**Supporting Information**

**Asymmetric Total Synthesis of Mycobacterial Diacyl Trehaloses Demonstrates a Role for Lipid Structure in Immunogenicity**

Mira Holzheimer, a Josephine F. Reijneveld, a, b, c, ‡ Alexandria Ramarine, b, ‡ Georgios Misiakos, a David C. Young, b Eri Ishikawa, d, e Tan-Yun Cheng, b Sho Yamasaki, d, e D. Branch Moody, b Ildiko Van Rhijn b, c, * and Adriaan J. Minnaard a, *

a Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands.

b Brigham and Women’s Hospital Division of Rheumatology, Immunology and Allergy and Harvard Medical School, Boston, MA, 02115, USA.

c Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL, Utrecht, The Netherlands.

d Department of Molecular Immunology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, 565-0871, Japan.

e Laboratory of Molecular Immunology, Immunology Frontier Research Center, Osaka University, Suita, Osaka, 565-0871, Japan.

‡ These authors contributed equally to this work.

Corresponding authors:
a.j.minnaard@rug.nl
i.vanrhijn@uu.nl

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**General methods and materials**

All reactions were performed using flame-dried glassware under N₂-atmosphere (unless specified otherwise) by Schlenk techniques, using anhydrous solvents. Reaction temperatures refer to the temperature of the heating mantle or cooling bath.

Anhydrous solvents (MTBE, CH₂Cl₂, THF, toluene) were taken from a MBraun solvent purification system (SPS-800). Other anhydrous solvents were purchased from Sigma Aldrich or Acros Organics and used without further purification. Other reagents were purchased and used without further purification.

TLC analysis was performed on silica gel 60/Kieselguhr F254, 0.25 mm (Merck). Compounds were visualized using elemental iodine followed by either Seebach stain or anis aldehyde stain.

Flash chromatography was performed using silica gel type SiliaFlash P60 (230 – 400 mesh). The eluent composition stated as v/v.

¹H and ¹³C NMR spectra were recorded on an Agilent 400 NMR spectrometer at 400 and 100.59 MHz, respectively, using CDCl₃ or CD₃OD as the solvent. Chemical shifts are reported in ppm with the solvent resonance as the internal standard (for CDCl₃: δ 7.26 ppm for ¹H, δ 77.16 ppm for ¹³C, CD₃OD δ 3.31 ppm for ¹H, δ 49.00 ppm for ¹³C). Data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, dd = double doublet, ddd = double double doublet, ddp = double double pentet, td = triple doublet, t = triplet, q = quartet, b = broad, m = multiplet), coupling constant J (Hz), and integration value.

Enantiomeric excesses were determined by chiral HPLC analysis on a Shimadzu FPLC equipped with a diode-array detector. Integration at three different wavelengths (254, 225, 190 nm) was performed and the reported enantiomeric excess is an average of the three integrations. Retention times are reported in minutes.

High resolution mass spectra (HRMS) were recorded on a Thermo Scientific LTQ Orbitrap XL mass spectrometer with electron spray ionization (ESI) in positive or negative mode.

Optical rotations were measured on a polarimeter (Schmidt+Haensch Polartronic MH8) with a 10 cm long cell (c given in g/100 mL) at ambient temperature (±20 °C).
Synthesis

*S*-ethyl (*S*-4-((*tert*-butyldiphenylsilyl)oxy)-3-methylbutanethioate (6)

\[ \text{EtS} \text{\_}_\text{\_} \text{OTBDPS} \]

A flame-dried Schlenk flask was placed under N₂-atmosphere and charged with (*R,S*-*Josiphos*-CuBr-complex (78.4 mg, 0.1 mmol, 0.01 eq) under N₂-flow and dissolved in 60 mL dry MTBE. The resulting solution was cooled to −75 °C. MeMgBr (3 M in Et₂O, 4 mL, 12 mmol, 1.2 eq) was added dropwise over 5 min. The resulting mixture was stirred for 30 min. at −75 °C. Then *S*-ethyl (*E*)-4-((*tert*-butyldiphenylsilyl)oxy)but-2-enethioate 5 (3.85 g, 10.0 mmol, 1 eq) was dissolved in 15 mL dry MTBE and added dropwise over 2 h using a syringe pump. The reaction mixture was stirred for additional 20 h at −75 °C. The reaction was quenched by addition of 10 mL MeOH at −75 °C, the cooling bath was then removed, 10 mL sat. aq. NH₄Cl was added and the mixture was allowed to come to room temperature. The phases were separated and the aqueous layer extracted with Et₂O (3 x 30 mL) and the combined organic phases were washed with water (50 mL) and brine (50 mL), dried over MgSO₄ and concentrated. The crude was purified by flash chromatography (pentane/Et₂O 75/1) to give the product 6 (3.259 g, 8.13 mmol, 81% yield, 98% ee) as colorless oil.

**1H NMR (400 MHz, Chloroform-\(\text{d}\)) \(\delta\) 7.71 – 7.63 (m, 4H), 7.47 – 7.35 (m, 6H), 3.56 (ddd, \(J = 9.9, 5.2, 1.0\) Hz, 1H), 3.47 (ddd, \(J = 9.9, 6.3, 1.0\) Hz, 1H), 2.93 – 2.79 (m, 3H), 2.38 (ddd, \(J = 14.5, 8.4, 1.0\) Hz, 1H), 2.29 (dqd, \(J = 8.2, 6.5, 5.3\) Hz, 1H), 1.25 (td, \(J = 7.4, 0.8\) Hz, 3H), 1.07 (d, \(J = 1.1\) Hz, 9H), 0.96 (dd, \(J = 6.6, 0.9\) Hz, 3H).

**13C NMR (101 MHz, Chloroform-\(\text{d}\)) \(\delta\) 199.23, 135.73, 133.80, 133.77, 129.75, 127.78, 68.06, 47.91, 33.92, 27.00, 23.43, 19.45, 16.57, 14.94.

HRMS (ESI+) calcd. for [M+H+] 401.1965; found 401.1957.

Optical Rotation: \([\alpha]_{D}^{23} = -7.4^\circ\) (c = 0.283, CHCl₃).

The analytical data is in agreement with previous reports.¹

**Determination of enantiomeric excess:** ²

A flame-dried Schlenk flask was placed under N₂-atmosphere and charged with thioester 6 (60.1 mg, 0.15 mmol, 1 eq) dissolved in 2.5 mL dry THF. The solution was cooled to 0 °C and LiAlH₄ (1 M in THF, 0.45 mL, 0.45 mmol, 3 eq) was added.
The reaction mixture was allowed to warm to room temperature and stirred for additional 24 h. The solution was then cooled again to 0 °C and quenched by addition of 1 mL water followed by 1 mL 1 M aq. NaOH. The aqueous phase was extracted with EtOAc (3 x 10 mL) and the combined organic extracts were washed with brine, dried over MgSO₄ and concentrated. The crude diol was then transferred into a flask, placed under N₂-atmosphere (3 x evacuation and N₂ backfilling) and dissolved in 3 mL dry pyridine followed by addition of benzoyl chloride (0.06 mL, 0.525 mmol, 3.5 eq). The resulting mixture was refluxed for 15 h. After cooling to room temperature the reaction mixture was concentrated (repeated co-evaporation with toluene) to afford the crude product, which was purified by flash chromatography (pentane/Et₂O 50/3).

The enantiomeric excess was determined by chiral HPLC:

Chiracel OD-H column, n-heptane : i-PrOH = 95:5, 40 °C, flow = 0.5 mL/min, UV detection at 190 nm, 220 nm, 254 nm, retention times for racemate (min): 12.5 (major) and 13.4 (minor).
Racemate:

PDA Ch1 254nm

| Peak# | Ret. Time | Area  | Height | Area% |
|-------|-----------|-------|--------|-------|
| 1     | 12,498    | 934477| 61443  | 50,134|
| 2     | 13,424    | 929499| 56496  | 49,866|
| Total |           | 1863975| 117939| 100,000|

PDA Ch2 220nm

| Peak# | Ret. Time | Area  | Height | Area% |
|-------|-----------|-------|--------|-------|
| 1     | 12,498    | 13666330| 911435| 50,045|
| 2     | 13,424    | 13661621| 837892| 49,955|
| Total |           | 27347951| 1749326| 100,000|

PDA CH3 190nm

| Peak# | Ret. Time | Area  | Height | Area% |
|-------|-----------|-------|--------|-------|
| 1     | 12,496    | 26707288| 2299919| 50,582|
| 2     | 13,424    | 28046596| 2129243| 49,418|
| Total |           | 56753884| 4429162| 100,000|
Enantiomerically enriched product

**PDA Ch1 254nm**

| Peak# | Ret. Time | Area   | Height | Area% |
|-------|-----------|--------|--------|-------|
| 1     | 12.495    | 814008 | 53227  | 98.701|
| 2     | 13.421    | 10715  | 749    | 1.299 |
| Total |           | 824724 | 53977  | 100.000|

**PDA Ch2 220nm**

| Peak# | Ret. Time | Area   | Height | Area% |
|-------|-----------|--------|--------|-------|
| 1     | 12.495    | 1181513| 765394 | 98.695|
| 2     | 13.428    | 156203 | 10497  | 1.305 |
| Total |           | 1196716| 795891 | 100.000|

**PDA Ch3 190nm**

| Peak# | Ret. Time | Area | Height | Area% |
|-------|-----------|------|--------|-------|
| 1     | 12.499    | 24348956| 2076825| 99.482|
| 2     | 13.437    | 126802 | 9721   | 0.518 |
| Total |           | 24475798| 2086548| 100.000|
(S)-4-((tert-butyldiphenylsilyl)oxy)-3-methylbutanal (S1)

A flame-dried Schlenk flask was placed under N₂-atmosphere and charged with thioester 6 (8.96 g, 22.35 mmol, 1 eq) dissolved in 125 mL dry CH₂Cl₂. The solution was cooled to -65 °C. Diisobutylaluminium hydride (1 M in CH₂Cl₂, 27.0 mL, 27.0 mmol, 1.2 eq) was added slowly over ~15 min. The resulting mixture was stirred for another 2 h at -65 °C and then quenched by addition of 75 mL saturated Rochelle’s salt. The cooling bath was removed and the reaction mixture was allowed to come to room temperature and stirred until complete phase separation. The aqueous layer was extracted with Et₂O (3 x 75 mL). The combined organic extracts were washed with brine (100 mL), dried over MgSO₄ and concentrated. The crude product S₁ (7.637 g) was obtained as yellow oil, which was used in the next step without further purification.

