We started the synthesis of mycolipenic acid with two consecutive 1,4-addition reactions for the introduction of the syn di-methyl motif. This methodology is described in chapter 2 and is based on iterative copper-catalyzed asymmetric 1,4-addition reactions with MeMgBr. The synthesis starts with bifunctional unsaturated thioester 20 (Chapter 2). \((R,S)\)-Josiphos/CuBr was used as the catalyst in combination with MeMgBr and 30 was obtained as the S-enantiomer in high yield and excellent enantiomeric excess (ee), of 95\% and 98\%, respectively. After reduction of thioester 30 followed by olefination with HWE reagent \((\text{EtO})_2\text{P(O)}\text{CH}_2\text{COSEt}\), unsaturated thioester 31 was obtained in 80\% yield over two steps. The second asymmetric 1,4-addition reaction was performed under the same conditions as the first addition. Syn-product 21 was obtained in 90\% yield with an excellent dr of 45:1. Thioester 21 was subsequently reduced to alcohol 22 with DIBAL-H in 89\% over two steps. Tosylation of alcohol 22 to 23 (85\%) followed by a copper-catalyzed Grignard cross-coupling with \(n\)-C\(_{16}\)H\(_{33}\)MgBr resulted in silyl ether 24 (95\%). Deprotection with tetrabutylammonium fluoride (TBAF) in THF resulted in alcohol 25 in 87\% yield. Alcohol 25 was oxidized to aldehyde 26 under neutral conditions with N-methylmorpholine oxide (NMO) and tetrapropylammonium perruthenate (TPAP)\(^{15}\) in 90\% yield. No epimerization of the alpha methyl stereo center was observed during this transformation as evident from extensive NMR analysis.
Scheme 5: Total synthesis of mycolipenic acid (3).

The introduction of the $\alpha,\beta$-unsaturated system was first investigated for hexanal and 2-methylbutanal with Wittig reagent 27 and the Horner-Wadsworth-Emmons (HWE) equivalent of 27, (EtO)$_2$P(O)CH(CH$_3$)$_2$COOEt. The reaction between the lithium salt of the HWE-reagent and hexanal proceeded with an E:Z selectivity of 9:1 at 50°C. However, when a more appropriate test substrate with a methyl substituent on the alpha position was used, 2-methylbutanal, the selectivity dropped to 1:1. Selectivity for the E-olefin with 2-methylbutanal could be improved to 4:1 by switching to acetonitrile as the solvent and diazobicycloundecene (DBU) as the base in combination with LiCl.\textsuperscript{16} Wittig reagent 27, however, was found to be superior in comparison to the HWE
reagent favoring the desired E-isomer in a 97 : 3 ratio in the reaction with 2-methylbutanal.

Therefore, aldehyde 26 was treated with Wittig reagent 27, which resulted in an E/Z ratio of 9:1, and the desired E-isomer 32 was isolated in 65% yield. In the final step, ethyl ester 32 was hydrolyzed in a H₂O/THF mixture with LiOH and mycolipenic acid 3 was obtained in 85% yield with an optical rotation of +16.4° (c = 1.96, CHCl₃). The optical rotation is comparable to that reported in the literature (+19°) for the natural product isolated from M. tuberculosis.¹⁷

The methyl ester of mycolipenic acid was prepared by treatment of 3 with (trimethylsilyl)diazomethane in methanol and its optical rotation (+15°, c = 0.47, CHCl₃) is comparable to the values of +14.7° and +16.8° found in the literature for the methyl ester of 3.¹⁷,¹⁸

### 3.4.2 Total synthesis of mycolpanolic acid

The synthesis of mycolpanolic acid started from aldehyde 26, described in section 3.3.1. We envisioned the use of an Evans aldol reaction using enantiopure oxazolidones as the chiral auxiliary for the introduction of the syn methylalcohol unit in mycolpanolic acid.¹⁹

Evans and co-workers developed a widely used method for the construction of the syn methyl-hydroxy motif based on the aldol reaction between an acyl oxazolidone enolate and an aldehyde.¹⁹ The chiral acyl oxazolidone enolate determines the stereochemical outcome of the aldol reaction. The chiral auxiliary can be removed after the reaction, resulting in the desired acid and the recovered chiral oxazolidone which can in principle be re-used.

