Chapter 4
A Glycerophospholipid from M. tuberculosis; Structure Elucidation by MS/MS Analysis and Asymmetric Synthesis

In this chapter the isolation, synthesis and characterization of a phospholipid from M. tuberculosis is described. This newly discovered glycerophospholipid contains two different acyl residues of which the relative position is unknown until now. The synthesis of both possible regioisomers is described together with the first enantioselective and fully catalytic synthesis of tuberculostearic acid. The position of the acyl residues is determined by comparison of spectroscopic and MS/MS data of the two synthetic compounds to the structural data of the isolated natural phospholipid.*

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4.1 Introduction

Glycerophospholipids can be divided in two major classes, namely the phosphatidylethanolamines and phosphatidylcholines, also known as cephalins and lecithins, respectively (Figure 1).\(^1\) Phosphatidylcholines contain a trimethylammonium group instead of the ammonium group found in the phosphatidylethanolamines. Both structures exist in their zwitterionic form resulting in a polar headgroup and long apolar aliphatic side chains.

![Figure 1: General structure of phosphatidylethanolamine and phosphatidylcholine.](image)

Glycerophospholipids are found in all biological membranes and play an essential role in the structure and functioning of cellular membranes.\(^1\) Phosphatidylethanolamines are commonly found in all living cells but are predominantly found in the plasma membrane of bacteria. Stereospecific numbering (sn-numbering)\(^2\) is used for the assignment of the absolute configuration of the glycerol backbone. The general structure of glycerophospholipids consists of a glycerol core with a phosphate group localized at the sn-3 position. The absolute configuration of the glycerol backbone for the phospholipid types given in Figure 1 is R, i.e. a phosphate group at the sn-3 position. Archaea bacteria are the only examples found in nature which contain phospholipids with a sn-1 phosphorylated glycerol backbone (the opposite enantiomer). Besides this remarkable difference they often have ether-linked side chains instead of ester linkages.\(^3\)

The two remaining hydroxyl functionalities of the glycerol backbone are substituted with at least one O-acyl, O-alkyl or O-alk-1'-enyl residue. The two acyl-substituents can be either identical or different and affect membrane packing in ways that influence bacterial survival and drug transport. Some bacteria, such as *Mycobacterium tuberculosis* (M.
tuberculosis), have very specific glycerophospholipids which can in principle be used to detect and identify a specific species. For physiological studies and potential early detection of tuberculosis disease, identification of pure cell wall and cell membrane components is of paramount importance.

As part of ongoing research on immunologically active compounds from *M. tuberculosis*, C. Seshadri and D. B. Moody (Harvard Medical School) isolated an abundant glycerophospholipid from a virulent reference strain of *M. tuberculosis* known as H37Rv. The isolate was consistent with phosphatidylethanolamine (PE) glycerol having two acyl residues, tuberculostearoyl ((R)-10-methyloctadecyl) and palmitoyl, Figure 2, compound 1 or 2.

![Figure 2: The two possible structures of the natural phospholipid; 1-O-TBSA-2-O-palmitoyl-sn-phospholipid (1) and 1-O-palmitoyl-2-O-TBSA-sn-phospholipid (2).](image)

This glycerophospholipid could be distinguished from mammalian phosphatidyl ethanolamines by mass analysis based on ions that contain a C19 tuberculostearoyl (TBSA) unit. Lipidomic surveys of mycobacterial lipids for candidate biomarkers are focused on ions that are abundant, universally present in pathogenic mycobacterial species and absent in mammalian lipids. Mycobacterial phosphatidylethanolamines (PE) fit all three criteria because a large subset of the PE molecular species carries a C19 tuberculostearic acid, which is not made by mammalian cells. Negative-mode mass spectrometry of the bacterial extracts from *M. tuberculosis*, detected several ions corresponding to the expected mass of tuberculostearic acid containing PE, with the strongest being m/z 732.5535 (Figure 3a). Because mammalian cells rarely produce fatty acids with an odd
number of methylene units, it was expected that mammalian PE would not contain a compound with the same mass. Confirming this, both the mass spectrum (not shown) and the extracted chromatogram (Figure 3b) showed lack of a molecule with $m/z$ 732.5535 in mammalian extracts eluting in the time range expected for PE, indicating that this molecule is a candidate biomarker of infection by mycobacteria.

Figure 3: A: Negative mode mass analysis of detected M. tuberculosis phosphatidylethanolamines (PE). B: Extracted ion chromatograms of PE's detected in M. tuberculosis compared to mammalian PE's from non-infected mouse lung homogenate, no ion at $m/z$ 732.55 was detected in this region of the chromatogram for the mammalian PE's.

Because the relative position (sn-1 or sn-2) of the tuberculostearic acyl (TBSA) and palmitic acyl residue could not be determined directly, we decided to elucidate the structure of the isolated phospholipid by synthesizing the two possible structures (Figure 2). We started with the synthesis of (R)-TBSA (3), a methyl branched fatty acid which is found in M.
tuberculosis and other Mycobacteria. Several synthetic routes (vide infra) have been described for (R)-TBSA (3), but all routes are based on chiral pool strategies\(^8,9\) and there is no catalytic enantioselective route until now. After the synthesis of (R)-TBSA (3) we planned to synthesize both possible regioisomers of the isolated phospholipid. MS/MS analysis will be used to elucidate the structure of the isolate from \textit{M. tuberculosis} by comparison of the natural product to the two synthetic compounds.

### 4.2 Previously reported syntheses of tuberculostearic acid

Tuberculostearic acid (3) was reported by Anderson\(^4\) already in 1927, isolated from a phosphatide fraction of the H.37 strain of \textit{M. tuberculosis}. Spielman confirmed the complete structure and assigned the methyl branching at the 10-position seven years later by oxidation studies.\(^5\) The same paper reported the first racemic synthesis of tuberculostearic acid. A difference in melting point between the natural product and the racemic synthetic compound led the authors to the conclusion that the natural product was not a racemate. Optical rotation of the natural compound, however, was found to be too small to measure. In 1947 and 1948 Prout and co-workers\(^6a,b\) reported a synthesis which started from optically pure 2-decanol, obtained by resolution. Both enantiomers of tuberculostearic acid were synthesized and the absolute configuration of the natural compound was found to be R. In the following decades, several groups reported the synthesis of tuberculostearic acid, either by racemic synthesis or based on kinetic resolution strategies.\(^7\) More recently, in 2006 and 2007, three groups\(^8,9,10\) reported different strategies for the synthesis of (R)-tuberculostearic acid (3) based on chiral pool starting materials.