¹H NMR (400 MHz, Chloroform-d) δ 9.79 (s, 1H), 7.65 (dt, \( J = 7.8, 1.9 \) Hz, 4H), 7.47 – 7.36 (m, 6H), 3.59 (dd, \( J = 10.5, 3.6 \) Hz, 1H), 3.44 (dd, \( J = 9.0, 7.1 \) Hz, 1H), 2.61 (ddd, \( J = 15.7, 5.5, 2.9 \) Hz, 1H), 2.39 – 2.23 (m, 2H), 1.05 (s, 9H), 0.95 (d, \( J = 6.7 \) Hz, 3H).

¹³C NMR (101 MHz, Chloroform-d) δ 202.83, 135.74, 135.72, 133.63, 133.61, 129.84, 127.84, 68.51, 48.29, 31.42, 26.96, 19.39, 16.91.

HRMS (ESI+) calcd. for [M+H⁺] 341.1931; found 341.1925.

The analytical data is in agreement with previous reports.¹

S-ethyl (S,E)-6-((tert-butyldiphenylsilyl)oxy)-5-methylhex-2-enethioate (7)

A flame-dried Schlenk flask was placed under N₂-atmosphere and charged with crude aldehyde S₁ (8.60 g, 35.79 mmol, 1.6 eq) dissolved in 150 mL dry THF and cooled to 0 °C. Then n-BuLi (1.6M in hexanes, 18.5 mL, 29.6 mmol, 1.3 eq) was added dropwise and the resulting mixture was stirred for additional 30 min. at 0 °C. Crude (S)-4-((tert-butyldiphenylsilyl)oxy)-3-methylbutanal (7.612 g) dissolved in 60 mL dry THF was slowly added over 10 min. at 0 °C. After complete addition, the ice bath was removed and the reaction mixture was stirred at room temperature for additional 2.5 h. Then the solution was cooled to 0 °C and quenched by addition of 85 mL sat. aq. NH₄Cl. The layers were separated and the aqueous layer was extracted
with Et₂O (3 x 80 mL). The combined organic layers were washed with brine (150 mL), dried over MgSO₄ and concentrated. The crude was purified by flash chromatography (pentane/Et₂O 99/1) to give the product 7 (8.95 g, 20.97 mmol, 94% yield over 2 steps) as colourless oil.

\[ \text{1H NMR (400 MHz, Chloroform-}d\text{)} \delta 7.70 - 7.63 (m, 4H), 7.48 - 7.35 (m, 6H), 6.88 (dt, J = 15.0, 7.5 Hz, 1H), 6.13 (d, J = 15.5 Hz, 1H), 3.54 (dd, J = 10.0, 5.4 Hz, 1H), 3.47 (dd, J = 10.0, 6.4 Hz, 1H), 2.96 (q, J = 7.4 Hz, 2H), 2.49 - 2.41 (m, 1H), 2.06 (dtt, J = 14.2, 7.8, 1.1 Hz, 1H), 1.94 - 1.81 (m, 1H), 1.30 (t, J = 7.4 Hz, 3H), 1.07 (s, 9H), 0.92 (d, J = 6.8 Hz, 3H).

\[ \text{13C NMR (101 MHz, Chloroform-}d\text{)} \delta 190.08, 144.02, 135.72, 133.70, 133.34, 133.81, 130.09, 129.75, 127.79, 68.21, 36.12, 35.57, 27.01, 23.18, 19.44, 16.62, 14.98.

HRMS (ESI+) calcd. for [M+Na+] 449.1941; found 449.1931.

Optical Rotation: \([\alpha]^{23}_D = -5.7^\circ (c = 0.070, \text{CHCl}_3)\).

The analytical data is in agreement with previous reports.¹

\[ \text{S-ethyl (3S,5S)-6-((} \text{tert-butyldiphenylsilyl})oxy)-3,5-dimethylhexanethioate (8)\]

A flame-dried Schlenk flask was placed under N₂-atmosphere and charged with (R,S)-Josiphos-CuBr-complex (498.7 mg, 0.636 mmol, 0.03 eq) dissolved in 150 mL dry MTBE and the solution cooled to –78 °C. MeMgBr (3 M in Et₂O, 8.5 mL, 25.5 mmol, 1.3 eq) was added dropwise over ~5 min. and the resulting mixture was stirred for 30 min. Then thioester 7 (8.52 g, 19.97 mmol, 1 eq) dissolved in 50 mL dry MTBE was added –78 °C over 3 h using a syringe pump. The mixture was then stirred for additional 16 h at –78 °C and then quenched by addition of 35 mL MeOH followed by addition of 120 mL sat. aq. NH₄Cl. The reaction mixture was allowed to come to room temperature. The layers were separated and the aqueous layer was extracted with Et₂O (3 x 100 mL) and the combined organic layers were washed with brine (200 mL), dried over MgSO₄ and concentrated. The crude orange oil was purified by flash chromatography (pentane/Et₂O 98.5/1.5) to afford the product 8 (8.074 g, 18.237 mmol, 92% yield) as colorless oil.

The diastereomeric ratio was determined by NMR:

\[ \text{syn (2.51 ppm) : anti (2.36 ppm)} = 1 : 0.05; \text{dr} = 95/5\]

\[ \text{1H NMR (400 MHz, Chloroform-}d\text{)} \delta 7.73 - 7.61 (m, 4H), 7.48 - 7.32 (m, 6H), 3.50 (dd, J = 9.8, 5.5 Hz, 1H), 3.42 (dd, J = 9.8, 6.3 Hz, 1H), 2.86 (q, J = 7.4 Hz, 2H), 2.51
(dd, J = 14.3, 5.0 Hz, 1H), 2.24 (dd, J = 14.3, 8.8 Hz, 1H), 2.16 – 2.00 (m, 1H), 1.79 – 1.63 (m, J = 6.5 Hz, 1H), 1.40 (dt, J = 13.7, 6.8 Hz, 1H), 1.24 (t, J = 7.4 Hz, 3H), 1.06 (s, 9H), 1.00 (dt, J = 14.0, 7.3 Hz, 1H), 0.94 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H).

$^{13}$C NMR (101 MHz, Chloroform- $d$) δ 199.32, 135.77, 134.11, 134.10, 129.66, 127.73, 68.89, 51.35, 40.95, 33.32, 28.85, 27.04, 23.41, 20.44, 19.45, 17.57, 14.95.

HRMS (ESI$^+$) calcd. for [M+Na$^+$] 465.2254; found 465.2243.

Optical Rotation: $[\alpha]_D^{23} = -7.2^\circ$ (c = 0.223, CHCl$_3$).

The analytical data is in agreement with previous reports.$^1$

$^{(3R,5S)}$-6-((tert-butyldiphenylsilyl)oxy)-3,5-dimethylhexan-1-ol (S2)

A flame-dried Schlenk flask was placed under N$_2$-atmosphere and charged with thioester 8 (3.925 g, 8.867 mmol, 1 eq) dissolved in 50 mL dry CH$_2$Cl$_2$. The solution was cooled to –65 °C. Diisobutylaluminium hydride (1 M in CH$_2$Cl$_2$, 12.0 mL, 12.0 mmol, 1.4 eq) was added slowly over ~2 min. The reaction mixture was then stirred for additional 1.5 h at –65 °C and quenched with 17 mL sat. Rochelle salt. The cooling bath was removed and the reaction mixture was allowed to come to room temperature. After stirring at room temperature until complete phase separation, the layers were separated and the aqueous phase was extracted with Et$_2$O (3 x 30 mL). The combined organic layers were washed with brine (50 mL) and concentrated to afford the crude aldehyde (3.417 g) as yellow oil.

$^1$H NMR (400 MHz, Chloroform- $d$) δ 9.73 (s, 1H), 7.72 – 7.63 (m, 4H), 7.49 – 7.34 (m, 6H), 3.53 (dd, J = 9.8, 5.5 Hz, 1H), 3.47 (dd, J = 9.9, 6.1 Hz, 1H), 2.40 – 2.30 (m, 1H), 2.20 – 2.04 (m, 2H), 1.72 (h, J = 5.7 Hz, 1H), 1.48 – 1.39 (m, 1H), 1.08 (s, 9H), 1.05 – 1.00 (m, 1H), 0.97 – 0.92 (m, 6H).

$^{13}$C NMR (101 MHz, Chloroform- $d$) δ 203.06, 135.76, 135.74, 134.06, 129.71, 127.75, 127.74, 68.79, 51.03, 41.09, 33.31, 27.04, 25.92, 20.81, 19.44, 17.60.

HRMS (ESI$^+$) calcd. for [M+H$^+$] 383.2401; found 383.2394.