The relative stereochemical (syn vs anti) outcome of the aldol reaction is dependent on the enolate geometry, Z-enolates result in the syn-product and E-enolates result in the anti-product. This relative stereochemical relation can be explained by the Zimmerman-Traxler model (Figure 3) in which the substituents are preferably in an equatorially position in the six-membered transition state thereby avoiding syn-pentane interactions.²⁰
To obtain the desired syn-product from the aldol reaction, the $Z$-boron-enolate is first formed selectively.\textsuperscript{19} The absolute configuration of the syn-product in the Evans aldol reaction is dependent on the stereochemistry of the auxiliary. Figure 4 shows that with the use of a chiral auxiliary S-acyl-oxazolidone 28, si-face attack is favored over the re-face because of sterical hinderance of the benzyl substituent on the re-face in the six-membered ring transition state.
Figure 4: Zimmerman-Traxler transition state model for the explanation of the stereoselective outcome of the Evans aldol reaction.

First we investigated the Evans aldol reaction with enantiopure oxazolidinone 28 and racemic 2-methylbutanal. Bu$_3$BOTf and triethylamine were used at $-78^\circ$ C to form the Z-enolate of 28 were upon 2-methylbutanal was added to the mixture (Scheme 6).$^{19a,b}$ The reaction mixture was brought to room temperature and was quenched with a phosphate buffer (pH = 7).

Scheme 6: Evans aldol reaction of S-28 with racemic 2-methylbutanal.

The crude $^1$H-NMR revealed the two expected syn-diastereomeric products (Figure 5, out of the possible eight, no sets of enantiomers because of the fixed stereocenter on the oxazolidine group). The observed ratio of diastereomers was approximately 2:3 as can be expected because the reaction had not reached full completion and both enantiomers of 2-methylbutanal react with different rates with S-28. By comparison with literature data, we could determine which absorptions could be assigned to which diastereomer.$^{21}$
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Figure 5: $^1$H-NMR spectrum of the two diastereomeric products of the Evans aldol reaction on racemic 2-methylbutanal.

Figure 6: $^{13}$C-APT spectrum of the two diastereomeric products of the Evans aldol reaction on racemic 2-methylbutanal.

The two diastereomers could also be clearly distinguished by $^{13}$C-NMR (Figure 6). So the aldol reaction with the Z-enolate of S-28 is highly
selective in the formation of the syn-methyl-hydroxy relationship, independent of the stereocenter present in the electrophilic aldehyde.

Next, the aldol reaction under these conditions was used on dimethyl aldehyde 26. Starting with freshly prepared aldehyde 26 by oxidation of alcohol 25 with NMO/TPAP followed by quick purification over silica to prevent over-oxidation and/or epimerization of the alpha stereocenter (Scheme 7). After work up syn-product 29 was isolated in a moderate yield of 45% with perfect stereocontrol. The expected syn-methyl-hydroxy relationship of 29 was confirmed by comparison with a closely related compound reported by Dias and Meira.\textsuperscript{21}

\[ \text{HO} \quad \text{N} \quad \text{C} \quad \text{H}_{35} \quad \text{25} \quad 87\% \quad \text{NMO} \quad \text{TPAP} \quad \text{H} \quad \text{O} \quad \text{C} \quad \text{H}_{35} \quad \text{26} \quad 90\% \quad \text{Bn} \quad \text{N} \quad \text{O} \quad \text{OH} \quad \text{29} \quad 45\% \quad \text{H}_{2}O_{2} \quad \text{LiOH} \quad \text{THF/H}_{2}O \quad \text{4} \quad 16\% \quad (90\% \text{ crude}) \quad \text{See text for details} \]

\textbf{Scheme 7: Total synthesis of mycolipanolic acid (4).}

Although a minor diastereomer was observed in the \textsuperscript{1}H- and \textsuperscript{13}C-NMR spectra (Figure 7 and Figure 8), this is most likely due to the minor diastereomer already present in 26 as a result of the two consecutive enantioselective 1,4 addition reactions (dr 45 : 1). Removal of the chiral auxiliary with \text{H}_{2}O_{2} and LiOH yielded desired mycolipanolic acid 4 in 90% crude yield. Unfortunately we lost material during chromatography and only 16% of the desired mycolipanolic acid was isolated. Later it was found that isolation over silica with pentane/ether 5/1 containing 1% of acetic acid gave perfect separation and no problems were observed during the isolation process.
**Figure 7:** $^1$H-NMR spectrum showing 29 with traces of a minor diastereomer.