#### 4.2.1 Chiral pool strategy based on Roche ester

Seeberger and co-workers\(^8\) published a nine step synthesis of tuberculostearic acid (3, Scheme 1) in 2006 based on a chiral pool strategy starting from Roche ester 4. The alcohol function of Roche ester is first protected as a THP (tetrahydropyran) ether (5). Reduction of the methyl ester to the corresponding alcohol followed by treatment with tosyl chloride yielded tosylate 6 in 88% over 3 steps. THP ether 7 was obtained by a copper-catalyzed Grignard coupling. Deprotection of the THP ether
and tosylate formation resulted in compound 8. Tosylate 8 was treated with a functionalized Grignard reagent in a second copper-catalyzed Grignard coupling to obtain THP ether 9. Acidic hydrolysis of the THP ether followed by oxidation resulted in (R)-tuberculostearic acid 3.

\[ \text{(S)-Roche ester (4)} \]

\[ \text{CrH}_2\text{MgBr} \]

\[ \text{Li}_2\text{CuCl}_4 (\text{cat.}) \]

\[ \text{5} \]

\[ \text{6} \]

\[ \text{80\% (3 steps)} \]

\[ \text{8} \]

\[ \text{71\% (3 steps)} \]

\[ \text{(R)-tuberculostearic acid (3)} \]

**Scheme 1:** Chiral pool strategy used by Seeberger *et al.*

### 4.2.2 Chiral pool strategy based on citronellol

Larsen and co-workers published a chiral-pool based synthesis of tuberculostearic acid (Scheme 2) in 2007 as part of the synthesis of a phosphatidylinositol mannoside containing TBSA. This route starts with commercially available citronellol 10. After tosylation of the alcohol, the C-6 of 11 chain was introduced by a copper-catalyzed Grignard addition. Ozonolysis of alkene 11 led to aldehyde 12 in 76% starting from (S)-citronellol. Benzyl-ether functionalized Grignard reagent 13 was added to aldehyde 12 to yield alcohol 14 in 78%. The alcohol was subsequently removed in a two step protocol. Tosylation followed by elimination led to a mixture of olefins 15 and 16. Simultaneous removal of the alkene and benzyl group was achieved by treatment with hydrogen and Pd/C to give alcohol 17 in 58% yield from 14. Oxidation of alcohol 17 with acidified potassium permanganate afforded (R)-tuberculostearic acid (3) in 98% yield.
4.2.3 Chiral pool strategy based on citronellyl bromide

Although the synthesis of Larsen is only eight steps compared to the nine steps required by Seeberger and co-workers, an even shorter 4 step synthesis was published one year earlier by the group of Baird.\(^{10}\) Here, the synthesis starts with commercially available (S)-citronellyl bromide (18). Direct Grignard coupling of hexylmagnesium bromide with (S)-citronellyl bromide (18) catalyzed by Li\(_2\)CuCl\(_4\) afforded olefin 19 in 95\% yield. Also in this case the olefin was oxidized by ozonolysis to aldehyde 20, which is then treated with an acid-functionalized Wittig reagent and the corresponding olefin 21 was isolated in 65\% yield. Hydrogenation of 21 afforded the (R)-tuberculostearic acid 3 in 94\% yield.
4.3 Results and discussion

4.3.1 Isolation of phosphatidylethanolamine from *M. tuberculosis* (performed at Harvard Medical School)

Total lipid extracts of *M. tuberculosis* strain H37Rv were prepared as previously described. One gram of lipid extracted into 2:1 (v/v) of chloroform : methanol was separated over a 75 mL open silica column (Alltech, Deerfield, IL) by sequential elution with five column volumes of chloroform, acetone, and methanol, respectively. The methanol eluting lipids were recovered, and 15 mg were separated by one dimensional thin layer chromatography on 200 µm silica plates resolved with chloroform, methanol, and water (v/v/v 60:30:6). Bands were visualized by spraying with water prior to scraping. Lipids were serially extracted from scraped bands with chloroform and methanol (v/v 2:1, 1:2) and methanol alone. Lipid content was analyzed by positive-mode electrospray ionization mass spectrometry (ThermoFinnigan LCQ Advantage). Phosphatidylethanolamine molecular species were detected in a band with RF of 0.47. Further purification of molecular species of phosphatidylethanolamine was achieved by high performance liquid chromatography/mass spectrometry. Approximately 50 µg of phosphatidylethanolamine containing palmitic and tuberculostearic acids was obtained at >95% purity as assessed by MS and 1H-NMR.
4.3.2 Catalytic asymmetric synthesis of (R)-tuberculostearic acid

As a prelude to TBSA containing glycerophosphatidylethanolamine we started with a novel synthesis of (R)-TBSA. Although the existing synthetic routes are versatile, they are all based on chiral pool strategies. We anticipated that the copper-catalyzed asymmetric conjugate addition (ACA) with methyl magnesium bromide could be applied in the synthesis of methyl branched (R)-TBSA also. This approach gives more flexibility and allows the preparation of derivatives, labeled compounds and analogues.

Unsaturated thioester 24 was obtained in 92% yield by a ruthenium-catalyzed olefin metathesis of olefin 22 and S-ethyl thioacrylate (23) using Hoveyda-Grubbs second generation catalyst 28. This strategy for the construction of unsaturated thioesters from terminal olefins was recently reported by our group.\(^\text{12}\) The use of copper-catalyzed ACA on unsaturated thioesters has recently been applied in a number of natural product syntheses by our group (Chapter 1)\(^\text{13a-f}\).

Thioester 24 was subjected to a copper-catalyzed ACA with methylmagnesium bromide and (S,R\(_6\))-Josiphos (30) as the ligand.\(^\text{13e}\) Methyl-branched thioester 25 was isolated in 85% yield with an ee of 90% (see section 4.3.3 for ee determination).\(^\text{14}\) The thioester functionality was selectively reduced in the presence of the iso-propyl oxo-ester to the corresponding aldehyde under Fukuyama conditions.\(^\text{15}\) Wittig reagent \(\text{C}_2\text{H}_4\text{CH} = \text{PPh}_3\) was added to the reaction mixture and a mixture of \(E\)- and \(Z\)-isomers of 26 was obtained in 92% yield from 25. Olefin 26 was reduced under transition-metal free conditions to avoid any double bond isomerization and thereby potentially epimerization of the methyl branched stereocenter. Flavin catalyst 29 in combination with hydrazine produced diimide \textit{in situ} which reduced olefin 26 in a concerted fashion. This catalytic methodology was recently published by our group.\(^\text{16}\) Saturated propylester 27 was hydrolyzed with KOH, leading to (R)-TBSA 3 in 5 steps starting from 22 with an overall yield of 67% (92% on average per step).
Scheme 4: Total synthesis of (R)-TBSA (3).

4.3.3 Ee determination of thioester 25

The ee determination of 25, described in the previous paragraph, deserves special attention. No direct method could be found to determine the ee of the 1,4-addition product by either chiral GC or HPLC. Reduction of the thioester to the alcohol followed by Mosher ester formation did not show separated visible signals for the two diastereomers in the $^1$H-, $^{13}$C- or $^{19}$F-NMR spectra. Enantiomeric excess and absolute configuration of
stereocenters bearing a methyl group on alkyl chains, however, has been resolved in the case where the alcohol functionality is positioned on the CH$_2$ next to the methyl branch.$^{17}$ In the case of 25 (after reduction of the thioester to the corresponding alcohol) there is an additional methylene moiety between the alcohol and the methyl substituent. Therefore we decided to remove one CH$_2$ between the methyl branch and the thioester which would lead to the desired alcohol after reduction. This shortening strategy is depicted in Scheme 5.

A small sample (100 mg) of 25 was turned into the corresponding methyl ketone 31 selectively by treatment of the thioester with Gilman reagent.$^{18}$ The methyl ketone was subsequently transformed into the acetate ester 32 by means of a Baeyer-Villiger reaction (which is regioselective for the internal position). Both ester functionalities were hydrolyzed and the remaining acid was directly converted into a methyl ester by treatment with (trimethylsilyl)diazomethane in MeOH. Alcohol 33 was then treated with (S)-(+) alpha-methoxy-alpha-trifluoromethylphenylacetyl chloride to give the (R)-Mosher ester 34 (priorities change!)$^{19}$ which was compared to the racemic product.