The crude aldehyde was subjected to another round of diisobutylaluminium hydride reduction (same conditions as described above). The crude alcohol was purified by flash chromatography (pentane/Et$_2$O 95/5) to give the product S2 (3.362 g, 8.741 mmol, 98% yield over 2 steps) as colorless oil.
\begin{align*}
\text{\textsuperscript{1}H NMR} \ (400 \text{ MHz, Chloroform-}\delta) & \ \delta \ 7.71 - 7.63 \text{ (m, 4H), 7.47 - 7.33 \text{ (m, 6H), 3.73 - 3.56 \text{ (m, 2H), 3.51 (dd, } J = 9.8, 5.3 \text{ Hz, 1H), 3.43 (dd, } J = 9.8, 6.4 \text{ Hz, 1H), 1.75 (dh, } J = 13.3, 6.6 \text{ Hz, 1H), 1.66 - 1.49 \text{ (m, 2H), 1.39 (dt, } J = 13.4, 6.6 \text{ Hz, 1H), 1.33 - 1.25 \text{ (m, 2H), 1.06 (s, 9H), 0.94 (d, } J = 6.7 \text{ Hz, 3H), 0.86 (d, } J = 6.5 \text{ Hz, 3H).} \\
\text{\textsuperscript{13}C NMR} \ (101 \text{ MHz, Chloroform-}\delta) & \ \delta \ 135.79, 135.77, 134.21, 129.65, 127.72, 68.95, 61.28, 41.38, 39.96, 33.29, 27.17, 27.05, 20.46, 19.47, 17.85. \\
\text{HRMS (ESI\textsuperscript{+}) calcd. for [M+H\textsuperscript{+}] 385.2557; found 385.2551.} \\
\text{Optical Rotation: } \ [\alpha]_{D}^{23} = -3.1^\circ \ (c = 0.065, \text{ CHCl}_3). \\
\text{The analytical data is in agreement with previous reports.}^1 \\
(3R,5S)-6-((\text{tert-butyl}diphenylsilyl)oxy)-3,5-\text{dimethylhexyl 4-methylbenzenesulfonate (9)} \\
\begin{center}
\begin{tikzpicture}
\node[above] at (0,0) {TsO}; \\
\node[below] at (0,-1) {OTBDPS};
\end{tikzpicture}
\end{center}
\text{A flask was charged with alcohol S2 (975 mg, 2.53 mmol, 1 eq) dissolved in 2.5 mL CHCl}_3. \text{ The solution was cooled to 0 \textdegree C and pyridine (0.41 mL, 5.36 mmol, 2.12 eq) was added. After stirring for 15 min at 0 \textdegree C, TsCl (724 mg, 3.80 mmol, 1.5 eq) was added as a solid in small portions over 10 min. After complete addition, the ice bath was removed and the resulting solution was stirred for additional 23 h at room temperature. The solvent was then evaporated and the crude product was purified by flash chromatography (pentane/Et}_2\text{O 95/5). The product 9 (1.229 g, 2.282 mmol, 90\% yield) was obtained as colorless oil.} \\
\text{\textsuperscript{1}H NMR} \ (400 \text{ MHz, Chloroform-}\delta) & \ \delta \ 7.77 \text{ (d, } J = 8.2 \text{ Hz, 2H), 7.69 - 7.61 \text{ (m, 4H), 7.46 - 7.34 \text{ (m, 6H), 7.30 (d, } J = 8.0 \text{ Hz, 2H), 4.09 - 3.98 \text{ (m, 2H), 3.46 (dd, } J = 9.8, 5.4 \text{ Hz, 1H), 3.37 (dd, } J = 9.8, 6.4 \text{ Hz, 1H), 2.43 (s, 3H), 1.73 - 1.61 \text{ (m, 2H), 1.60 - 1.49 \text{ (m, 1H), 1.38 - 1.24 \text{ (m, 2H), 1.04 (s, 9H), 0.95 - 0.86 \text{ (m, 4H), 0.77 (d, } J = 6.6 \text{ Hz, 3H).} \\
\text{\textsuperscript{13}C NMR} \ (101 \text{ MHz, Chloroform-}\delta) & \ \delta \ 144.72, 135.75, 135.74, 134.10, 134.08, 133.43, 129.92, 129.69, 128.00, 127.76, 127.74, 69.17, 68.81, 41.08, 35.68, 33.11, 27.02, 26.94, 21.75, 19.90, 19.42, 17.68. \\
\text{HRMS (ESI\textsuperscript{+}) calcd. for [M+H\textsuperscript{+}] 539.2646; found 539.2633.} \\
\text{The analytical data is in agreement with previous reports.}^1
\end{align*}
A flame-dried Schlenk flask was placed under N$_2$-atmosphere and charged with freshly ground Mg turnings (1.413 g, 58.144 mmol, 6.7 eq), a crystal of I$_2$ and some crushed glass under N$_2$-flow. The flask was heated using a heatgun to evaporate the I$_2$. Then 75 mL dry THF was added followed by slow addition of 1-bromohexadecane (16.0 mL, 52.35 mmol, 6 eq) over 5 min. The resulting mixture was stirred for additional 30 min. (a heterogeneous slurry formed) and then cooled to 0 °C. CuBr·SMe$_2$ (906 mg, 4.41 mmol, 0.5 eq) was added as solid under N$_2$-flow and the suspension was stirred for 10 min. Then tosylate 9 (4.70 g, 8.72 mmol, 1 eq) was added dissolved in 40 mL dry THF. The ice bath was removed and the reaction was stirred for 16 h at room temperature, then cooled again to 0 °C and quenched by addition of 40 mL sat. aq. NH$_4$Cl. The reaction mixture was allowed to warm to room temperature and then diluted with water (20 mL). The layers were separated and the aqueous phase was extracted with Et$_2$O (3 x 50 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL), dried over MgSO$_4$ and concentrated. The crude was purified by flash chromatography (pentane 100%) to afford the product 10 (4.974 g, 8.387 mmol, 96% yield) as colorless oil.

$^1$H NMR (400 MHz, Chloroform-d) δ 7.71 – 7.64 (m, 4H), 7.45 – 7.34 (m, 6H), 3.51 (dd, $J = 9.7, 5.2$ Hz, 1H), 3.41 (dd, $J = 9.7, 6.5$ Hz, 1H), 1.73 (dq, $J = 12.9, 6.5$ Hz, 1H), 1.47 – 1.40 (m, 1H), 1.40 – 1.32 (m, 2H), 1.26 (s, 33H), 1.06 (s, 9H), 1.04 – 0.98 (m, 1H), 0.93 (d, $J = 6.7$ Hz, 3H), 0.89 (t, $J = 6.7$ Hz, 3H), 0.81 (d, $J = 6.3$ Hz, 3H).

$^{13}$C NMR (101 MHz, Chloroform-d) δ 135.79, 134.31, 129.60, 127.69, 69.09, 41.31, 37.04, 33.36, 32.10, 30.24, 30.23, 29.92, 29.88, 29.83, 29.53, 27.05, 27.01, 22.86, 20.47, 19.48, 17.93, 14.29.

HRMS (ESI+) calcd. for [M+H$^+$] 593.5112; found 593.5097.

Optical Rotation: $[\alpha]_{D}^{23} = -4.5^\circ$ (c = 0.221, CHCl$_3$).

The analytical data is in agreement with previous reports.$^1$

(2S,4S)-2,4-dimethyldocosan-1-ol (S3)
Silylether 10 (5.616 g, 9.47 mmol, 1 eq) was dissolved in 260 mL THF. To the stirred solution TBAF (1 M in THF, 15.5 mL, 15.5 mmol, 1.6 eq) was added dropwise at room temperature. After 17 h stirring at room temperature, the solvent was evaporated and the crude product was purified by flash chromatography (pentane/Et2O 95/5). The product S3 (4.237 g, 9.366 mmol, 99% yield) was obtained as colourless waxy solid in 78.4% purity by weight containing 21.7 mol% siloxanes (MW 494.825).

1H NMR (400 MHz, Chloroform-d) δ 3.52 (dd, J = 10.5, 5.1 Hz, 1H), 3.38 (dd, J = 10.5, 6.8 Hz, 1H), 1.73 (hept, J = 6.6 Hz, 1H), 1.53 – 1.44 (m, 1H), 1.26 (d, J = 1.9 Hz, 35H), 1.05 – 0.99 (m, 1H), 0.92 (dd, J = 6.7, 1.7 Hz, 3H), 0.88 (td, J = 6.7, 1.7 Hz, 6H).

13C NMR (101 MHz, Chloroform-d) δ 68.58, 41.22, 36.83, 33.27, 32.09, 30.21, 30.19, 29.88, 29.86, 29.82, 29.52, 27.04, 22.85, 20.52, 17.45, 14.28.

HRMS (ESI+) calcd. for [M+H+]·H2O 337.3829; found 337.3827.

Optical Rotation: [α]D23 = -7.3° (c = 0.110, CHCl3).

The analytical data is in agreement with previous reports.1

(2S,4S)-2,4-dimethyldocosanal (11)

A flask was placed under N2-atmosphere (3 x evacuation and N2 backfilling) and charged with alcohol S3 (4.237 g, 9.36 mmol, 78% purity by wt., 1 eq) and Dess-Martin Periodinane (5.174 g, 12.20 mmol, 1.3 eq) under N2 flow. 100 mL dry CH2Cl2 was added and the resulting mixture was stirred for 3.5 h at room temperature. The reaction mixture was concentrated, the heterogeneous crude was suspended in pentane and filtered over a glass filter. The filtrate was concentrated to afford the product 11 (3.704 g, 9.20 mmol, 88% purity by wt., 98% yield) as colourless waxy solid.

1H NMR (400 MHz, Chloroform-d) δ 9.58 (s, 1H), 2.50 – 2.38 (m, 1H), 1.71 (dt, J = 12.4, 6.2 Hz, 1H), 1.54 – 1.46 (m, 1H), 1.26 (s, 35H), 1.17 – 1.11 (m, 1H), 1.08 (dd, J = 6.9, 1.6 Hz, 3H), 0.92 – 0.86 (m, 6H).

13C NMR (101 MHz, Chloroform-d) δ 205.55, 44.30, 38.46, 36.90, 32.08, 30.54, 30.07, 29.85, 29.83, 29.82, 29.52, 26.96, 22.84, 19.97, 14.30, 14.25.
HRMS (ESI+) calcd. for [M+H+] 353.3778; found 353.3777.

The analytical data is in agreement with previous reports.¹
To a stirred solution of aldehyde **11** (1.85 g, 4.59 mmol, 1 eq, 88% purity by wt.) and 2-methyl-2-butene (4.8 mL, 45.38 mmol, 10 eq) in 30 mL t-BuOH was added a solution of NaClO₂ (4.11 g, 45.41 mmol, 10 eq) and NaH₂PO₄·H₂O (2.49 g, 18.04 mmol, 4 eq) in 12 mL water. The reaction mixture was left stirring for 16 h at room temperature. The organic solvents were evaporated and the aqueous residue was extracted with CH₂Cl₂ (3 x 15 mL), washed with brine (20 mL), dried over MgSO₄ and concentrated. The crude product was purified by flash chromatography (pentane/Et₂O/AcOH 90/10/1) to afford mycosanoic acid **2** (1.549 g, 4.202 mmol, 91% yield) as pale yellow waxy solid.

**1H NMR (400 MHz, Chloroform-d)** δ 2.57 (dt, \( J = 8.7, 6.5 \) Hz, 1H), 1.73 (ddd, \( J = 14.0, 8.9, 5.5 \) Hz, 1H), 1.54 – 1.41 (m, 1H), 1.26 (s, 33H), 1.18 (d, \( J = 6.9 \) Hz, 3H), 1.12 (ddd, \( J = 11.6, 7.9, 4.6 \) Hz, 2H), 0.94 – 0.83 (m, 6H).

**13C NMR (101 MHz, Chloroform-d)** δ 184.01, 41.41, 37.51, 37.18, 32.12, 30.87, 30.13, 29.90, 29.88, 29.85, 29.56, 26.94, 22.87, 19.73, 17.94, 14.28.

HRMS (ESI-) calculated for [M-H⁻] 367.3571; found 367.3582.

Optical Rotation: \([α]_{D}^{25} = +4.5^° \) (c = 1.1, CHCl₃).