**Figure 8:** APT spectrum showing 29 with traces of a minor diastereomer.

A sample of 4 was converted to the corresponding methyl ester by using excess trimethylsilyldiazomethane in MeOH to compare the optical rotation to the literature value. The optical rotation was determined to be
–7.0°, (c = 0.20, CHCl₃), which is in perfect comparison with the literature value of –7.19°.²²

3.5 Conclusions

Mycolipenic and mycolipanolic acid, two natural compounds from M. tuberculosis, have been successfully prepared. Mycolipenic acid was prepared with an overall yield of 5% in 11 steps with an average of 84% yield per step. Iterative copper-catalyzed asymmetric 1,4-addition reactions were used for the introduction of the two methyl substituents in a highly enantioselective fashion. The α,β-unsaturated acid moiety was introduced using a Wittig reaction which provided the E-isomer with high selectivity.

The closely related mycolipanolic acid was prepared enantioselectively with an overall yield of 2% in 11 steps with an average yield of 75% per step. The enantioselective Evans aldol reaction was a key step in the synthesis and allowed us to introduce the methyl-hydroxy-methyl unit in a completely stereoselective all-syn manner.

Mycolipenic and mycolipanolic acid can now be obtained via a catalytic enantioselectively route in sufficient quantities and can readily be used in the synthesis of PAT(1) and DAT(2).
3.6 Experimental

For general experimental procedures, see chapter 2; Experimental Section.

**S-Ethyl 2-(diethoxyphosphoryl)ethanethioate**

\[
\begin{align*}
\text{EtO} & \quad \text{O} \\
\text{P} & \quad \text{O} \\
\text{SeEt} & \\
\end{align*}
\]

A solution of 5.0 mL (30.4 mmol) diethylphosphonoacetic acid, 2.8 mL (30.4 mmol) ethanethiol and 371 mg (0.1 eq.) DMAP in 60 mL CH\(_2\)Cl\(_2\) under nitrogen was cooled with an ice bath. DCC (6.3 g, 30.4 mmol) was added in portions. The reaction mixture was allowed to warm up to rt overnight. The mixture was filtered through Celite which was subsequently washed with 50 mL CH\(_2\)Cl\(_2\). The organic layer was washed with aqueous sodium bicarbonate, water and brine. After the organic layer was dried with sodium sulfate, the solvent was evaporated under reduced pressure. The crude product was further purified by flash chromatography (ether : pentane 2:1) to yield a colorless oil (5.52 g, 76% yield). \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta\) ppm 4.19-4.10 (m, 4H), 3.18 (d, \(J = 7.41\) Hz, 2H), 1.34-1.29 (m, 6H), 1.24 (t, \(J = 7.43\) Hz, 3H). \(^13\)C-NMR (100.6 MHz, CDCl\(_3\)) \(\delta\) ppm 190.17 (s), 62.73 (t), 62.67 (t), 43.36 (t), 42.05 (t), 24.07 (t), 16.22 (q), 16.16 (q), 14.31 (q). \(^31\)P-NMR (400 MHz, CDCl\(_3\)) \(\delta\) ppm 19.3.

**(-)-(3R,5S)-6-(t-Butyldiphenylsilyloxy)-3,5-dimethylhexan-1-ol (22)**

\[
\begin{align*}
\text{TBDSO} & \quad \text{OH} \\
\end{align*}
\]

To a stirred solution of 21 (455 mg, 1.028 mmol) in CH\(_2\)Cl\(_2\) (5 mL) was added DIBAL-H (1.336 mL, 1.336 mmol, 1.0 M solution in CH\(_2\)Cl\(_2\)) at –20 °C under nitrogen. Stirring was continued until the reduction was complete (3-4 h). The reaction mixture was quenched with 30 mL saturated Rochelle solution (potassium sodium tartrate) and stirred for 30 min. The phases were separated and the aqueous layer was extracted with three portions of 30 mL CH\(_2\)Cl\(_2\). The combined organic phases were dried over MgSO\(_4\) and concentrated under reduced pressure to yield crude aldehyde. The above reduction/work-up procedure was repeated to yield crude alcohol 22 as a colorless oil which was purified by flash
chromatography (pentane/diethyl ether 5:1) to afford 22 as a colorless oil (348 mg, 89% yield). [α]D = −3.4° (c = 1.00, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 7.68 (dd, J = 7.8, 1.7 Hz, 4H), 7.40 (m, 6H), 3.65 (s, 2H), 3.56-3.48 (m, 1H), 3.44 (dd, J = 9.8, 6.4 Hz, 1H), 1.76 (td, J = 13.2, 6.6 Hz, 1H), 1.59 (tt, J = 12.1, 5.9 Hz, 2H), 1.46-1.18 (m, 3H), 1.07 (s, 9H), 0.95 (d, J = 6.7 Hz, 4H), 0.87 (d, J = 6.4 Hz, 3H). ¹³C NMR (100.6 MHz, CDCl₃): δ 135.60, 135.58, 133.99, 129.47, 127.54, 77.31, 77.00, 76.68, 68.74, 61.05, 41.18, 39.72, 33.07, 26.95, 26.86, 20.27, 19.28, 17.67. HRMS(El+) calculated for C₂₀H₂₂O₂Si (M – t-butyl) 327.1780, found 327.1775.