Scheme 5: Derivatization of thioester 25 and Mosher ester formation.

Derivatization of racemic 25 as depicted in Scheme 5 showed that the diastereomers are clearly visible by $^1$H-NMR (Figure 4) as has been described for a related compound.$^{17}$ The chemical shift of the CH$_2$-group
next to the Mosher ester moiety of both diastereomers is different and can be used to compare to the Mosher ester of the enantiomerically-enriched product.

Figure 4: $^1$H-NMR of the diastereomers of Mosher ester 34.

The diastereomeric ratio determined by $^1$H-NMR was 95 : 5 which results in an ee of 90% for the product of the 1,4-addition on thioester 24. The major diastereomer also matched with the expected absolute configuration of the methyl branch stereocenter as was reported for similar compounds by several groups.\(^{20}\)

4.3.4 Synthesis of tuberculostearic acid containing phosphatidylethanolamine.

Commercially available (R)-benzyl glycidyl ether (35) was heated to 100 °C together with one equivalent of (R)-TBSA (3) as a melt with catalytic amounts of Bu$_4$NBr.\(^5\) Ester 36 was obtained in 81% yield together with a small amount of the other regioisomer which could be separated by column chromatography. The 2-position was acylated with palmitic acid (C$_{15}$H$_{29}$COOH) and DCC as the coupling agent to provide 37 in 70% yield.\(^9\) Pd-catalyzed hydrogenolysis of the benzyl ether in a 1:1 mixture of
ethanol and acetic acid resulted in alcohol 38 in 76% yield. Basic conditions could easily lead to undesired acyl migration. We only observed traces of the 1,3-sn-diacyl glycerol product, which could be separated by flash chromatography. Initially, we tried different methods for the introduction of the phosphatidylethanolamine group starting from phosphoryl chloride which always gave unsatisfactory results.\(^1\)

\[
\begin{align*}
\text{Scheme 6: Synthesis of 1-O-TBSA-2-O-palmitoyl-sn-phospholipid (1).}
\end{align*}
\]
The phosphatidylethanolamine group was introduced in a two step one pot procedure. Phosphoramidite reagent 39 was added to 38 together with 5-phenyl-1H-tetrazole. The reaction was followed by TLC and after full conversion of the starting material to 40, m-chloroperbenzoic acid (mCPBA) was added as the oxidant. Benzyl and carboxybenzyl (Cbz) protected 41 was obtained in 75%. Finally, phosphate 41 was exposed to hydrogenolysis conditions to remove the benzyl ether and Cbzprotecting groups in one step to yield the desired product 1-O-TBSA-2-O-palmitoyl-sn-phospholipid (1) in 73% yield.

The regioisomer of 1-O-TBSA-2-O-palmitoyl-sn-phospholipid (1), that is 1-O-palmitoyl-2-O-TBSA-sn-phospholipid (2) was synthesized in the same way, the only difference being the order of the introduction of the acyl residues. Palmitic acid was first introduced followed by (R)-TBSA (3). Regioisomer 2 was obtained in 5 steps, (R)-TBSA synthesis not included, with an over yield of 19%.

![Structure of regioisomer 1-O-palmitoyl-2-O-TBSA-sn-phospholipid (2).](image)

**Figure 5:** Structure of regioisomer 1-O-palmitoyl-2-O-TBSA-sn-phospholipid (2).

### 4.3.5 Structural assignment of the isolated phospholipid

The structural assignment of phospholipids with two different acyl residues has been studied by Smith et al. in 1995. Negative-ion electrospray ionization tandem mass spectrometry was used to compare the fragmentation pattern of two commercially available regioisomers, 1-O-palmitoyl-2-O-oleoyl-sn-glycero-3-phosphoethanolamine and 1-O-oleoyl-2-O-palmitoyl-sn-glycero-3-phosphoethanolamine. As was already suggested by Gross et al., cleavage of the acyl residues occurred preferentially at the sn-2 position of the glycerol backbone resulting in a higher abundance of the sn-2 positioned carboxylic acid residue (approximately in a 2:1 ratio).
A mechanistic study by Hsu and Turk suggests that this preferred cleavage at the sn-2 position is the result of a sterically less hindered nucleophilic attack of the anionic phosphate onto the C-2 of the glycerol backbone compared to the C-1 position (Scheme 7).

Scheme 7: Nucleophilic attack of the anionic phosphate group at sn-2 compared to sn-1 suggested by Hsu and Turk.

This hypothesis suggests that the sn-2 position is less sterically hindered compared to the sn-1 position which to our knowledge is not correct. The preferred cleavage of the sn-2 position could, however, be explained by a more stable carbocation intermediate. This suggests a $S_{n1}$ type mechanism with a preference for the more stable secondary carbocation compared to the primary one.

However, Gilleron et al. used the same principle in 2001 to determine the fatty acid distribution of mycobacterial phosphatidylyl-myo-inositol mannosides by observing of what they thought was the preferred sn-2-glycerol cleavage in mass analysis and assigned a TBSA residue to the sn-2 position of the glycerol backbone. They later observed that their analysis was wrong and re-assigned the TBSA residue to the sn-1 position. Painter et al. published the synthesis of phosphatidylinositol dimannoside, a natural product from mycobacteria, in 2007. The synthesis of sn-1 palmitoyl and sn-2 tuberculosteryl containing glycerophospholipid synthesis was based on the earlier work by Gilleron et al. (2001) and
therefore they unfortunately made the wrong regioisomer of the natural product!

The two synthetic isomers 1 and 2 were compared with the natural product by MS/MS analysis in order to establish the correct structure of the natural isolate. The mass of the natural isolate \((m/z = 732.550)\) and the synthetic compounds 1-O-TBSA-2-O-palmitoyl-sn-phospholipid (1) \((m/z = 732.551)\) and 1-O-palmitoyl-2-O-TBSA-sn-phospholipid (2) \((m/z = 732.552)\) agreed well and were within 6 ppm of the calculated value for \(C_{40}H_{79}NO_8P\) \((m/z = 732.555)\). Additional information, confirming the presence of the TBSA acyl residue, was found by comparing the \(^1H\)-NMR spectrum of the natural product with that of the synthetic compounds. The \(\text{CH}_3\) branch has a distinct chemical shift and coupling constant in \(^1H\)-NMR. For both the natural isolate and the synthetic compounds a doublet at 0.83 ppm was observed with \(J = 6.4\) Hz.

To determine the position of the acyl residues in the natural product we compared the fragmentation pattern of the natural compound to each of the two synthetic regioisomers 1 and 2 (Figure 6). MS/MS (ESI) in the negative mode shows a clear difference in the fragmentation pattern. The acyl groups at the \(sn\)-2-position of the glycerol backbone are preferentially cleaved, as predicted. Cleavage can occur between the carbonyl carbon atom and the oxygen atom with transfer of hydrogen to leave a hydroxy group on the resulting fragment ion (phosphatidyl fragment), \(m/z\) 494 and \(m/z\) 452 for regioisomer 1 and 2, respectively. Alternatively, cleavage occurs between the oxygen and glycerol backbone resulting in a negatively-charged carboxylate fragment \((m/z\) 255 and \(m/z\) 297 for palmitic acid and TBSA, respectively). These types of fragmentation occur preferentially at the \(sn\)-2-position of the glycerol backbone compared to the \(sn\)-1-position. The fragmentation pattern of the natural product matches with synthetic 1, whereas that of isomer 2 clearly does not match with the natural product.
Figure 6: MS/MS analysis (ESI) negative mode, fragmentation patterns for the natural isolate and regioisomers 1 and 2.