**A flame-dried Schlenk flask was placed under N₂-atmosphere and charged with solid (S)-4-benzyloxazolidin-2-one (1.24 g, 7.00 mmol, 1 eq) under N₂ flow. The flask was again subjected to three cycles of evacuation and N₂ purging. Then 25 mL dry THF was added and the resulting solution was cooled to –78 °C. n-BuLi (2.5M in hexanes, 2.83 mL, 7.07 mmol, 1.01 eq) was added dropwise and the solution was stirred for 30 min at –78 °C. Then neat propionyl chloride (0.67 mL, 7.7 mmol, 1.1 eq) was added and the reaction mixture was stirred for 1 h at –78 °C, then allowed to warm to room temperature and then quenched by addition of sat. aq. NH₄Cl (25 mL) and extracted with CH₂Cl₂ (3 x 15 mL). The organic solvents were evaporated and the resulting residue was purified by flash chromatography (pentane/Et₂O/CH₃CO₂H 90/10/1) to afford the desired product (S)-4-benzyl-3-propionyloxazolidin-2-one (S4).**

**A flame-dried Schlenk flask was placed under N₂-atmosphere and charged with solid (S)-4-benzyloxazolidin-2-one (1.24 g, 7.00 mmol, 1 eq) under N₂ flow. The flask was again subjected to three cycles of evacuation and N₂ purging. Then 25 mL dry THF was added and the resulting solution was cooled to –78 °C. n-BuLi (2.5M in hexanes, 2.83 mL, 7.07 mmol, 1.01 eq) was added dropwise and the solution was stirred for 30 min at –78 °C. Then neat propionyl chloride (0.67 mL, 7.7 mmol, 1.1 eq) was added and the reaction mixture was stirred for 1 h at –78 °C, then allowed to warm to room temperature and then quenched by addition of sat. aq. NH₄Cl (25 mL) and extracted with CH₂Cl₂ (3 x 15 mL). The organic solvents were evaporated and the resulting residue was purified by flash chromatography (pentane/Et₂O/CH₃CO₂H 90/10/1) to afford the desired product (S)-4-benzyl-3-propionyloxazolidin-2-one (S4).**
mL). The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 20 mL), the combined organic layers were washed with 1 M aq. NaOH (100 mL) and brine, dried over MgSO₄ and concentrated. The crude was purified by flash chromatography (pentane/EtOAc 9/1) to give the product S₄ (1.621 g, 6.95 mmol, 99%) as colourless oil which solidified upon standing.

¹H NMR (400 MHz, Chloroform-d) δ 7.36 – 7.23 (m, 3H), 7.22 – 7.17 (m, 2H), 4.66 (ddt, J = 10.4, 7.0, 3.5 Hz, 1H), 4.23 – 4.13 (m, 2H), 3.29 (dd, J = 13.4, 3.3 Hz, 1H), 3.05 – 2.84 (m, 2H), 2.77 (dd, J = 13.4, 9.6 Hz, 1H), 1.19 (t, J = 7.3 Hz, 3H).

¹³C NMR (101 MHz, Chloroform-d) δ 174.13, 153.58, 135.42, 129.49, 129.01, 127.40, 66.29, 55.23, 37.99, 29.27, 8.38.

Optical Rotation: [α]D²³ = +61.9° (c = 2.0, CHCl₃).

The analytical data is in agreement with previous reports.³

(S)-4-benzyl-3-[[2S,3R,4S,6S)-3-hydroxy-2,4,6-trimethyltetrasosanoyl]oxazolidin-2-one (13)

A flame-dried Schlenk flask was placed under N₂-atmosphere and charged with chiral auxiliary S₄ (47.3 mg, 0.203 mmol, 1 eq) under N₂ flow and dissolved in 0.8 mL dry CH₂Cl₂. The solution was cooled to 0 °C. Then Bu₂BOTf (1 M in CH₂Cl₂, 0.24 mL, 0.24 mmol, 1.18 eq) was added dropwise over 3 min. (the colorless solution turned orange/brown). After stirring for 5 min., dry Et₃N (37 µL, 0.264 mmol, 1.3 eq) was added. Upon addition, the solution turned colorless again, was stirred for another 45 min. at 0 °C and then cooled down to −78 °C in an acetone/liquid N₂ bath. Aldehyde 11 (79 mg, 0.223 mg, 1.1 eq) was suspended in 0.2 mL dry CH₂Cl₂ and added. This was repeated 3 x with a total volume of 0.6 mL dry CH₂Cl₂. Upon addition of the aldehyde, a white suspension formed and was stirred for another hour at −78 °C, then the cooling bath was exchanged for an ice bath and the suspension was stirred for 1 h at 0 °C (suspension turned to solution). The reaction was quenched by addition of 3 mL aq. 1 M KH₂PO₄ solution, followed by addition of 2 mL MeOH and 2 mL MeOH/50% H₂O₂ 2/1. The resulting mixture was stirred for 1 h at room temperature and then diluted with water (10 mL). The layers were separated and the aqueous phase was extracted with Et₂O (3 x 10 mL). The combined organic layers were washed with sat. aq. NaHCO₃ (30 mL) and brine (30 mL), dried over MgSO₄ and concentrated. After purification by flash chromatography
(pentane/EtOAc 9/1) the product 13 (103 mg, 0.166 mmol, 82% yield) was obtained as colourless waxy solid in 94% purity by weight containing 16 mol% (S)-4-benzyl-3-propionyloxazolidin-2-one.

The diastereomeric ratio was determined by NMR:
syn : anti = 1 : 0.01; dr = >20/1 as established for the Evans Aldol Reaction (J. Am. Chem. Soc. 1981, 103, 2127-2129).

\[^1\text{H} \text{NMR} (400 \text{ MHz, Chloroform-}d) \delta 7.37 \sim 7.27 (m, 3H), 7.21 (d, J = 6.8 Hz, 2H), 4.74 \sim 4.64 (m, 1H), 4.25 \sim 4.16 (m, 2H), 3.99 (qd, J = 6.9, 4.2 Hz, 1H), 3.67 (dd, J = 6.6, 4.3 Hz, 1H), 3.25 (dd, J = 13.4, 3.4 Hz, 1H), 2.84 \sim 2.73 (m, 1H), 2.44 (s, 1H), 1.74 \sim 1.60 (m, 1H), 1.59 \sim 1.47 (m, 1H), 1.34 \sim 1.22 (m, 37H), 1.07 \sim 0.99 (m, 1H), 0.98 \sim 0.92 (m, 4H), 0.90 \sim 0.85 (m, 6H).

\[^{13}\text{C} \text{NMR} (101 \text{ MHz, Chloroform-}d) \delta 177.51, 152.99, 135.19, 129.57, 129.09, 127.55, 75.31, 66.22, 55.28, 40.95, 40.19, 37.89, 36.03, 33.29, 32.07, 30.20, 29.96, 29.88, 29.85, 29.80, 29.50, 26.92, 22.83, 20.69, 15.54, 14.26, 11.76.

HRMS (ESI+) calcd. for [M+H\(^+\)] 586.4830; found 586.4810.

Optical Rotation: \([\alpha]_D^{23} = +19.2^\circ \ (c = 0.151, \text{CHCl}_3)\).

The analytical data is in agreement with previous reports.\(^1\)

\((2S,3R,4S,6S)-3\text{-hydroxy-2,4,6-trimethyltetracosanoic acid (3)}\)

A flask was charged with oxazolidinone 13 (62.1 mg, 0.10 mmol, 1 eq). The substrate was dissolved in 1.5 mL THF/water 4/1 and cooled to 0 °C. Then aq. 50% H\(_2\)O\(_2\) (0.08 mL, 1.4 mmol, 14 eq) was added followed by LiOH (3.6 mg, 0.15 mmol, 1.5 eq). After addition, the ice bath was removed and the solution was stirred for 2 h at room temperature. The reaction was quenched by slow and careful addition of 1.5 mL sat. aq. NaHSO\(_3\) (exothermic reaction!) and stirred for another hour. Then 2.5 mL sat. aq. NH\(_4\)Cl was added and the aqueous phase was extracted with EtOAc (3 x 5 mL). The combined organic extracts were washed with brine (25 mL), dried over MgSO\(_4\) and concentrated. Purification by flash chromatography (pentane/EtOAc/AcOH 85/14/1) gave mycolipanolic acid 3 (45.6 mg, 0.10 mmol, 98% purity by weight, quant. yield) as colourless waxy solid.
$^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 6.48 (s, 1H), 3.69 (t, $J = 5.7$ Hz, 1H), 2.71 (p, $J = 6.6$ Hz, 1H), 1.68 (hept, $J = 5.9$, 5.1 Hz, 1H), 1.57 – 1.45 (m, 1H), 1.35 – 1.20 (m, 37H), 1.05 – 0.96 (m, 2H), 0.96 – 0.92 (m, 3H), 0.91 – 0.81 (m, 6H).

$^{13}$C NMR (101 MHz, Chloroform-$d$) $\delta$ 181.90, 75.35, 42.52, 41.21, 36.27, 33.12, 32.08, 30.18, 29.91, 29.89, 29.86, 29.82, 29.52, 26.96, 22.84, 20.51, 14.91, 14.26, 11.76.

HRMS (ESI$^+$) calcd. for [M+H$^+$] 427.4146; found 427.4137.

Optical Rotation: [$\alpha$]$_{D}^{23} = -32^\circ$ (c = 0.97, CHCl$_3$).

The analytical data is in agreement with previous reports.$^1$

**Ethyl (4S,6S,E)-2,4,6-trimethyltetracos-2-enoate (12)**

$$\text{C}_{16}\text{H}_{33}^+$$

In a pressure tube ethyl 2-(triphenylphosphoranylidene) propionate (2.449 g, 6.758 mmol, 1.5 eq) and aldehyde 11 (1.854 g, 4.606 mmol, 88% purity by wt., 1 eq) were dissolved in 15 mL dry toluene. The mixture was stirred under reflux for 16 h. The solvent was evaporated and the crude product ($E/Z$ 15/1) was purified by flash chromatography (pentane/Et$_2$O 99/1) to give the product 12 (196 mg, 0.426 mmol, $E/Z > 20/1$, 85%) as colourless waxy solid.

$^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 6.50 (d, $J = 10.1$ Hz, 1H), 4.18 (q, $J = 7.2$ Hz, 2H), 2.70 – 2.50 (m, 1H), 1.84 (s, 3H), 1.40 – 1.33 (m, 2H), 1.32 – 1.20 (m, 36H), 1.15 – 1.06 (m, 2H), 0.97 (d, $J = 6.6$ Hz, 3H), 0.88 (t, $J = 6.7$ Hz, 3H), 0.82 (d, $J = 6.2$ Hz, 3H).

$^{13}$C NMR (101 MHz, Chloroform-$d$) $\delta$ 168.68, 148.46, 126.25, 60.55, 44.67, 37.72, 32.08, 31.01, 30.85, 30.15, 29.86, 29.81, 29.52, 27.03, 22.85, 20.71, 19.73, 14.45, 14.29, 12.63.

HRMS (ESI$^+$) calculated for [M+H$^+$] 437.4353; found 437.4355.

Optical Rotation: [$\alpha$]$_{D}^{23} = +15.5^\circ$ (c = 1.05, CHCl$_3$).