(--)-(2S,4S)-t-Butyl-diphenyl-(2,4-dimethyl-docosyloxy)-silane (24)

22 (340 mg, 0.88 mmol) was dissolved in 5 mL CH₂Cl₂ under nitrogen and the mixture cooled to 0 °C. p-Toluensulfonyl chloride (340 mg, 2 equiv.) and pyridine (0.150 mL, 2 equiv.) were added and the mixture was stirred overnight. The solvent was evaporated and the crude product was purified by flash chromatography (pentane/Et₂O 10:1) (366 mg, 80% yield). ¹H NMR (400 MHz, CDCl₃) δ ppm 7.76 (d, J = 8.3 Hz, 2H), 7.64 (dd, J = 7.8, 1.6 Hz, 4H), 7.46-7.3 (m, 6H), 7.30 (d, J = 8.3 Hz, 2H), 4.09-3.98 (m, 2H), 3.52-3.33 (m, 2H), 2.42 (s, 3H), 1.74-1.60 (m, 2H), 1.54 (s, 1H), 1.37-1.23 (m, 2H), 1.23-1.18 (m, 1H), 1.04 (s, 9H), 0.88 (dd, J = 6.8, 3.8 Hz, 3H), 0.76 (d, J = 6.6 Hz, 3H).

A crystal of iodine was added together with crushed glass to 98 mg (4.08 mmol, 6.5 equiv.) of magnesium turnings under nitrogen. The vessel was heated to vaporize the iodine. 1-Bromohexadecane (1.2 g, 4.08 mmol, 6 equiv.) in 2 mL THF was added, the mixture heated to 45 °C for 4 h and then cooled to 0 °C. Copper bromide-dimethyl sulfide complex (28 mg, 0.14 mmol, 0.2 equiv.) was added, followed by 366 mg (0.68 mmol) of 23 as a solution in THF. The mixture was stirred overnight after which it was quenched with a saturated aq. solution of NH₄Cl. The organic layer was separated and the aqueous layer was extracted with three portions of diethyl ether. The combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography to afford 24 as a colorless oil (80 mg, 94% yield). [α]D = −4.7° (c = 1.83, CHCl₃). ¹H-NMR (400 MHz, CDCl₃) δ ppm 7.67 (dd, J = 7.8, 1.6 Hz, 4H), 7.47-7.31 (m, 6H), 3.50 (dd, J = 9.8, 5.2 Hz, 1H), 3.40 (dd, J = 9.8, 6.5 Hz, 1H), 1.80-1.66 (m, 1H), 1.49-1.40 (m, 1H), 1.40-
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1.32 (m, 2H), 1.32-1.13 (m, 34H), 1.05 (s, 9H), 0.92 (d, J = 6.7 Hz, 3H), 0.88 (t, J = 6.3, 6.3 Hz, 3H), 0.81 (d, J = 6.5 Hz, 3H). $^{13}$C-NMR (100.6 MHz, CDCl$_3$) δ ppm, 135.9 (d), 134.4 (s), 129.7 (d), 127.8 (d), 69.1 (t), 41.4 (t), 37.1 (t), 33.4 (d), 32.2 (t), 30.4 (t), 30.3 (d), 30.0 (t), 29.6 (t), 27.1 (q), 23.0 (t), 20.6 (q), 19.6 (s), 18.0 (q), 14.4 (q). HRMS(EI+) calculated for $C_{36}H_{59}OSi (M – H$_2$O) 535.4335, found 535.4311.