With both synthetic 1-O-TBSA-2-O-palmitoyl-sn-phospholipid (1) and 1-O-palmitoyl-2-O-TBSA-sn-phospholipid (2) in hand, we conclude that it is straightforward to determine the position of two acyl side chains in diacylglycerol-phosphatidylethanolamine lipids by MS/MS analysis. The acyl residue at the sn-2-position will give a higher abundance in the ESI mass spectrum compared to the acyl residue at the sn-1-position. This is consistent with the reports of Hsu and Turk.25
4.4 Conclusions

We have developed the first catalytic enantioselective synthesis of (R)-tuberculostearic acid (3). State of the art catalytic methods have been applied in this synthesis (cross-metathesis, enantioselective 1,4-addition and organo-catalytic alkene reduction). R-TBSA (3) was synthesized in 5 steps starting from iso-propyl ester 22 with an overall yield of 67%.

A newly isolated glycerophospholipid from *M. tuberculosis* was characterized and analyzed by MS/MS analysis. Two regioisomers of the phospholipid were successfully synthesized and compared to the natural compound. MS/MS analysis was used to determine the position of the acyl residues. The natural product was identified as 1-O-TBSA-2-O-palmitoyl-sn-phosphatidylethanolamine (1). Additional evidence was obtained by ¹H-NMR. Different di-acyl phosphatidylethanolamines can quickly be analyzed and identified by mass analysis and the relative position of the acyl residues can be determined by comparison of the mass fragmentation patterns. Production of this synthetic version of mycobacterial PE, with a stereochemically correct and pathogen-specific fatty acyl group in high yield, can be used as a standard in LC-MS based lipidomic analyses to detect trace amounts of mycobacterial PE in human blood, sputum or tissues as a marker of infection by mycobacteria.
4.5 Experimental

General remarks:

For general experimental procedures, see chapter 2, experimental section.

Tandem Mass Spectrometry (performed at Harvard Medical School)

Analytical MS/MS was performed on LCQ Advantage (Thermo Finnigan, Waltham, MA). Compounds were analyzed by nanospray ESI-MS using borosilicate glass pipettes pulled to a 2-µm orifice (internal electrode 1.2 kV). Ions were detected in the negative mode and collided at 20% energy.

Chromatography of natural and synthetic phospholipids (performed at Harvard Medical School)

Natural and synthetic compounds were analyzed on a 1200 series HPLC with a quadrupole time of flight (Q-TOF) mass spectrometer (Agilent Technologies, Santa Clara, CA). Voltage 3.5 kV, source temperature 325 °C, drying gas 5 L/min, nebulizer pressure 30 psi, and ions were detected in the negative mode. Approximately 2 µg of each of the synthetic compounds and 1 µg of purified natural phospholipid was injected via an autoinjector. A 250 mm x 4.6 mm C\textsubscript{18}-bonded silica column (Vydac, Deerfield, IL) was used with a gradient elution program in which solvent A consists of 50% (v/v) methanol, 30% (v/v) acetonitrile, 20% (v/v) water, and solvent B consists of 90% (v/v) isopropanol and 10% (v/v) hexanes (additives were 0.1% (v/v) formic acid, 0.01% (v/v) ammonium acetate, 0.02% (v/v) trifluoroacetic acid, 0.02% (v/v) hexafluoroisopropyl alcohol). Solvent B was run at 60% (v/v) from 0 to 4 min and then increased to 100% (v/v) from 16 min to 28 min.

Preparative HPLC purification of the isolated phospholipid (performed at Harvard Medical School)

HPLC/MS (Thermo Scientific LXQ, Waltham, MA) using a 4.6 mm x 250 mm C\textsubscript{18}-bonded silica column (Vydac, Deerfield, IL) as the stationary phase and isocratic elution with a mobile phase consisting of 63% (v/v) isopropanol, 15% (v/v) methanol, 9% (v/v) acetonitrile, 7% (v/v) hexanes, and 6% (v/v) water (additives were 0.1% (v/v) formic acid, 0.01% (v/v)
ammonium acetate, 0.02% (v/v) trifluoroacetic acid, 0.02% (v/v) hexafluoroisopropylalcohol).

(E)-iso-Propyl 12-(ethylthio)-12-oxododec-10-enoate (24)

Unsaturated thioester 24 was prepared from commercially available undec-10-enoic acid in two steps. First undec-10-enoic acid (10 g, 54.27 mmol) was refluxed overnight in 100 mL isopropyl alcohol with a catalytic amount (2 mol%) of p-toluenesulfonic acid monohydrate (206 mg, 1.085 mmol). The reaction mixture was cooled down to rt and concentrated under reduced pressure. The crude product was flushed over a small plug of silica with pentane/diethyl ether (9:1). The filtrate was concentrated to yield 7.08 g (58%) of pure isopropyl ester. $^1$H-NMR (400 MHz, CDCl$_3$): δ 5.77 (td, $J = 6.7, 10.2, 16.9$ Hz, 1H), 5.02-4.87 (m, 3H), 2.22 (t, $J = 7.5$ Hz, 2H), 2.02-1.97 (m, 2H), 1.61-1.54 (m, 2H), 1.37-1.22 (br, 10H), 1.19 (d, $J = 6.3$ Hz, 6H); $^{13}$C-NMR (CDCl$_3$, 100.6 MHz) δ 173.23 (s), 139.00 (d), 114.05 (t), 67.16 (d), 34.61 (t), 33.71 (t), 29.21 (t), 29.13 (t), 29.01 (t), 28.97 (t), 28.81 (t), 24.94 (t), 21.76 (q, 2 x C). The isopropyl ester (4.00g, 17.54 mmol) was dissolved in 30 mL dry CH$_2$Cl$_2$ and nitrogen gas was bubbled through for 30 min. Thioacrylate 23 (1.344 mL, 11.69 mmol) was added via a syringe, 1 mol% of Hoveyda-Grubbs second-generation catalyst 28 was added in one portion and the mixture was stirred for 1 d under nitrogen. The solution was concentrated under reduced pressure and the crude material was purified by flash chromatography (pentane/diethyl ether, 20:1) to afford unsaturated thioester 24 as a colorless oil (3.382 g, 92% yield, the product contains small amounts of homo-coupled isopropyl ester, which can be separated in the next step). $^1$H-NMR (400 MHz, CDCl$_3$): δ 6.86 (td, $J = 6.9, 15.4$ Hz, 1H), 6.07 (d, $J = 15.1$ Hz, 1H), 4.98 (heptet, $J = 6.3$ Hz, 1H), 2.91 (q, $J = 7.4$ Hz, 2H), 2.23 (t, $J = 7.5$ Hz, 3H), 2.16 (m, 2H), 1.58(m, 2H), 1.43(m, 2H), 1.37-1.22 (br, 10H), 1.19 (d, $J = 6.3$ Hz, 6H); $^{13}$C-NMR (CDCl$_3$, 100.6 MHz) δ 190.07, 173.29, 145.29, 128.60, 67.24, 34.61, 32.07, 29.11, 29.05, 29.01, 28.97, 27.88, 24.92, 22.95, 21.79 (2 x C), 14.78. HRMS(ESI+) calculated for C$_{17}$H$_{30}$O$_3$S (M + Na$^+$) 337.1813, found 337.1805.
A Glycerophospholipid from M. tuberculosis; Structure Elucidation by MS/MS Analysis and Asymmetric Synthesis