The analytical data is in agreement with previous reports.$^1$

**(4S,6S,E)-2,4,6-trimethyltetracos-2-enoic acid (4)**
A solution of ethyl ester 12 (1.498 g, 3.259 mmol, 1 eq) in 30 ml THF was cooled to 0 °C, tetrabutylammonium hydroxide was added (1 M in THF, 4.4 mL, 6.60 mmol, 2 eq). After addition, the cooling bath was removed and the solution was stirred at room temperature for 17 h. The reaction was acidified with 1 M aq. HCl to pH 3. The aqueous layer was extracted with EtOAc (3 x 50 mL), the combined organic extracts were dried over MgSO4 and concentrated. The crude was purified by flash chromatography (pentane/Et2O/AcOH 90/10/1) to afford mycolipenic acid 4 (1.256 g, 3.073 mmol, 94% yield) as colourless waxy solid.

1H NMR (400 MHz, Chloroform-d) δ 6.67 (d, J = 10.2 Hz, 1H), 2.73 – 2.56 (m, 1H), 1.86 (s, 3H), 1.26 (s, 37H), 0.99 (d, J = 6.6 Hz, 3H), 0.88 (t, J = 6.7 Hz, 3H), 0.83 (d, J = 6.2 Hz, 3H).

13C NMR (101 MHz, Chloroform-d) δ 174.20, 151.33, 125.60, 44.60, 37.76, 32.11, 31.29, 30.97, 30.16, 29.88, 29.86, 29.84, 29.54, 27.07, 22.86, 20.54, 19.74, 14.28, 12.22.

HRMS (ESI +) calculated for [M+H+] 409.4040; found 409.4038.

Optical Rotation: [α]D23 = +17.1° (c = 0.94, CHCl3).

The analytical data is in agreement with previous reports.1
solid.

$^1$H NMR (400 MHz, Methanol-$d_4$) δ 7.50 (tt, $J$ = 4.3, 2.1 Hz, 5H), 7.34 (dd, $J$ = 5.2, 1.9 Hz, 7H), 5.57 (s, 2H), 5.13 (d, $J$ = 4.0 Hz, 1H), 4.23 (dd, $J$ = 10.0, 5.0 Hz, 2H), 4.13 (td, $J$ = 10.0, 5.1 Hz, 2H), 4.03 (t, $J$ = 9.4 Hz, 1H), 3.73 (t, $J$ = 10.1 Hz, 2H), 3.63 (dd, $J$ = 9.4, 3.8 Hz, 2H), 3.49 (t, $J$ = 9.5 Hz, 2H).

$^{13}$C NMR (101 MHz, Methanol-$d_4$) δ 139.22, 129.89, 129.03, 129.01, 127.54, 103.05, 96.40, 83.03, 73.78, 71.51, 69.95, 64.20.
A flask was placed under N₂ atmosphere (3 x evacuation and N₂ backfilling) and charged with dibenzylidene trehalose S5 (3.0 g, 5.8 mmol, 1 eq) and DMAP (709 mg, 5.8 mmol, 1 eq) under N₂ flow. The solids were dissolved in 9.5 mL dry pyridine. Palmitoyl chloride (2.3 mL, 7.54 mmol, 1.3 eq) was added dropwise at rt and the resulting mixture was stirred for 24 h. The reaction was quenched by addition of 40 mL water and the aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with brine (40 mL), dried over MgSO₄ and concentrated. The crude product was purified by flash chromatography (pentane/EtOAc 3/2 to 1/1) to give the product S6 (1.2484 g, 1.65 mmol, 28% yield) as colourless waxy solid.

¹H NMR (400 MHz, Chloroform-d) δ 7.52 – 7.42 (m, 4H), 7.39 – 7.31 (m, 6H), 5.54 (s, 1H), 5.48 – 5.43 (m, 1H), 5.37 – 5.33 (m, 1H), 5.11 – 5.03 (m, 1H), 4.89 (dt, J = 9.7, 3.1 Hz, 1H), 4.31 (dd, J = 10.3, 4.9 Hz, 1H), 4.24 (t, J = 9.6 Hz, 1H), 4.16 – 4.05 (m, 2H), 4.01 – 3.92 (m, 1H), 3.86 – 3.55 (m, 5H), 3.49 – 3.38 (m, 1H), 2.44 (h, J = 8.6 Hz, 2H), 1.67 – 1.59 (m, 2H), 1.25 (s, 24H), 0.88 (t, J = 6.6 Hz, 3H).

¹³C NMR (101 MHz, Chloroform-d) δ 173.59, 137.11, 136.94, 129.47, 129.40, 128.49, 128.40, 126.43, 126.41, 102.05, 101.94, 95.02, 92.63, 81.36, 80.83, 73.02, 72.13, 71.22, 68.85, 68.69, 68.45, 63.36, 62.96, 34.11, 32.06, 29.85, 29.83, 29.80, 29.73, 29.67, 29.51, 29.42, 29.40, 29.28, 24.85, 22.83, 14.28.

The analytical data is in agreement with previous reports.⁴
A flask was charged with palmitoyl trehalose S6 (1.23 g, 1.625 mmol, 1 eq) and placed under N₂ atmosphere (3 x evacuation and N₂ backfilling). The starting material was dissolved in 16 mL dry pyridine and cooled to 0 ºC. TIPSCl₂ (0.63 mL, 1.969 mmol, 1.2 eq) was added and after stirring for 5 min at 0 ºC, the reaction was stirred at room temperature for 3 days. The reaction mixture was poured onto ice water (100 mL) and the aqueous phase was extracted with EtOAc (3 x 75 mL). The combined organic extracts were washed with brine (20 mL), dried over MgSO₄ and concentrated. The crude was purified by flash chromatography (pentane/EtOAc 2/1) to give the product 1 (540.5 mg, 0.541 mmol, 33% yield) as colourless waxy solid.

¹H NMR (400 MHz, Chloroform-d) δ 7.51 – 7.42 (m, 4H), 7.41 – 7.29 (m, 6H), 5.54 (s, 2H), 5.37 (d, J = 3.7 Hz, 1H), 5.12 (d, J = 4.0 Hz, 1H), 4.87 (dd, J = 9.7, 3.8 Hz, 1H), 4.24 (t, J = 9.2 Hz, 3H), 4.18 – 4.10 (m, 2H), 3.86 (ddd, J = 27.3, 9.2, 4.3 Hz, 2H), 3.72 (dt, J = 15.1, 11.0 Hz, 2H), 3.59 (t, J = 9.2 Hz, 1H), 3.52 (t, J = 9.2 Hz, 1H), 2.40 (hept, J = 8.0, 7.5 Hz, 2H), 1.60 (p, J = 7.4 Hz, 2H), 1.26 (s, 10H), 1.15 – 1.02 (m, 40H), 0.98 – 0.91 (m, 2H), 0.88 (t, J = 6.6 Hz, 3H).

¹³C NMR (101 MHz, Chloroform-d) δ 173.71, 137.79, 137.16, 129.50, 128.74, 128.46, 128.11, 126.61, 126.02, 102.47, 101.19, 94.57, 91.98, 81.55, 81.16, 75.25, 73.60, 73.15, 68.96, 68.86, 68.78, 62.97, 62.47, 34.18, 32.08, 29.86, 29.83, 29.82, 29.71, 29.63, 29.52, 29.33, 29.17, 24.85, 22.85, 17.59, 17.54, 17.48, 17.45, 17.32, 17.30, 17.28, 17.22, 17.21, 17.19, 14.29, 13.15, 12.96, 12.88, 12.40, 11.89.

The analytical data is in agreement with previous reports.⁴
A flask was charged with mycosanoic acid 2 (98.1 mg, 0.266 mmol, 1.3 eq) and placed under N₂ atmosphere (3 x evacuation and N₂ backfilling). Then 8 mL dry toluene was added and to the resulting solution was added dry Et₃N (70 μL, 0.504 mmol, 2.4 eq) followed by 2,4,6-trichlorobenzoyl chloride (50 μL, 0.321 mmol, 1.5 eq) at room temperature. After 45 min, palmitoyl trehalose 1 (210.4 mg, 0.211 mmol, 1 eq) and DMAP (31.2 mg, 0.255 mmol, 1.2 eq) were added as solids under N₂ flow. The reaction was left stirring at room temperature for 5.5 h and then quenched with 10 mL sat. aq. NaHCO₃. The aqueous layer was extracted with EtOAc (3 x 30 mL), the combined organic extracts were dried over MgSO₄ and concentrated. The crude product was purified by flash chromatography (pentane/Et₂O 95/5) and the product 14a (199 mg, 0.147 mmol, 70% yield) was obtained as colorless oil.

¹H NMR (400 MHz, Chloroform-d) δ 7.51 – 7.46 (m, 2H), 7.43 – 7.40 (m, 2H), 7.37 – 7.30 (m, 5H), 5.68 (t, J = 9.8 Hz, 1H), 5.55 (s, 1H), 5.49 (s, 1H), 5.40 (d, J = 3.8 Hz, 1H), 5.15 (d, J = 4.1 Hz, 1H), 5.04 (dd, J = 9.9, 3.8 Hz, 1H), 4.33 (td, J = 9.9, 4.9 Hz, 1H), 4.27 – 4.19 (m, 2H), 4.15 (dd, J = 10.1, 4.7 Hz, 1H), 3.93 (dd, J = 8.4, 4.1 Hz, 1H), 3.84 (td, J = 10.0, 4.7 Hz, 1H), 3.79 – 3.62 (m, 3H), 3.53 (t, J = 9.2 Hz, 1H), 2.63 – 2.51 (m, 1H), 2.34 (td, J = 7.8, 6.2 Hz, 2H), 1.71 (ddd, J = 14.0, 9.6, 5.0 Hz, 1H), 1.64 – 1.51 (m, 2H), 1.41 – 1.04 (m, 88H), 1.04 – 0.99 (m, 3H), 0.90 (t, J = 6.6 Hz, 6H), 0.79 (d, J = 6.6 Hz, 3H).

¹³C NMR (101 MHz, Chloroform-d) δ 175.44, 173.26, 137.74, 137.20, 128.97, 128.71, 128.13, 128.07, 126.27, 126.05, 101.65, 101.26, 94.62, 92.14, 81.26, 79.61, 75.43, 73.60, 71.04, 68.92, 68.87, 68.48, 62.91, 62.61, 41.18, 37.82, 37.54, 34.08, 32.08, 30.85, 30.30, 29.88, 29.83, 29.82, 29.72, 29.65, 29.53, 29.52, 29.32, 29.28, 26.99, 24.80, 22.84, 19.46, 18.38, 17.58, 17.55, 17.47, 17.35, 17.30, 17.26, 17.13, 14.26, 13.05, 12.80, 12.41, 11.83.