(–)-(25,4S)-2,4-Dimethyldocosan-1-ol (25)

Silyl ether 24 (350 mg, 0.59 mmol) was dissolved in 15 mL THF under nitrogen and 0.95 mL of TBAF (0.95 mmol) was added. The progress of the reaction was followed by TLC and the mixture filtered over a silica plug after completion (3-4 h). The crude product, still containing siloxanes was used directly in the oxidation step without further purification. ([α]$_D$ = –6.6º (c = 1.40, CHCl$_3$)). $^1$H-NMR (400 MHz, CDCl$_3$) δ ppm 3.50 (dd, J = 9.8, 5.2 Hz, 1H), 3.40 (dd, J = 9.8, 6.5 Hz, 1H), 1.80-1.65 (m, 1H), 1.47-1.33 (m, 2H), 1.33-1.14 (m, 36H), 0.92 (d, J = 6.7 Hz, 3H), 0.88 (t, J = 6.8 Hz, 3H), 0.81 (dd, J = 6.5, 2.7 Hz, 3H), $^{13}$C-NMR (100.6 MHz, CDCl$_3$) δ ppm, 68.7 (t), 41.3 (t), 36.9 (t), 33.3 (d), 32.2 (t), 30.3 (t), 30.2 (d), 29.9 (t), 29.6 (t), 27.1 (t), 22.9 (t), 20.6 (q), 17.5 (q), 14.4 (q). HRMS(EI+) calculated for $C_{36}H_{59}OSi (M – H$_2$O) 336.3756, found 336.3761.

(+)-(4S,6S,E)-Ethyl 2,4,6-trimethyltetrasco-2-enoate (32)

Crude alcohol 25 (180 mg, 0.508 mmol) was dissolved in 4 mL dry CH$_2$Cl$_2$ under nitrogen in the presence of crushed molecular sieves (4 Å). 4-Methylmorpholine N-oxide 91 mg (0.67 mmol) and 11 mg (0.031 mmol) tetrapropylammonium perruthenate were added to this mixture. The reaction mixture was stirred at rt till completion of the reaction (by TLC, pentane/diethyl ether 10:1, 3-4 h). The crude product was purified by flash chromatography (pentane : diethyl ether 10:1) and aldehyde 26 was obtained as a colorless oil (105 mg, 59% yield). $^1$H-NMR (400 MHz, CDCl$_3$) δ ppm 9.58 (d, J = 2.5 Hz, 1H), 2.49-2.37 (m, 1H), 1.76-1.63 (m, 1H), 1.54-1.15 (m, 36H), 1.08 (d, J = 6.9 Hz, 3H), 0.88 (m, 6H). The aldehyde was
directly used in the Wittig reaction in order to prevent oxidation or degradation. Aldehyde 26 (105 mg 0.300 mmol) was dissolved in 3 mL CH₂Cl₂ under nitrogen. After adding 130 mg (0.360 mmol) of Wittig reagent 27 the reaction mixture was stirred for 24 h. The organic solvent was removed under reduced pressure and the crude product was purified by flash chromatography (pentane/Et₂O 40:1) to afford 32 as a colorless oil (85 mg, 65% yield, [α]D = +14.5º (c = 1.22, CHCl₃)). ¹H-NMR (300 MHz, CDCl₃) δ ppm 6.50 (d, J = 10.2 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 2.70-2.50 (m, 1H), 1.84 (s, 3H), 1.45-1.01 (m, 40H), 0.97 (d, J = 6.5 Hz, 3H), 0.85 (m, 6H), ¹³C-NMR (100.6 MHz, CDCl₃) δ ppm 168.7 (s), 148.5 (d), 126.3 (s), 60.6 (t), 44.8 (t), 37.8 (t), 32.1 (t), 31.1 (d), 30.9 (d), 30.2 (t), 29.9 (t), 29.6 (t), 27.1 (t), 22.9 (t), 20.8 (q), 19.8 (q), 14.5 (q), 14.3 (q), 12.7 (q), HRMS(EI+) calculated for C₂₉H₅₅O₂ 436.4280, found 436.4302.