(-)-(S)-iso-Propyl 12-(ethylthio)-10-methyl-12-oxododecanoate (25)

(S,R<sub>en</sub>)-Josiphos•CuBr complex (23 mg, 0.032 mmol, 1 mol%) was dissolved in t-BuOMe (30 mL) under nitrogen. The solution was cooled to –75°C and methylmagnesium bromide (4.141 mmol, solution in diethyl ether) was added dropwise over 10 min. After stirring for 10 min, a solution of thioester 24 (1.000 g, 3.185 mmol) in t-BuOMe (6.0 mL) was added via syringe pump over 30 min. The reaction mixture was stirred at –75 °C for 17 h, then quenched by the addition of MeOH and allowed to warm to room temperature. A saturated aqueous NH<sub>4</sub>Cl solution was then added. After phase separation and extraction of the aqueous phase with 3 portions of diethyl ether (30 mL), the combined organic phases were dried over MgSO<sub>4</sub>, concentrated under reduced pressure and purified by flash chromatography (pentane/diethyl ether, 20:1) to afford 25 as a colorless oil (956 mg, 91% yield, 90% ee, [α]<sub>D</sub> = –3.1º (c = 1.31, CHCl<sub>3</sub>). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ 4.97 (heptet, <i>J</i> = 6.3 Hz, 1H), 2.84 (q, <i>J</i> = 7.4 Hz, 2H) 2.49 (dd, <i>J</i> = 6.0, 14.4 Hz, 1H) 2.30 (dd, <i>J</i> = 8.1, 14.4 Hz, 1H), 2.22 (t, <i>J</i> = 7.5 Hz, 3H), 2.04-1.90 (br, 1H), 1.62-1.51 (br, 2H), 1.34-1.20 (br, 14 H), 1.20 (d, <i>J</i> = 6.3 Hz, 6H), 0.89 (d, <i>J</i> = 6.7 Hz, 6H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100.6 MHz) δ 199.21 (s), 173.30 (s), 67.20 (d), 51.31 (t), 36.51 (t), 34.63 (t), 30.99 (t), 29.55 (t), 29.32 (t), 29.15 (t), 29.02 (t), 26.73 (t), 24.95 (t), 23.18 (t), 21.79 (q), 19.45 (q), 14.75 (q). HRMS(ESI+) calculated for C<sub>18</sub>H<sub>34</sub>O<sub>3</sub>S (M + Na<sup>+</sup>) 353.2126, found 353.2115.

(S)-iso-Propyl 10-methyloctadec-12-enoate (26)

To a stirred solution of thioester 25 (497 mg, 1.506 mmol) and 0.730 mL (8.853 mmol) triethylsilane in 3 mL CH<sub>2</sub>Cl<sub>2</sub>, 81 mg Pd/C (10%) (5 mol%) was added in one portion. The resulting mixture was stirred for 30 min at rt. After filtration over a small Celite plug, the reaction mixture was concentrated under reduced pressure and the crude aldehyde was directly used without further purification. Hexyltriphenylphosphonium bromide 1.287 g (2 equiv.,
3.012 mmol) in 15 mL THF was cooled down to –20°C and 1.5 equiv. of n-BuLi (1.412 mL, 1.6M in hexane) was added in a dropwise fashion. The reaction mixture was brought to rt. The crude aldehyde was dissolved in 2 mL THF and was added dropwise to the solution of the Wittig reagent. After 3 h the reaction was quenched with a saturated aq. solution of NH₄Cl and 20 mL ether was added. After phase separation and extraction of the aqueous phase with 3 portions of diethyl ether (30 mL), the combined organic phases were dried over MgSO₄, concentrated under reduced pressure and purified by flash chromatography (pentane/diethyl ether 9:1) to afford 26 as a mixture of E and Z stereo-isomers (468 mg, 92% yield).

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\text{\textsuperscript{1}H-NMR (400 MHz, CDCl}_3): \delta 5.41-5.29 (m, 2H), 4.99 (\text{heptet, } J = 6.3 \text{ Hz, 1H}), 2.24 (t, J = 7.5 \text{ Hz, 2H}), 2.01-1.58 (br, 2H), 1.84 (\text{quintet, } J = 7.4 \text{ Hz, 2H}), 1.63-1.55 (br, 2H), 1.45-1.17 (br, 19 H), 1.21 (d, J = 6.3 Hz, 6H), 0.97 (t, J = 8.0 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H).
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\(\text{\textsuperscript{13}C-NMR (CDCl}_3, 100.6 \text{ MHz}) \delta 173.38 (s), 67.24 (t), 37.08 (t), 34.71 (t), 32.73 (d), 31.92 (t), 30.03 (t), 29.93 (t), 29.68 (t), 29.50 (t), 29.36 (t), 29.27 (t), 29.12 (t), 27.07 (t), 27.03 (t), 26.99 (t), 25.03 (t), 22.67 (t), 21.83 (q, 2 x C), 19.69 (q), 14.10 (q). HRMS(El+) calculated for C₂₂H₄₄O₂ 340.3341, found 340.3356.

\(\text{\textsuperscript{1}H-NMR (400 MHz, CDCl}_3): \delta 5.00 (\text{heptet, } J = 6.3 \text{ Hz, 1H}), 2.25 (t, J = 7.5 \text{ Hz, 1H}), 1.63-1.56 (m, 2H), 1.42-0.99 (br, 28H), 1.22 (d, J = 6.4 \text{ Hz, 6H}), 0.87 (t, J = 6.8 \text{ Hz, 3H}), 0.83 (d, J = 6.5 \text{ Hz, 3H}); \text{\textsuperscript{13}C-NMR (CDCl}_3, 100.6 \text{ MHz}) \delta 173.38 (s), 67.24 (d), 37.08(t), 34.71 (t), 32.73 (d), 31.92 (t), 30.03 (t), 29.93 (t), 29.68 (t), 29.50 (t), 29.36 (t), 29.27 (t), 29.12 (t), 27.07 (t), 27.03 (t), 26.99 (t), 25.03 (t), 22.67 (t), 21.83 (q, 2 x C), 19.69 (q), 14.10 (q). HRMS(El+) calculated for C₂₂H₄₄O₂ 340.3341, found 340.3356.
(−)-(R)-10-Methyloctadecanoic acid (Tuberculostearic acid) (3)

mg, 0.606 mmol) was dissolved in a mixture of EtOH and H₂O (1:1, 5 mL) and 10 eq. KOH (340 mg, 6.06 mmol) was added. The mixture was stirred for 17 h at rt. Diethyl ether was added (10 mL) together with 5 mL H₂O. After phase separation and extraction of the aqueous phase with 3 portions of diethyl ether (15 mL), the combined organic phases were dried over MgSO₄, concentrated under reduced pressure and purified by flash chromatography (pentane/diethyl ether, 1:1 and one drop of acetic acid) to afford 3 as a colorless semisolid (180 mg, 95 % yield). [α]D = −0.31° (c = 2.10, CHCl₃). ¹H-NMR (400 MHz, CDCl₃): δ 3.25 (t, J = 7.5 Hz, 2H), 1.63 (m, 2H), 1.42-1.00 (m, 28H), 0.88 (t, J = 6.8 Hz, 3H). ¹³C-NMR (CDCl₃, 100.6 MHz) δ 180.39 (s), 37.11 (t), 37.09 (t), 34.10 (t), 32.75 (d), 31.93 (t), 30.04 (t), 29.93 (t), 29.69 (t), 29.47 (t), 29.37 (t), 29.25 (t), 29.07 (t), 27.09 (t), 27.04 (t), 24.67 (t), 22.69 (t), 19.70 (q), 14.10 (q). HRMS(El+) calculated for C₂₉H₄₉O₂: 298.2872, found 298.2885.