HRMS (ESI +) calculated for [M+H⁺] 1349.923; found 1349.925.

HRMS (ESI +) calculated for [M+Na⁺] 1371.905; found 1371.904.
HRMS (ESI +) calculated for [M+NH₄⁺] 1366.949; found 1366.950.

\[(2R,4aR,6R,7R,8S,8aR)-6-(((4aR,6R,7R,8S,8aS)-7,8-di\text{hydroxy}-2-\text{phenylhexahydropyrano}[3,2-d][1,3]dioxin-6-yloxy)-2-\text{phenylhexahydropyrano}[3,2-d][1,3]dioxin-8-yl (2S,4S)-2,4\text{-dimethyldecosanoate (15a)}\]

A mixture of glacial acetic acid (0.170 mL, 2.97 mmol, 40 eq) and TBAF (1 M in THF, 3.0 mL, 3.0 mmol, 40 eq) was added to a stirred solution of protected diacyl trehalose 14a (102 mg, 0.076 mmol, 1 eq) in 4 mL THF. After 3 h stirring at room temperature, the reaction mixture was diluted with 45 mL EtOAc, washed with water (15 mL) and brine (15 mL), dried over MgSO₄ and concentrated. The crude product was purified by flash chromatography (pentane/Et₂O 6/4) and the product 15a (80.0 mg, 0.072 mmol, 96% yield) was obtained as a colourless waxy solid.

\(^1\)H NMR (400 MHz, Chloroform-\(d\)) \(\delta\) 7.50 – 7.39 (m, 4H), 7.38 – 7.30 (m, 6H), 5.65 (t, \(J = 9.9\) Hz, 1H), 5.51 (s, 2H), 5.39 (d, \(J = 3.5\) Hz, 1H), 5.17 (d, \(J = 3.8\) Hz, 1H), 5.06 (dt, \(J = 10.1, 3.3\) Hz, 1H), 4.33 (dd, \(J = 10.2, 4.9\) Hz, 1H), 4.24 – 4.04 (m, 3H), 3.88 – 3.78 (m, 1H), 3.76 – 3.63 (m, 4H), 3.55 – 3.48 (m, 1H), 2.64 – 2.48 (m, 1H), 2.44 – 2.29 (m, 2H), 1.76 – 1.65 (m, 1H), 1.60 (p, \(J = 7.4\) Hz, 2H), 1.26 (s, 58H), 1.13 – 1.09 (m, 3H), 0.88 (t, \(J = 6.7\) Hz, 8H), 0.80 (d, \(J = 6.5\) Hz, 3H).

\(^13\)C NMR (101 MHz, Chloroform-\(d\)) \(\delta\) 175.82, 173.09, 137.08, 136.89, 129.38, 129.08, 128.39, 128.27, 126.45, 126.43, 126.18, 126.11, 102.11, 101.46, 94.93, 92.59, 80.98, 79.50, 72.37, 71.40, 70.80, 68.85, 68.78, 68.72, 68.53, 68.43, 63.42, 63.24, 41.19, 37.87, 37.65, 37.54, 34.09, 32.08, 30.93, 30.69, 30.47, 30.29, 30.21, 29.85, 29.83, 29.77, 29.74, 29.69, 29.65, 29.52, 29.45, 29.39, 29.37, 29.33, 27.02, 24.83, 22.85, 19.49, 18.44, 17.52, 14.27.

HRMS (ESI +) calculated for [M+H⁺] 1107.771; found 1107.771.

HRMS (ESI +) calculated for [M+Na⁺] 1129.753; found 1129.753.

HRMS (ESI +) calculated for [M+NH₄⁺] 1124.797; found 1124.798.
(2R,3R,4S,5R,6R)-3-hydroxy-2-(hydroxymethyl)-5-(palmitoyloxy)-6-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-4-yl (2S,4S)-2,4-dimethyldocosanoate (DAT\textsubscript{1})

Protected diacyl trehalose 15a (65.5 mg, 0.059 mmol, 1 eq) was dissolved in 3 mL CHCl\textsubscript{3} and 2.4 mL MeOH. 15% aq. H\textsubscript{2}SO\textsubscript{4} (0.3 mL, 0.123 mmol, 2 eq) was added and the reaction mixture was stirred at room temperature for 2 days. Upon completion, 7 mL water was added and the solution was neutralized by addition of sat. aq. NaHCO\textsubscript{3}. The aqueous layer was extracted with EtOAc (3x 15 mL) and the combined organic extracts were washed with brine (10 mL), dried over MgSO\textsubscript{4} and concentrated. The crude product was purified by flash chromatography (CH\textsubscript{2}Cl\textsubscript{2}/acetone/MeOH 8/1/1) to afford the product DAT\textsubscript{1} (24.5 mg, 0.026 mmol, 45% yield) as a colorless wax.

\textsuperscript{1}H NMR (400 MHz, Chloroform-d/Methanol-d\textsubscript{4} 4/1) δ 5.39 (t, \(J = 9.7 \) Hz, 1H), 5.24 (d, \(J = 3.3 \) Hz, 1H), 5.06 (d, \(J = 3.5 \) Hz, 1H), 4.82 (dd, \(J = 10.3, 3.3 \) Hz, 1H), 3.96 – 3.87 (m, 1H), 3.86 – 3.72 (m, 2H), 3.72 – 3.60 (m, 3H), 3.58 – 3.45 (m, 3H), 3.39 (t, \(J = 9.4 \) Hz, 1H), 2.63 – 2.44 (m, 1H), 2.34 – 2.12 (m, 2H), 1.69 – 1.59 (m, 1H), 1.51 (p, \(J = 7.5, 7.1 \) Hz, 2H), 1.21 (s, 58H), 1.11 (d, \(J = 6.9 \) Hz, 3H), 1.08 – 0.98 (m, 2H), 0.83 (ddt, \(J = 9.4, 6.7, 2.8 \) Hz, 9H).

\textsuperscript{13}C NMR (101 MHz, Chloroform-d/Methanol-d\textsubscript{4} 4/1) δ 177.57, 173.47, 94.63, 91.74, 73.42, 72.66, 72.62, 72.02, 71.77, 70.87, 70.24, 69.41, 61.66, 61.44, 41.39, 37.61, 37.24, 33.98, 32.08, 30.90, 30.31, 29.91, 29.88, 29.86, 29.85, 29.81, 29.69, 29.51, 29.40, 27.08, 24.75, 22.82, 19.59, 17.93, 14.13.

HRMS (ESI +) calculated for [M+Na\textsuperscript{+}] 953.6900; found 953.6900.

HRMS (ESI +) calculated for [M+NH\textsubscript{4}\textsuperscript{+}] 948.7346; found 948.7348.

Optical Rotation: \([\alpha]\text{D}\textsuperscript{23} = +74.4^\circ\) (\(c = 0.68, \text{CHCl}_3\)).
A flask was placed under N2-atmosphere and charged with mycolipanolic acid 3 (40.5 mg, 0.095 mmol, 1 eq). Dry THF (0.95 mL) was added and the substrate was dissolved followed by addition of Et3N (20 µL, 0.142 mmol, 1.5 eq) and 2,4,6-trichlorobenzoyl chloride (18 µL, 0.114 mmol, 1.2 eq). The resulting reaction mixture was stirred at room temperature for 1 h. Protected palmitoyl trehalose 1 (94.8 mg, 0.095 mmol, 1 eq) and DMAP (2.9 mg, 0.024 mmol, 0.25 eq) were dissolved in 0.95 mL and added. The resulting mixture was stirred at room temperature for another 18 h. Sat. aq. NH4Cl 10 mL was added and the aqueous phase was extracted with Et2O (3x 15 mL), washed with brine (5 mL), dried over MgSO4 and concentrated. The crude was purified by flash chromatography (pentane/EtOAc 95/5 to 92/8) and the product 14b (88.1 mg, 0.063 mmol, 66% yield) was obtained as colourless wax.

1H NMR (400 MHz, Chloroform-δ) δ 7.46 (dd, J = 6.8, 2.9 Hz, 2H), 7.40 (dd, J = 6.7, 3.1 Hz, 2H), 7.37 – 7.29 (m, 6H), 5.66 (t, J = 9.8 Hz, 1H), 5.53 (s, 1H), 5.47 (s, 1H), 5.36 (d, J = 3.8 Hz, 1H), 5.13 (d, J = 4.1 Hz, 1H), 5.05 (dd, J = 9.9, 3.8 Hz, 1H), 4.31 (td, J = 9.9, 4.9 Hz, 1H), 4.28 – 4.16 (m, 2H), 4.14 (dd, J = 10.1, 4.7 Hz, 1H), 3.91 (dd, J = 8.5, 4.1 Hz, 1H), 3.83 (td, J = 10.0, 4.7 Hz, 1H), 3.79 – 3.62 (m, 2H), 3.58 (t, J = 5.8 Hz, 1H), 3.52 (t, J = 9.2 Hz, 1H), 2.65 (p, J = 6.8 Hz, 1H), 2.39 – 2.28 (m, 2H), 1.69 – 1.60 (m, 1H), 1.58 – 1.45 (m, 3H), 1.31 – 1.19 (m, 52H), 1.16 – 1.11 (m, 15H), 1.10 – 1.04 (m, 18H), 1.03 – 0.99 (m, 3H), 0.99 – 0.95 (m, 2H), 0.88 (t, J = 6.7 Hz, 6H), 0.85 – 0.81 (m, 6H), 0.81 – 0.76 (m, 1H).

13C NMR (101 MHz, Chloroform-δ) δ 174.64, 173.50, 137.74, 137.08, 129.12, 128.75, 128.22, 128.11, 126.30, 126.06, 101.76, 101.28, 94.66, 92.14, 81.24, 79.44, 75.39, 74.79, 73.57, 70.89, 69.13, 68.88, 63.02, 62.68, 42.98, 40.96, 36.50, 34.08, 32.09, 30.19, 29.91, 29.87, 29.84, 29.82, 29.74, 29.64, 29.54, 29.52, 29.33, 29.24, 27.00, 24.82, 22.86, 20.49, 17.61, 17.56, 17.47, 17.37, 17.32, 17.31, 17.29, 17.19, 15.15, 14.28, 13.06, 12.82, 12.46, 11.89.
HRMS (ESI+) calcd. for [M+H\(^+\)] 1407.9647; found 1407.9665.

HRMS (ESI+) calcd. for [M+Na\(^+\)] 1429.9467; found 1429.9456.