(+)-(4S,6S,E)-2,4,6-Trimethyltetracos-2-enoic acid (3)

Ethyl ester 32 (85 mg, 0.20 mmol) was dissolved in 3 mL THF and 1.5 mL water. Lithium hydroxide (15 mg, 3 equiv.) was added and the reaction mixture was stirred for 72 h. The reaction mixture was acidified with 1 M aq. HCl and extracted with diethyl ether. The organic layer was dried on MgSO₄ and concentrated under reduced pressure. The crude product was purified using flash chromatography (pentane/Et₂O/AcOH 8:1:0.1) to afford 3 as a colorless semi-solid (57 mg, 70% yield, [α]D = +16.4º (c = 1.96, CHCl₃)). ¹H-NMR (400 MHz, CDCl₃) δ ppm 6.66 (d, J = 10.2 Hz, 1H), 2.70-2.56 (m, 1H), 1.85 (s, 3H), 1.40-1.10 (m, 38H), 0.99 (d, J = 6.5 Hz, 3H), 0.90-0.80 (m, 6H), ¹³C-NMR (100.6 MHz, CDCl₃) δ ppm 174.0 (s), 151.4 (d), 125.6 (s), 44.7 (t), 37.8 (t), 32.2 (t), 31.3 (d), 30.7 (t), 30.2 (d), 29.9 (t), 29.6 (t), 27.1 (t), 22.9 (t), 20.6 (t), 20.2 (q), 19.8 (q), 19.8 (t), 14.4 (t), 12.3 (q), HRMS(EI+) calculated for C₂₇H₅₀O₂ 408.3967, found 408.3964.

To compare the optical rotation to the literature, the corresponding methyl ester was prepared using (trimethylsilyl)diazomethane and 5 mg of 1 in MeOH. [α]D = +15º (c = 0.47, CHCl₃) was found compared to [α]D = +16º (c = 4.5, CHCl₃) reported in the literature.¹⁷,¹⁸

¹H-NMR (400 MHz, CDCl₃) δ ppm 6.51 (d, J = 10.3 Hz, 1H), 3.73 (s, 3H), 2.70-2.51 (m, 1H), 1.85 (s, 3H), 1.52-1.03 (m, 37H), 0.97 (d, J = 6.5 Hz, 3H,
(S)-4-Benzyl-3-propionyloxazolidin-2-one (28)

0.500 g (2.80 mmol) of (S)-4-benzylloxazolidin-2-one was dissolved in 10 mL THF under nitrogen and the solution cooled down to –78 °C. Then, 1.8 mL (1.01 equiv.) of 1.6 M BuLi was slowly added before 0.500 mL (0.270 mmol, 1.1 equiv.) propionyl chloride was added in one portion. The reaction mixture was stirred at –78 °C for another 30 min and then allowed to warm up to rt. The excess propionyl chloride was quenched with a saturated aq. NH₄Cl solution. The aqueous layer was extracted with two portions of 10 mL CH₂Cl₂ after which the combined organic layers were washed with an aq. 1 M sodium hydroxide solution and brine. The organic layer was dried over MgSO₄ and concentrated under reduced pressure. After recrystallization from EtOAc, 28 was obtained as a white solid (0.52 g, 80% yield). ¹H-NMR (400 MHz, CDCl₃) δ ppm 7.41-7.16 (m, 5H), 4.67 (ddd, J = 13.3, 6.9, 3.5 Hz, 1H), 4.18 (ddd, J = 13.3, 3.1 Hz, 2H), 3.30 (dd, J = 13.3, 3.1 Hz, 1H), 3.07-2.85 (m, 2H), 2.77 (dd, J = 13.3, 9.6 Hz, 1H), 1.20 (t, J = 6.9, 6.9 Hz, 3H). Spectral data identical to those in the literature.

(4S)-4-Benzyl-3-((2S,3R,4(R,S))-3-hydroxy-2,4-dimethylhexanoyl)oxazolidin-2-one (33)

Compound 28 (100 mg, 0.43 mmol) was dissolved in 2 mL of dry CH₂Cl₂ under nitrogen and the solution cooled to 0 °C. Dibutylboron triflate (0.46 mL 1.15 equiv.) was slowly added causing the solution to turn dark red. Upon slow addition of 78 µL (0.56 mmol, 1.3 equiv.) triethylamine the solution discolored and it was cooled down to –78 °C. Then, 48 µL (0.44 mmol, 1.05 equiv.) of (rac)-2-methylbutanal was slowly added and the reaction mixture was stirred for another 20 min before the solution was allowed to warm to 0 °C. After stirring for 1 h at 0
°C the reaction was quenched with 0.6 mL of a phosphate buffer (pH = 7) and 2 mL methanol. Then a 2 mL solution of 2:1 methanol - 30% aqueous hydrogen peroxide was slowly added. The aqueous layer was extracted with diethyl ether (3 x 5 mL). The combined organic layers were washed with a 5% aq. sodium bicarbonate solution and brine. The organic layer was dried on sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography (diethyl ether : pentane, 1:3) to yield the aldol product (two diastereomers) as a solid (59 mg, 43% yield).