(−)-(R)-3-(Benzyloxy)-2-hydroxypropyl 10-methyloctadecanoate (36)

(R)-(−)-Benzyl glycidyl ether 35 (49 µL, 0.321 mmol) and (R)-TBSA 3 (100 mg, 0.321 mmol) were heated to 100°C as a melt together with 3 mol% (2 mg) Et₃NBr for 3 h. The melt was cooled down to rt and was purified by flash chromatography (pentane/diethyl ether, 7:3) to afford 36 as a white wax (120 mg, 81 % yield). A trace amount of the undesired regioisomer could be separated from 36 in the chromatography step. [α]D = +1.1° (c = 2.25, CHCl₃). ¹H-NMR (400 MHz, CDCl₃): δ 7.37-7.27 (m, 5H), 4.55 (s, 2H), 4.19 (dd, J = 4.5, 11.5 Hz, 1H), 4.14 (dd, J = 6.0, 11.5 Hz, 1H), 4.03 (m, 1H) 3.55 (dd, J = 4.3, 9.6 Hz, 1H) 3.49 (dd, J = 6.1, 9.6 Hz, 1H), 2.66-2.62 (br, 1H, OH), 2.32 (t, J = 7.6 Hz, 2H), 1.35-1.00 (br, 29H), 0.88 (t, J = 6.8 Hz, 3H), 0.84 (d, J = 6.5 Hz, 3H); ¹³C-NMR (CDCl₃, 100.6 MHz) δ 173.82 (s), 137.64 (s), 128.40 (d), 127.80 (d), 127.68 (d), 73.45 (t), 70.86 (t), 68.88
(S)-3-(Benzyloxy)-2-hydroxypropyl palmitate (36b)

![Chemical Structure](image)

(R)-(−)-Benzylic glycidyl ether 35 (49 µL, 0.321 mmol) and palmitic acid (82 mg, 0.321 mmol) were heated to 100°C as a melt together with 3 mol% (2 mg) Et3NBr for 3 h. The melt was cooled down to rt and the product was purified by flash chromatography (pentane/diethyl ether, 7:3) to afford 36b as a white wax (113 mg, 84% yield). Spectral data as reported in the literature.27

(+)-(R)-(−)-(S)-3-(Benzyloxy)-2-(palmitoyloxy)propyl 10-methyloctadecanoate (37)

![Chemical Structure](image)

Palmitic acid (89 mg, 0.35 mmol) was dissolved in 4 mL CH2Cl2 with 71 mg DCC (0.35 mmol) and 1 mg DMAP. Compound 36 (80 mg, 0.17 mmol) was added in one portion and the mixture was stirred for 16 h. The mixture was filtered over Celite, the filtrate was concentrated under reduced pressure and the crude product was purified by flash chromatography (pentane/diethyl ether, 9:1) to yield 37 as a white waxy solid (85 mg, 70%, [α]D = +5.0° (c = 1.33, CHCl3)). 1H-NMR (300 MHz, CDCl3): δ 7.36-7.28 (m, 5H), 5.25 (m, 1H) 4.54 (d, J = 3.5 Hz, 2H) 4.35 (dd, J = 3.7, 11.9 Hz, 1H) 4.19 (dd, J = 6.4, 11.9 Hz, 1H) 3.59 (m, 1H) 2.32 (t, J = 7.4 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 1.68-1.51 (m, 4H), 1.40-1.00 (br, 52H), 0.88 (t, J = 6.6 Hz, 6H), 0.84 (d, J = 6.4 Hz, 3H); 13C-NMR (CDCl3),
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100.6 MHz) δ 173.32 (s), 173.02 (s), 137.65 (s), 128.35 (d), 127.71 (d), 127.56 (d), 73.24 (t), 69.93 (d), 68.19 (t), 62.59 (t), 37.05 (t, 2 x C), 34.27 (t), 34.06 (t), 32.71 (d), 31.89 (t), 30.00 (t), 29.94 (t), 29.66 (t, 9 x C), 29.63 (t), 29.60 (t), 29.49 (t), 29.45 (t), 29.33 (t, 2 x C), 29.26 (t, 2 x C), 29.09 (t), 29.05 (t), 27.07 (t), 27.03 (t), 24.91 (t), 24.83 (t), 22.65 (t), 19.65 (q), 14.08 (q, 2 x C). MS(EI+) for C_{45}H_{80}O_{5}: m/z(%) = 701.0 (0.8%, M + H^+), MS(Cl+) for C_{45}H_{80}O_{5}: m/z(%) = 718.0 (100%, M + NH_4^+).

(+)-(R)-(S)-2-(Benzyloxy)-3-(palmitoyloxy)propyl 10-methyloctadecanoate (37b)

R-TBSA 3 (13 mg, 0.042 mmol) was dissolved in 1 mL CH_2Cl_2 with 26 mg DCC (0.13 mmol) and 0.5 mg DMAP. Alcohol 36b (26 mg, 0.063 mmol) was added in one portion in 1 mL CH_2Cl_2 and the mixture was stirred for 16 h. The mixture was filtered over Celite, the filtrate was concentrated under reduced pressure and the crude product was purified by flash chromatography (pentane/diethyl ether, 9/1) to yield 37b as a white waxy solid (19 mg, 65%, [α]_D = +4.8° (c = 1.00, CHCl_3)). 1H-NMR (300 MHz, CDCl_3): δ 7.36-7.28 (m, 5H), 5.25 (m, 1H) 4.54 (d, J = 3.5 Hz, 2H) 4.35 (dd, J = 3.7, 11.9 Hz, 1H) 4.19 (dd, J = 6.4, 11.9 Hz, 1H) 3.59 (m, 1H), 2.32 (t, J = 7.4 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 1.68-1.51 (m, 4H), 1.40-1.00 (br, 52H), 0.88 (t, J = 6.6 Hz, 6H), 0.84 (d, J = 6.4 Hz, 3H); 13C-NMR (CDCl_3, 100.6 MHz) δ 173.32 (s), 173.02 (s), 137.65 (s), 128.35 (d), 127.71 (d), 127.56 (d), 73.24 (t), 69.93 (d), 68.19 (t), 62.59 (t), 37.05 (t, 2 x C), 34.27 (t), 34.06 (t), 32.71 (d), 31.89 (t), 30.00 (t), 29.94 (t), 29.66 (t, 9 x C), 29.63 (t), 29.60 (t), 29.49 (t), 29.45 (t), 29.33 (t, 2 x C), 29.26 (t, 2 x C), 29.09 (t), 29.05 (t), 27.07 (t), 27.03 (t), 24.91 (t), 24.83 (t), 22.65 (t), 19.65 (q), 14.08 (q, 2 x C). MS (EI+) for C_{45}H_{80}O_{5}: m/z(%) = 701.0 (0.8%, M + H^+), MS(Cl+) for C_{45}H_{80}O_{5}: m/z(%) = 718.0 (100%, M + NH_4^+)
(−)-(R)-((S)-3-Hydroxy-2-(palmitoyloxy)propyl) 10-methyloctadecanoate (38)