(4aR,6R,7R,8S,8aR)-6-(((4aR,6R,7R,8R,8aS)-7,8-dihydroxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-6-yl)oxy)-7-(palmitoyloxy)-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl (2S,3R,4S,6S)-3-hydroxy-2,4,6-trimethyltetradecanoate (15b)

Protected diacyl trehalose 14b (88.1 mg, 0.063 mmol, 1 eq) was dissolved in 0.2 mL THF. To this, TBAF (1 M in THF, 2.45 mL, 2.45 mmol, 38.9 mmol) and glacial AcOH (0.14 mL, 2.45 mmol, 38.9 eq) were added to the stirred solution at room temperature. After two hours, TLC monitoring showed the reaction was complete. The reaction was diluted with EtOAc (25 mL) and then washed with water (10 mL) and brine (10 mL), dried over MgSO\(_4\) and concentrated. The crude product was purified by flash chromatography (pentane/EtOAc 3/1 to 5/2) giving the product 15b (56.7 mg, 0.049 mmol, 78%) as colourless waxy solid.

\( ^1\)H NMR (400 MHz, Chloroform-\(d\)) \( \delta 7.50 - 7.39 (m, 4H), 7.39 - 7.30 (m, 6H), 5.66 (t, J = 9.9 Hz, 1H), 5.50 (d, J = 4.0 Hz, 2H), 5.37 (d, J = 3.9 Hz, 1H), 5.14 (d, J = 3.8 Hz, 1H), 5.08 (dd, J = 10.0, 3.9 Hz, 1H), 4.33 (dd, J = 10.3, 4.9 Hz, 1H), 4.21 (td, J = 9.8, 4.9 Hz, 1H), 4.12 (dd, J = 10.2, 4.7 Hz, 1H), 4.05 (t, J = 9.3 Hz, 1H), 3.82 (td, J = 9.9, 4.7 Hz, 1H), 3.78 - 3.65 (m, 4H), 3.61 (t, J = 5.8 Hz, 1H), 3.49 (t, J = 9.3 Hz, 1H), 2.68 (p, J = 6.8 Hz, 1H), 2.38 (t, J = 7.8 Hz, 2H), 1.71 - 1.56 (m, 3H), 1.48 (q, J = 7.3 Hz, 1H), 1.31 - 1.18 (m, 61H), 1.05 - 0.93 (m, 2H), 0.90 - 0.78 (m, 12H).

\( ^{13}\)C NMR (101 MHz, Chloroform-\(d\)) \( \delta 174.95, 173.27, 137.09, 136.79, 129.39, 129.17, 128.40, 128.32, 126.43, 126.11, 102.10, 101.50, 95.00, 92.62, 80.96, 79.35, 74.70, 72.27, 71.30, 70.68, 69.08, 68.80, 68.77, 63.45, 63.15, 43.04, 40.98, 36.55, 34.06, 32.92, 32.08, 30.19, 29.91, 29.86, 29.82, 29.81, 29.79, 29.74, 29.66, 29.52, 29.51, 29.38, 29.33, 27.01, 24.83, 22.84, 20.47, 15.07, 14.27, 12.15.

HRMS (ESI+) calcd. for [M+H\(^+\)] 1165.8125; found 1165.8145.

HRMS (ESI+) calcd. for [M+Na\(^+\)] 1187.7944; found 1187.7948.
(2R,3R,4S,5R,6R)-3-hydroxy-2-(hydroxymethyl)-5-(palmitoyloxy)-6-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-4-yl (2S,3R,4S,6S)-3-hydroxy-2,4,6-trimethyltetraicosanoate (DAT₂)

A flask was charged with protected diacyl trehalose 15b (10.0 mg, 9 µmol, 1 eq), Pd/C (10% Pd by weight, 8.9 mg, 8 µmol, 1 eq) and Pd(OH)₂ (20% Pd by weight, 6.4 mg, 9 µmol, 1 eq). Then 1 mL THF was added, the resulting suspension was placed under H₂-atmosphere and stirred at room temperature for 4 days. The reaction mixture was filtered over Celite using MeOH to fully elute the product and then purified by flash chromatography (CH₂Cl₂/acetone/MeOH 8/1/1). The product DAT₂ was obtained as colorless oil (5.6 mg, 6 µmol, 66% yield).

¹H NMR (400 MHz, Chloroform-d/Methanol-d₄ 4/1) δ 5.39 (t, J = 9.7 Hz, 1H), 5.23 (d, J = 3.7 Hz, 1H), 5.05 (d, J = 3.9 Hz, 1H), 4.85 (dd, J = 10.3, 3.6 Hz, 1H), 3.92 – 3.88 (m, 1H), 3.83 – 3.71 (m, 2H), 3.68 – 3.60 (m, 3H), 3.58 – 3.46 (m, 4H), 3.38 (t, J = 8.6 Hz, 1H), 2.64 (p, J = 5.4 Hz, 1H), 2.24 (t, J = 6.9 Hz, 2H), 1.64 – 1.43 (m, 5H), 1.20 (s, 58H), 1.12 (d, J = 7.0 Hz, 3H), 0.98 – 0.92 (m, 1H), 0.91 – 0.77 (m, 12H).

¹³C NMR (101 MHz, Chloroform-d/Methanol-d₄ 4/1) δ 176.20, 173.72, 94.64, 91.78, 75.30, 73.44, 72.75, 72.71, 72.49, 71.81, 70.73, 70.26, 69.34, 61.62, 61.45, 43.26, 41.25, 36.46, 34.01, 33.26, 32.11, 30.27, 29.96, 29.89, 29.88, 29.84, 29.83, 29.72, 29.55, 29.54, 29.38, 27.07, 24.84, 22.85, 20.44, 15.09, 14.16, 11.41.

HRMS (ESI⁺) calcd. for [M+NH₄⁺] 1006.7764; found 1006.7779.

HRMS (ESI⁺) calcd. for [M+Na⁺] 1011.7318; found 1011.7317.

Optical Rotation: [α]D²⁵ = +70° (c = 0.01, CHCl₃).
A flask was charged with mycolipenic acid 4 (121.8 mg, 0.298 mmol, 1.3 eq) and placed under N₂ atmosphere (3 x evacuation and N₂ backfilling). Then 8 mL dry toluene was added and to the resulting solution was added dry Et₃N (70 μL, 0.503 mmol, 2.3 eq) followed by 2,4,6-trichlorobenzoyl chloride (50 μL, 0.320 mmol, 1.4 eq). After 45 min. stirring at room temperature, palmitoyl trehalose 1 (222.6 mg, 0.223 mmol, 1 eq) and DMAP (33.1 mg, 0.271 mmol, 1.2 eq) were added as solids and the reaction was stirred at room temperature for 18 h. The reaction was quenched with 10 mL sat. aq. NaHCO₃ and the aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic extracts were washed with brine (20 mL), dried over MgSO₄ and concentrated. The crude product was purified by flash chromatography (pentane/Et₂O 95/5) and the product 14c (233 mg, 0.168 mmol, 75% yield) was obtained as a colorless oil.

**¹H NMR (400 MHz, Chloroform-d) δ 7.51 – 7.46 (m, 2H), 7.46 – 7.42 (m, 2H), 7.37 – 7.32 (m, 6H), 6.51 (d, J = 10.1 Hz, 1H), 5.74 (t, J = 9.8 Hz, 1H), 5.54 (s, 1H), 5.51 (s, 1H), 5.38 (d, J = 3.8 Hz, 1H), 5.16 (d, J = 4.1 Hz, 1H), 5.11 (dd, J = 9.9, 3.9 Hz, 1H), 4.37 (td, J = 10.0, 4.9 Hz, 1H), 4.30 – 4.20 (m, 2H), 4.15 (dd, J = 10.1, 4.8 Hz, 1H), 3.94 (dd, J = 8.4, 4.2 Hz, 1H), 3.88 (td, J = 10.0, 4.8 Hz, 1H), 3.82 – 3.66 (m, 3H), 3.53 (t, J = 9.3 Hz, 1H), 2.72 – 2.51 (m, 1H), 2.37 – 2.27 (m, 2H), 1.83 (d, J = 1.3 Hz, 3H), 1.53 (p, J = 7.5 Hz, 2H), 1.28 (s, 58H), 1.17 – 1.07 (m, 28H), 1.06 – 1.04 (m, 3H), 0.96 (d, J = 6.6 Hz, 3H), 0.90 (t, J = 6.7 Hz, 6H), 0.81 (d, J = 6.1 Hz, 3H).

**¹³C NMR (101 MHz, Chloroform-d) δ 173.37, 167.17, 149.25, 137.76, 137.31, 129.04, 128.73, 128.19, 128.08, 126.44, 126.12, 125.62, 101.84, 101.36, 94.76, 92.36, 81.26, 79.60, 75.48, 73.62, 71.18, 69.22, 68.95, 68.90, 62.85, 62.82, 44.50, 37.67, 34.17, 32.08, 30.94, 30.89, 30.26, 29.89, 29.87, 29.83, 29.82, 29.73, 29.59, 29.53, 29.52, 29.32, 29.28, 29.16, 27.06, 24.95, 22.84, 20.51, 19.73, 17.58, 17.57, 17.48, 17.36, 17.30, 17.23, 17.14, 14.26, 13.07, 12.79, 12.68, 12.39, 11.87.

HRMS (ESI +) calculated for [M+H⁺] 1389.9541; found 1389.9557.
A mixture of glacial acetic acid (175 µL, 3 mmol, 40 eq) and TBAF (1 M in THF, 3 mL, 3 mmol, 40 eq) was added to a stirred solution of protected diacyl trehalose 14c (142 mg, 0.102 mmol, 1 eq) in 4 mL THF. After 1.5 h stirring at room temperature, the reaction mixture was diluted with 25 mL EtOAc, washed with water (15 mL) and brine (15 mL), dried over MgSO₄ and concentrated. The crude product was purified by flash chromatography (Pentane/Et2O 1/1) and the product 15c (112 mg, 0.098 mmol, 96% yield) was obtained as a colourless wax.

1H NMR (400 MHz, Chloroform-d) δ 7.49 – 7.41 (m, 4H), 7.37 – 7.29 (m, 6H), 6.52 (d, J = 10.0 Hz, 1H), 5.73 (t, J = 9.9 Hz, 1H), 5.53 (s, 1H), 5.47 (s, 1H), 5.35 (d, J = 3.9 Hz, 1H), 5.15 – 5.09 (m, 2H), 4.34 (dd, J = 10.2, 4.9 Hz, 1H), 4.23 (td, J = 9.8, 4.9 Hz, 1H), 4.10 (dd, J = 10.3, 4.8 Hz, 1H), 4.04 (t, J = 9.2 Hz, 1H), 3.84 (td, J = 10.0, 4.8 Hz, 1H), 3.76 (t, J = 9.5 Hz, 2H), 3.72 – 3.61 (m, 2H), 3.48 (t, J = 9.4 Hz, 1H), 3.16 (s, 1H), 2.85 (s, 1H), 2.67 – 2.52 (m, 1H), 2.34 (t, J = 7.7 Hz, 2H), 1.83 (s, 3H), 1.63 – 1.49 (m, 2H), 1.25 (d, J = 11.4 Hz, 61H), 0.97 (d, J = 6.5 Hz, 3H), 0.89 (t, J = 6.7 Hz, 6H), 0.81 (d, J = 6.2 Hz, 3H).