**1H-NMR (400 MHz, CDCl₃)** δ ppm 7.25-7.73 (m, 3H), 7.23-7.18 (m, 2H), 4.74-4.65 (m, 1H), 4.27-4.13 (m, 2H), 4.02-3.90 (m, 1H), 3.69 (td, J = 7.2, 3.7, 3.7 Hz, 0.4H), 3.65-3.58 (dt, J = 9.1, 2.6, 2.6 , 0.6H), 3.26 (dd, J = 13.3, 3.1 Hz, 1H), 2.96 (d, J = 3.2 Hz, 0.6H), 2.79 (ddd, J = 13.3, 9.5, 2.2 Hz, 1H), 2.66 (d, J = 3.9 Hz, 0.3H), 1.87-1.74 (m, 0.7H), 1.56-1.44 (m, 1.3H), 1.25 (dd, J = 12.6, 7.0 Hz, 3 H), 1.21-1.05 (m, 1H), 0.98 (d, J = 6.6 Hz, 1H), 0.94-0.88 (m, 3H), 0.86 (d, J = 6.8 Hz, 2H).

**13C-NMR (100.6 MHz, CDCl₃)** δ ppm 178.3 (s), 177.9 (s), 153.1 (s), 135.3 (s), 129.7 (d), 129.2 (d), 127.7 (d), 75.2 (t), 75.0 (t), 66.4 (d), 55.4 (t), 40.0 (t), 39.7 (t), 38.0 (d), 37.3 (t), 37.2 (t), 25.8 (d), 25.4 (d), 15.0 (q), 14.9 (q), 11.5 (q), 11.4 (q), 11.1 (q), 9.8 (q).

(+)-(4R)-4-Benzyl-3-((2S,3R,4S,6S)-3-hydroxy-2,4,6-trimethyltetradecanoyl)dihydrofuran-2(3H)-one (29)

Crude alcohol 25 (220 mg, 0.620 mmol) was dissolved in 4 mL dry CH₂Cl₂ under a nitrogen atmosphere in the presence of crushed mol sieves (4 Å). To this mixture was added 109 mg (0.93 mmol, 1.5 equiv.) 4-methylmorpholine N-oxide and 13 mg (0.036 mmol, 6 mol%) tetrapropylammonium perruthenate. The reaction mixture was stirred at rt till it had reached full conversion as indicated by TLC (pentane : diethyl ether, 10:1, 3-4 h). The crude product was purified by flash chromatography (pentane : diethyl ether, 10:1) and aldehyde 26 was obtained in a moderate yield of 60% (128 mg). To avoid degradation, aldehyde 26 was directly used in the aldol reaction. Chiral auxiliary 28 (81 mg, 0.35 mmol) was dissolved in 1 mL of dry CH₂Cl₂ under nitrogen and cooled to 0 °C. Dibutylboron triflate (0.37 mL 1.06 equiv.) was slowly added to the solution causing the solution to turn dark red. Upon slow
addition of 48 µL (0.56 mmol, 1.3 equiv.) of triethylamine the solution discolored and was cooled down to –78 °C. Then 128 mg (0.36 mmol, 1.05 equiv.) of 26 was slowly added and the reaction was stirred for another 20 min before the solution was allowed to warm to 0 °C. After stirring for 1 h at 0 °C the reaction was quenched with 0.6 mL of a phosphate buffer (pH = 7) and 2 mL methanol. Additionally a 2 mL solution of 2:1 methanol - 30% aqueous hydrogen peroxide was slowly added. The aqueous layer was extracted with diethyl ether (3 x 5 mL). The combined organic layers were washed with a 5% aq. sodium bicarbonate solution and brine. The organic layer was dried on sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography (pentane/diethyl ether, 1:3) to give 29 as a white solid (90 mg, 43% yield, [α]D = +20º (c = 0.63, CHCl3)). 1H-NMR (400 MHz, CDCl3) δ ppm 7.37-7.28 (m, 3H), 7.27-7.23 (m, 2H), 4.71-4.64 (m, 1H), 4.24-4.13 (m, 2H), 4.12-3.99 (m, 1H), 3.70 (dt, J = 9.0, 2.5 Hz, 1H), 3.38 (dd, J = 13.4, 3.2 Hz, 1H), 2.74 (dd, J = 13.4, 9.9 Hz, 1H), 2.42 (d, J = 9.0 Hz, 1H), 1.86-1.75 (m, 1H), 1.56-1.48 (m, 1H), 1.48-1.39 (m, 1H), 1.37-1.17 (m, 33H), 1.16 (d, J = 6.8 Hz, 3H), 1.11-0.99 (m, 2H), 0.92 (d, J = 6.8 Hz, 3H), 0.89 (m, 6H). 13C-NMR (100.6 MHz, CDCl3) δ ppm 177.5 (s), 154.2 (s), 135.6 (s), 129.7 (d), 129.2 (d), 127.5 (d), 77.2 (d), 66.3 (t), 56.1 (d), 41.8 (t), 41.1 (d), 38.0 (t), 37.3 (t), 32.2 (t), 32.0 (d), 30.3 (t), 30.1 (d), 29.9 (t), 29.6 (t), 27.2 (t), 23.0 (t), 20.2 (q), 14.8 (q), 14.3 (q), 13.2 (q) (minor diastereomers: 40.9 (d), 37.9 (t), 32.5 (d), 19.8 (q), 14.9 (q)). HRMS(EI+) calculated for C37H63NO4 585.4757, found 585.4777.