Benzyl ether 37 (84 mg, 0.12 mmol) was dissolved in a 1/1 mixture of acetic acid and ethanol (13 mL) and 12.6 mg Pd/C (10%) was added. The reaction mixture was stirred for 17 h under a H₂-atmosphere (balloon). The reaction mixture was filtered over Celite and the filtrate was concentrated under reduced pressure. The crude material was purified by flash chromatography (pentane/diethyl ether, 1/1) to yield 38 as a white waxy solid (56 mg, 76%, [α]D = −2.9° (c = 0.90, CHCl₃)). ¹H-NMR (400 MHz, CDCl₃); δ 5.08 (m, 1H) 4.31 (dd, J = 6.8 Hz, 6H) 0.82 (d, J = 6.5 Hz, 1H) 1.61 (m, 4H) 1.41-1.00 (br, 51H) 0.87 (t, J = 6.8 Hz, 6H) 0.82 (d, J = 6.5 Hz, 3H); ¹³C-NMR (CDCl₃, 100.6 MHz) δ 174.03 (s), 173.67 (s), 72.30 (s), 62.24 (t), 61.72 (t), 37.32 (t), 34.51 (t), 34.33 (t), 32.98 (d), 32.15 (t), 30.27 (t), 30.20 (t), 29.93 (t, 8 x C), 29.89 (t), 29.85 (t), 29.75 (t), 29.71 (t), 29.60 (t, 2 x C), 29.51 (t, 2 x C), 29.36 (t), 29.32 (t), 27.32 (t), 27.29 (t), 25.16 (t), 25.11 (t), 22.92 (t), 19.92 (q), 14.35 (q, 2 x C). HRMS(El⁺) calculated for C₂₈H₄₇O₄ (M – H₂O) 592.5431, found 592.5417.

(−)-(R)-((S)-2-Hydroxy-3-(palmitoyloxy)propyl) 10-methyloctadecanoate (38b)

Benzyl ether 37b (19 mg, 0.027 mmol) was dissolved in a 1/1 mixture of acetic acid and ethanol (3 mL) and 5 mg Pd/C (10%) was added. The reaction mixture was stirred for 24 h under a H₂-atmosphere (balloon). The reaction mixture was filtered over Celite and the filtrate was concentrated under reduced pressure. The crude material was purified by flash chromatography (pentane/diethyl ether, 1/1) to yield 38b as a white
waxy solid (9 mg, 55%, $[\alpha]_D = -3.2^\circ \; (c = 0.30, \text{CHCl}_3))$. $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 5.08 (m, 1H) 4.31 (dd, $J = 4.4$, 11.9 Hz, 1H) 4.22 (dd, $J = 5.7$, 12.0 Hz, 1H) 3.72 (m, 2H) 2.32 (m, 4H) 2.17 (t, $J = 6.5$ Hz, 1H) 1.61 (m, 4H) 1.41-1.00 (br, 51H) 0.87 (t, $J = 6.8$ Hz, 6H) 0.82 (d, $J = 6.5$ Hz, 3H); $^{13}$C-NMR (CDCl$_3$, 100.6 MHz) $\delta$ 174.03 (s), 173.67 (s), 72.30 (d), 62.24 (t), 61.72 (t), 37.32 (t), 34.51 (t), 34.33 (t), 32.98 (d), 32.15 (t), 30.27 (t), 30.20 (t), 29.93 (t, 8 x C), 29.89 (t), 29.85 (t), 29.75 (t), 29.71 (t), 29.60 (t, 2 x C), 29.51 (t, 2 x C), 29.36 (t), 29.32 (t), 27.32 (t), 27.29 (t), 25.16 (t), 25.11 (t), 22.92 (t), 19.92 (q), 14.35 (q, 2 x C). HRMS (EI+) calculated for C$_{38}$H$_{72}$O$_4$ (M – H$_2$O) 592.5431, found 592.5417.

(10R)-((2R)-3-((2-Amino(Cbz)ethoxy)(benzyloxy)phosphoryloxy)-2-(palmitoyloxy)propyl) 10-methyloctadecanoate (41a)

5-Phenyl-1H-tetrazole (13 mg, 0.090 mmol) was added to a stirred solution of alcohol 38 (55 mg, 0.090 mmol) in CH$_2$Cl$_2$ (3 mL) under nitrogen at 0 ºC. Reagent 39 (47 mg, 0.11 mmol) was added in one portion and the reaction mixture was slowly brought to rt. When the starting material was completely consumed (TLC, pentane/diethyl ether 1/1, 1 h), the reaction mixture was cooled down to 0 ºC and mCPBA (75 mg, 3 equiv., 70% purity) was added. The reaction was followed by TLC (pentane/diethyl ether, 1/1) until the reaction was completed (product on baseline TLC, 10 min). The reaction was quenched with 5 mL of saturated aq. NaHCO$_3$ solution. The aqueous layer was extracted with three portions of diethyl ether (3 x 10 mL). The combined organic layers were dried over MgSO$_4$ and concentrated under reduced pressure and purified by flash chromatography (diethyl ether) to afford 41a as a white solid (22 mg, 73%). $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 7.33 (m, 10H) 5.33 (m, 1H) 5.19 (m, 1H) 5.09 (s, 2H) 5.06 (dd, $J = 4.7$, 9.0 Hz, 2H) 4.27-4.10 (m, 6H) 3.43 (br, 2H) 2.28 (t, $J = 7.5$ Hz, 4H) 1.58 (m, 4H) 1.43-1.00 (br, 51H) 0.88 (t, $J = 6.8$ Hz, 3H).
(10R)-((2R)-2-((2-Amino(Cbz)ethoxy)(benzyloxy)phosphoryloxy)-3-(palmitoyloxy)propyl) 10-methyloctadecanoate (41b)