13C NMR (101 MHz, Chloroform-d) δ 173.16, 167.39, 149.64, 137.17, 137.02, 129.29, 129.07, 128.33, 128.27, 126.51, 126.24, 125.50, 102.08, 101.57, 95.15, 92.73, 80.95, 79.50, 72.28, 71.23, 70.92, 69.15, 68.87, 68.77, 63.35, 44.46, 37.59, 34.17, 32.07, 30.99, 30.88, 30.46, 30.26, 29.88, 29.85, 29.81, 29.80, 29.74, 29.62, 29.51, 29.50, 29.33, 29.24, 27.05, 24.98, 22.83, 20.46, 19.77, 14.26, 12.69.

HRMS (ESI +) calculated for [M+H⁺] 1147.802; found 1147.803.

HRMS (ESI +) calculated for [M+Na⁺] 1169.784; found 1169.784.

HRMS (ESI +) calculated for [M+NH₄⁺] 1164.828; found 1164.830.
Protected diacyl trehalose 15c (63.3 mg, 0.055 mmol, 1 eq) was dissolved in 2.8 mL chloroform and 2.2 mL methanol and 15% aq. H$_2$SO$_4$ (0.3 mL, 0.123 mmol, 2.2 eq) was added. The reaction mixture was stirred at room temperature overnight. Upon completion, 7 mL water was added and the reaction mixture was neutralized by addition of sat. aq. NaHCO$_3$. The aqueous layer was extracted with EtOAc (3 x 25 mL), washed with brine (20 mL), dried over MgSO$_4$ and concentrated. The crude product was purified by flash chromatography (CH$_2$Cl$_2$/Acetone/MeOH 8/1/1) to afford the product DAT$_3$ as a colorless wax (41.5 mg, 0.043 mmol, 78% yield).

$^1$H NMR (400 MHz, Chloroform-$d$/Methanol-$d_4$ 4/1) δ 6.50 (d, $J = 10.1$ Hz, 1H), 5.46 (dd, $J = 10.3, 3.6$ Hz, 1H), 5.24 (d, $J = 3.6$ Hz, 1H), 5.08 (d, $J = 3.7$ Hz, 1H), 4.93 (dd, $J = 10.3, 3.6$ Hz, 1H), 3.95 (dd, $J = 10.1, 5.4, 2.5$ Hz, 1H), 3.83 (dd, $J = 12.1, 2.6$ Hz, 1H), 3.77 (t, $J = 9.4$ Hz, 1H), 3.73 – 3.65 (m, 3H), 3.63 – 3.55 (m, 2H), 3.51 (dd, $J = 9.8, 3.8$ Hz, 1H), 3.39 (t, $J = 9.4$ Hz, 1H), 2.65 – 2.51 (m, 1H), 2.20 (td, $J = 7.4, 1.7$ Hz, 2H), 1.80 (s, 3H), 1.54 – 1.41 (m, 2H), 1.22 (s, 6H), 0.93 (d, $J = 6.6$ Hz, 3H), 0.84 (t, $J = 6.8$ Hz, 6H), 0.79 (d, $J = 6.1$ Hz, 3H).

$^{13}$C NMR (101 MHz, Chloroform-$d$/Methanol-$d_4$ 4/1) δ 173.60, 168.80, 150.23, 125.62, 94.83, 92.04, 73.56, 72.96, 72.87, 72.77, 71.90, 70.84, 70.39, 69.33, 61.71, 61.55, 44.60, 37.70, 34.21, 32.16, 31.11, 31.03, 30.39, 29.97, 29.93, 29.90, 29.88, 29.71, 29.60, 29.59, 29.51, 29.33, 27.16, 25.09, 22.90, 20.40, 19.71, 14.19, 12.55.

HRMS (ESI +) calculated for [M+H$^+$] 993.7213; found 993.7212

Optical Rotation: $[\alpha]_D^{23} = +88.2^\circ$ (c = 1.39, CHCl$_3$).
Analysis of *M. tuberculosis* lipids by mass spectrometry

Analysis of *Mtbc* lipid extracts by HPLC-MS

Solvents used for extraction were HPLC grade 2:1 chloroform : methanol (C:M; Merck), 1:1 C:M, and 1:2 C:M. The three supernatants were pooled and dried and lipids were dissolved and stored in 1:1 C:M. Extracts were analyzed on Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS. Reversed phase liquid chromatography (LC) used a C-18 HPLC column (Agilent Poroshell 120, 2.7 µm, 4.6 mm x 100 mm) and a gradient method with 7:3 methanol:water (solvent A) and 85:15 1-propanol:cyclohexane (solvent B). Both solvents contained 2.0 mM ammonium formate. 0.1% water was added to solvent B to aid dissolution. The solvent gradient used a 0.5 mL/min flowrate throughout and started at 60% solvent B, increased linearly starting at 1.0 min and ending at 100% solvent B at 10.0 min, holding at 100% B until 15.0 min. To prepare samples 200µL of 1 mg/mL *M. tuberculosis* lipid extracts of strain H37Rv, and clinical isolates j257, j011, and j117 were dried under nitrogen and reconstituted in 200µL of starting mobile phase. Runs were initiated with 40-µL sample injections. Detected ions were analyzed using Agilent Technologies MassHunter Qualitative Analysis B.07.00 software. For MSMS (collisionally induced dissociation 15 V, nitrogen collision gas) analysis the same chromatography was carried out with an Agilent 6546 High Resolution QTOF mass spectrometer and a 10-µL injection for natural extracts and 2-µL injection for the more concentrated standards.

Synthetic DAT standards were spiked in 1:10 to the H37Rv sample solution to determine if they coeluted with the putative DAT compounds in the natural lipid mixture using the same injection conditions.

High resolution CID of DAT standards and natural compounds yielded the ions shown in the Figures below and in Figure 2 in the main manuscript. Calculated masses for ions not shown in Figure 2 appear below. Errors are shown in the parenthesis as ppm.

|        | M+NH₄⁺ | loss of ammonia | loss of H₂O | loss of hexose | loss of palmitoyl | loss of H₂O |
|--------|--------|----------------|------------|----------------|------------------|------------|
| DAT1   | 948.7345 | 931.7080 | 913.6974 | 751.6446 | 513.4149 | 495.4044 |
| DAT2   | 1006.7764 | 989.7499 | 971.7393 | 809.6865 | 571.4568 | 553.4462 |
| DAT3   | 988.7658 | 971.7393 | 953.7287 | 791.6759 | 553.4462 | 535.4357 |

hexose-palmitoly (H⁺) 401.2898 loss of water 383.2792
Collision-Induced Dissociation of DATs

DAT₁, DAT₂, and DAT₃ from synthetic standards and bacteria were collided at 15V via HPLC-MS. The spectra for the bacterial sample is compared to CID spectra for the respective synthesized DAT to rule in structural matches based on more than one fragment match with better than acceptable mass accuracy. For DAT₁ and DAT₃, bacterial samples and synthetic standards have the same parent ion m/z window collided and the same retention time. DAT₂ from Mtb and from synthetic sources show different retention times and different CID patterns.
Thin-layer chromatography of synthetic DAT₁-₃

Silica-coated glass TLC plates were precleared with CHCL₃:MeOH:H₂O (60:30:6 v:v:v) and dried. Synthetic diacyltrehaloses and glucose monomycolate (10 µg) were applied and resolved with CHCL₃:MeOH:H₂O (60:16:2 v:v:v). The plates were dried and sprayed with 3% copper acetate monohydrate (Sigma-Aldrich) in 8% phosphoric acid (Merck) on the plate and baking at 140 °C.

Mincle activation and binding assays

For the cellular Mincle assays TDM (Sigma T3034), DAT₁, DAT₂, and DAT₃ dissolved in chloroform/methanol at 1 mg/mL were diluted in isopropanol and added to a 96-well plate at 20 µL/well. After evaporation of the isopropanol, 30,000 2B4-NFAT-GFP reporter cells expressing mouse Mincle or human Mincle were added to each well in 100 µL medium. After incubation at 37 °C for 24 hours the expression of NFAT-GFP was analyzed by flow cytometry. For the ELISA-based binding assay, plates were coated with DAT₁, DAT₂, DAT₃ or TDM as above. After blocking with 5% BSA, 3 µg/mL of mouse Mincle-human immunoglobulin Fc (hIg) fusion proteins were added followed by detection of binding with anti-human Ig-horse radish peroxidase (HRP).
Comparison of NMR signals of natural and synthetic DAT

| Proton | Chemical shift natural DAT₁ [ppm] | Chemical shift synthetic DAT₁ [ppm] |
|--------|-----------------------------------|------------------------------------|
| H-1    | 5.25                              | 5.24                               |
| H-2    | 4.83                              | 4.82                               |
| H-3    | 5.40                              | 5.39                               |
| H-4    | 3.46                              | 3.58-3.45 (3H)                     |
| H-5    | 3.90                              | 3.96-3.87                          |
| H-6a   | 3.65                              | 3.72-3.60 (3H)                     |
| H-6b   | 3.80                              | 3.86-3.72 (2H)                     |
| H-1'   | 5.05                              | 5.06                               |
| H-2'   | 3.47                              | 3.58-3.45 (3H)                     |
| H-3'   | 3.75                              | 3.86-3.72 (2H)                     |
| H-4'   | 3.35                              | 3.39                               |
| H-5'   | 3.55                              | 3.58-3.45 (3H)                     |
| H-6a'  | 3.68                              | 3.72-3.60 (3H)                     |
| H-6b'  | 3.68                              | 3.72-3.60 (3H)                     |
Comparison of $^1$H NMR signals from natural DAT\(_1\) and synthetic DAT\(_1\). NMR spectra of synthetic and natural DAT\(_1\) were recorded using CDCl\(_3/CD_3OD\) 4/1 as solvent.

Comparison of the $^{13}$C NMR signals of natural DAT\(_1\) and synthetic DAT\(_1\):

$^{13}$C NMR spectrum of natural DAT\(_1\) as reported by Besra et al.\(^5\)

$^{13}$C NMR spectrum of synthetic DAT\(_1\)
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NMR spectra