(−)-(2S,3R,4S,6S)-3-Hydroxy-2,4,6-trimethyltetracosanoic acid (4)

Compound 29 (88 mg, 0.15 mmol) was dissolved in 1.4 mL of a 1:4 water/THF mixture under a nitrogen atmosphere and the solution cooled with an ice bath. To this solution was added 0.07 mL (4 equiv.) 30% aqueous hydrogen peroxide and 6 mg lithium hydroxide in 2 mL water. The solution was stirred till the reaction was complete (by TLC, pentane : diethyl ether, 3:1, 1.5-2 h) and then quenched with saturated aqueous sodium sulfite solution (3 mL). The chiral auxiliary was extracted from the mixture with CH2Cl2 before the aqueous layer was acidified to pH 3 with 6 M aq. HCl. Next the water layer was extracted with EtOAc (3 x 3 mL), the combined organic layers were dried over
MgSO₄ and concentrated under reduced pressure to yield the crude acid (60 mg, 94% yield). The crude material was purified by column chromatography (pentane/diethyl ether, 2:1). Unfortunately, only 11 mg of 4 was recovered. Finally, 4 was successfully purified by flash chromatography (pentane : diethyl ether, 5:1 containing 1% of acetic acid), and isolated as a white solid (10 mg, 16% yield, [α]D = –3.0º (c = 0.10, CHCl₃)). ¹H-NMR (400 MHz, CDCl₃) δ ppm 6.13-5.55 (br, 1H), 3.64 (dd, J = 8.60, 3.06 Hz, 1H), 2.66 (qd, J = 14.28, 7.16 Hz, 1H), 1.82-1.66 (m, 1H), 1.57-1.46 (m, 1H), 1.46-1.36 (m, 2H), 1.30-1.10 (m, 33H), 1.19 (d, J = 7.17 Hz, 3H), 1.11-1.01 (m, 2H), 0.91-0.83 (m, 9H). ¹³C-NMR (100.6 MHz, CDCl₃) δ ppm 75.4 (d), 43.4 (d), 41.6 (t), 37.1 (t), 32.2 (t), 31.8 (d), 30.3 (d), 30.0 (q), 29.9 (t), 29.6 (d), 27.2 (t), 22.9 (t), 20.3 (q), 14.3 (t), 13.1 (q). HRMS(EI+) calculated for C₂₇H₅₂O₂ (M – H₂O) 408.3967, found 408.3956.

A small sample of 4 was converted into its methyl ester using (trimethyl)diazosilane in MeOH for comparison of the optical rotation to the literature value ([α]D = –7.0º (c = 0.20, CHCl₃), lit: [α]D = –7.19º). ¹H-NMR (400 MHz, CDCl₃) δ ppm 3.71 (s, 3H), 3.63-3.55 (m, 1H), 2.70-2.58 (m, 1H), 2.34 (d, J = 6.05 Hz, 1H), 1.77-1.60 (m, 1H), 1.53-1.46 (m, 1H), 1.46-1.36 (m, 2H), 1.30-1.10 (m, 32H), 1.16 (d, J = 7.14 Hz, 3H), 1.09-0.96 (m, 2H), 0.90-0.83 (m, 9H). HRMS(EI+) calculated for C₂₈H₅₅O₃ 440.4238, found 440.4208.
Total Synthesis of Mycolipenic and Mycolipanolic acid

3.7 References