5-Phenyl-1H-tetrazole (2.7 mg, 0.018 mmol) was added to a stirred solution of alcohol 38b (9.3 mg, 0.015 mmol) in CH₂Cl₂ (2 mL) under nitrogen at 0 °C. Reagent 39 (13.1 mg, 0.030 mmol) was added in one portion and the reaction mixture was slowly brought to rt. When the starting material was completely consumed (TLC, pentane/diethyl ether 1/1, 1 h) the reaction mixture was cooled down to 0 °C and mCPBA (19 mg, 5 equiv., 70% purity) was added. The reaction was followed by TLC (pentane/diethyl ether 1/1) until the reaction was completed (product on baseline TLC, 10 min). The reaction was quenched with 2 mL of saturated aq. NaHCO₃ solution. The water layer was extracted with three portions of diethyl ether (3 x 5 mL). The combined organic layers were dried over MgSO₄ and concentrated under reduced pressure and purified by flash chromatography (diethyl ether) to afford 41b as a white solid (14 mg, 95%). $^1$H-NMR (400 MHz, CDCl₃): δ 7.33 (m, 10H) 5.33 (m, 1H) 5.19 (m, 1H) 5.09 (s, 2H) 5.06 (dd, J = 4.7, 9.0 Hz, 2H) 4.27-4.10 (m, 6H) 3.43 (br, 2H) 2.28 (t, J = 7.5 Hz, 4H) 1.58 (m, 4H) 1.43-1.00 (br, 51H) 0.88 (t, J = 6.8 Hz, 6H) 0.83 (d, J = 6.5 Hz, 3H); $^{13}$C-NMR (CDCl₃, 100.6 MHz) 173.17 (s), 172.82 (s), 156.27 (s), 136.31 (s), 135.31 (s), 128.76 (d, 2 x C), 128.62 (d, 2 x C), 128.42 (d, 2 x C), 128.05 (d, 2 x C), 128.03 (d), 127.99 (d), 69.72 (br, t), 69.20 (br, d), 67.03 (br, t), 66.90 (br, t), 65.48 (br, t), 61.51 (br, t), 41.30 and 41.23, t), 37.06 (t), 34.07 (t), 32.72 (d), 31.88 (t), 30.00 (t), 29.94 (t), 29.65 (t, 11 x C), 29.62 (t), 29.59 (t), 29.50 (t), 29.45 (t), 29.32 (t, 2 x C), 29.26 (t), 29.24 (t), 29.08 (t), 29.03 (t), 27.05 (t), 24.78 (t), 22.65 (t), 19.66 (q), 14.08 (q, 2 x C); $^{31}$P-NMR (162 MHz, CDCl₃) δ 0.31. HRMS (ESI+) calculated for C₅₂H₉₀NO₅P (M + Na⁺) 980.6357, found 980.6346.
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t), 69.20 (br, d), 67.03 (br, t), 66.90 (br, t), 65.48 (br, t), 61.51 (br, t), (41.30 and 41.23, t), 37.06 (t), 34.07 (t), 32.72 (d), 31.88 (t), 30.00 (t), 29.94 (t), 29.65 (t, 11 x C), 29.62 (t), 29.59 (t), 29.50 (t), 29.45 (t), 29.32 (t, 2 x C), 29.26 (t), 29.24 (t), 29.08 (t), 29.03 (t), 27.05 (t), 24.78 (t), 22.65 (t), 19.66 (q), 14.08 (q, 2 x C); $^{31}$P-NMR (162 MHz, CDCl$_3$) δ 0.31. HRMS (ESI+) calculated for C$_{55}$H$_{92}$NO$_{10}$P (M + Na$^+$) 980.6357, found 980.6346.

(+)1-O-TBSA-2-O-palmitoyl-sn-phospholipid (1)

To a solution of 41a (40 mg, 0.041 mmol) in CHCl$_3$/MeOH/H$_2$O (95/35/2, 10 mL), 30 mg of Pd/C (10%) was added and the mixture was stirred under a H$_2$-atmosphere for 16 h. The reaction mixture was filtered over Celite and the filtrate was concentrated under reduced pressure. The crude material was purified by flash chromatography (CHCl$_3$/MeOH/H$_2$O, 95/35/2) to afford 1 as a white solid (22 mg, 73%, [α]$_D$ = +6.9º (c = 0.32, CHCl$_3$). $^1$H-NMR (400 MHz, CDCl$_3$): δ 8.36 (m, 2H) 5.21 (m, 1H), 4.42-4.03 (m, 4H), 3.94 (br s, 2H), 3.47 (s, 1H), 3.15 (br s, 2H), 2.29 (m, 4H), 1.58 (br s, 4H), 1.40-1.00 (br, 51H), 0.88 (t, J = 6.7 Hz, 6H) 0.83 (d, J = 6.4 Hz, 3H); $^{13}$C-NMR (CDCl$_3$, 100.6 MHz): 173.63 (s), 173.34 (s), 70.50 (d), 64.13 (t), 62.78 (t), 62.45 (t), 40.67 (t), 37.40 (t), 37.38 (t), 34.50 (t), 34.31 (t), 33.03 (d), 32.16 (t, 2 x C), 30.29 (t, 2 x C), 29.98 (t, 2 x C), 29.94 (t), 29.92 (t), 29.88 (t), 29.85 (t), 29.65 (t, 5 x C), 29.61 (t, 2 x C), 29.47 (t), 29.42 (t), 27.38 (t), 27.35 (t), 25.18 (t), 25.11 (t), 22.92 (t, 2 x C), 19.90 (q), 14.35 (q, 2 x C). $^{31}$P-NMR (162 MHz, CDCl$_3$) δ 1.16. High resolution MALDI TOF calculated for C$_{40}$H$_{81}$NO$_8$P (M + H$^+$), 734.5694 found 734.5703.
(+)-1-O-Palmitoyl-2-O-TBSA-sn-phospholipid (2)

To a solution of 41b (14 mg, 0.014 mmol) in CHCl₃/MeOH/H₂O (95/35/2, 10 mL), 10 mg of Pd/C (10%) was added and the mixture was stirred under a H₂-atmosphere for 16h. The reaction mixture was filtered over Celite and the filtrate was concentrated under reduced pressure. The crude material was purified by flash chromatography (CHCl₃/MeOH/H₂O, 95/35/2) to afford 2 as a white solid (7 mg, 66%, [α]D = +5.8° (c = 0.12, CHCl₃)). 

¹H-NMR (400 MHz, CDCl₃): δ 8.36 (m, 2H) 5.21 (m, 1H), 4.42-4.03 (m, 4H), 3.94 (br s, 2H), 3.47 (s, 1H), 3.15 (br s, 2H), 2.29 (m, 4H), 1.58 (br s, 4H), 1.40-1.00 (br, 51H), 0.88 (t, J = 6.7 Hz, 6H) 0.83 (d, J = 6.4 Hz, 3H); 

¹³C-NMR (CDCl₃, 100.6 MHz): 173.63 (s), 173.34 (s), 70.50 (d), 64.13 (t), 62.78 (t), 62.45 (t), 40.67 (t), 37.40 (t), 37.38 (t), 34.50 (t), 34.31 (t), 33.03 (d), 32.16 (t, 2 x C), 30.29 (t, 2 x C), 29.98 (t, 2 x C), 29.94 (t), 29.92 (t), 29.88 (t), 29.85 (t), 29.65 (t, 5 x C), 29.61 (t, 2 x C), 29.47 (t), 29.42 (t), 27.38 (t), 27.35 (t), 25.18 (t), 25.11 (t), 22.92 (t, 2 x C), 19.90 (q), 14.35 (q, 2 x C). 

³¹P-NMR (162 MHz, CDCl₃) δ 1.16. High resolution MALDI TOF calculated for C₄₀H₈₁NO₈P (M + H⁺), 734.5694 found 734.5697.
4.6 References

1 For a review on glycerolipids, see: Vance, J. E. J. Lipid Res, 2008, 49, 1377-1387.

2 sn = stereospecific numbering. The carbon atom that appears on top in the Fischer projection of the phospholipid that shows a vertical carbon chain with the hydroxyl group at carbon-2 to the left is designated as C-1.


14 The enantiomeric excess of compound 25 is somewhat low (90% ee) compared to closely related substrates obtained from the 1,4-addition reaction as was reported in ref 12e (typically 95-96% ee).

The acid chloride group has priority over the CF<sub>3</sub>-group, whereas the newly formed oxo-ester does not have priority over the CF<sub>3</sub>-group (Cl > F). Cahn, R. S.; Ingold, C.; Prelog, V. Angew. Chem. Int. Ed. 2003, 5, 385-415.