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Synthesis of different Bis(monoacylglycero)phosphate derivatives as probes for metabolic processes

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1 Introduction

Lipids are besides nucleic acids, proteins and carbohydrates a very interesting and important class of biomolecules with a large diversity. Among them, the subclass of phospholipids (PLs) plays an important role in cell morphology due to their unique structural properties.[1] Unlike other lipids like triacylglycerols (TAGs), which are important in nutrition and energy storage, PLs have biological relevance in membrane formations and cell structure. Due to the polar anionic phosphate head group and the non-polar fatty acid residues, they have an amphiphilic structure, which enables the formation of bilayers in aqueous environments.[2]

In 1967 Gray and Body discovered a minor PL from pig lung, which they named lysobisphosphatidic acid (LBPA).[3] Nowadays it is strongly recommended to use its more systematic name bis(monoacylglycero)phosphate (BMP), because its large structural difference to phosphatidic acid and the resulting confusion concerning its structure. In healthy mammalian tissues, BMP is a minor lipid, which comprises 1-2% of the total phospholipid mass. Its concentration is much higher in specialized cells like alveolar macrophages of the lung, where it accounts for 18% of the found phospholipids and in cells affected by lysosomal disorders.[1b]

The fatty acid (FA) composition of BMP is very interesting. It might be responsible for some important biophysical properties, which could also influence its functions. Oleic acid (18:1n-9, OA) is the most often esterified FA in BMP. The other major FAs are docosahexanoic acid (22:6n-3, DHA) and arachidonic acid (20:4n-6, AA).[4]

BMP seems to have an in nature unique structure. It simply consists of a phosphate group, two glycerin units and two esterified FA acyl chains, as you can see in figure 1. Therefore, it belongs to the group of polyglycerophospholipids, which simply means, that more than one (in the case of BMP: two) glycerol units are attached to the phosphate moiety.[1b] Because one phosphate oxygen atom is not esterified, it has a negative charge in aqueous environment. It has a peculiar structure due to its unique constitution and configuration. On the one hand, the constitution is unusual, because the two FAs are esterified on the so called *sn*-2:*sn*-2' positions of glycerol.[5] On the other hand the stereo configuration of BMP is probably also unique in nature, because its two glycerol units are esterified with their *sn*-1:*sn*-1' hydroxyl moieties at the phosphate.[6] This

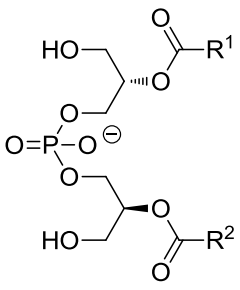


Figure 1: Structure of 2,2' diacyl-*sn*-1:*sn*-1' BMP

uncommon configuration leads to some questions about its biosynthesis and biodegradation.

A simple reason for the importance of a different configuration might be the localization of BMP in the internal membrane of late endosomes and lysosomes. These organelles contain among other hydrolyzing enzymes lipid degrading lipases. Due to BMP's stereoconfiguration and constitution it is not degraded by these enzymes and can act as an inert structural element in late endosomes and lysosomes.

To gain better insight in the biodegradative pathway(s) of BMP, it would be interesting to show, which hydrolases are able to degrade it. The aim of this thesis is the synthesis of different BMP derivatives and the identification of active hydrolases for this very uncommon PL.

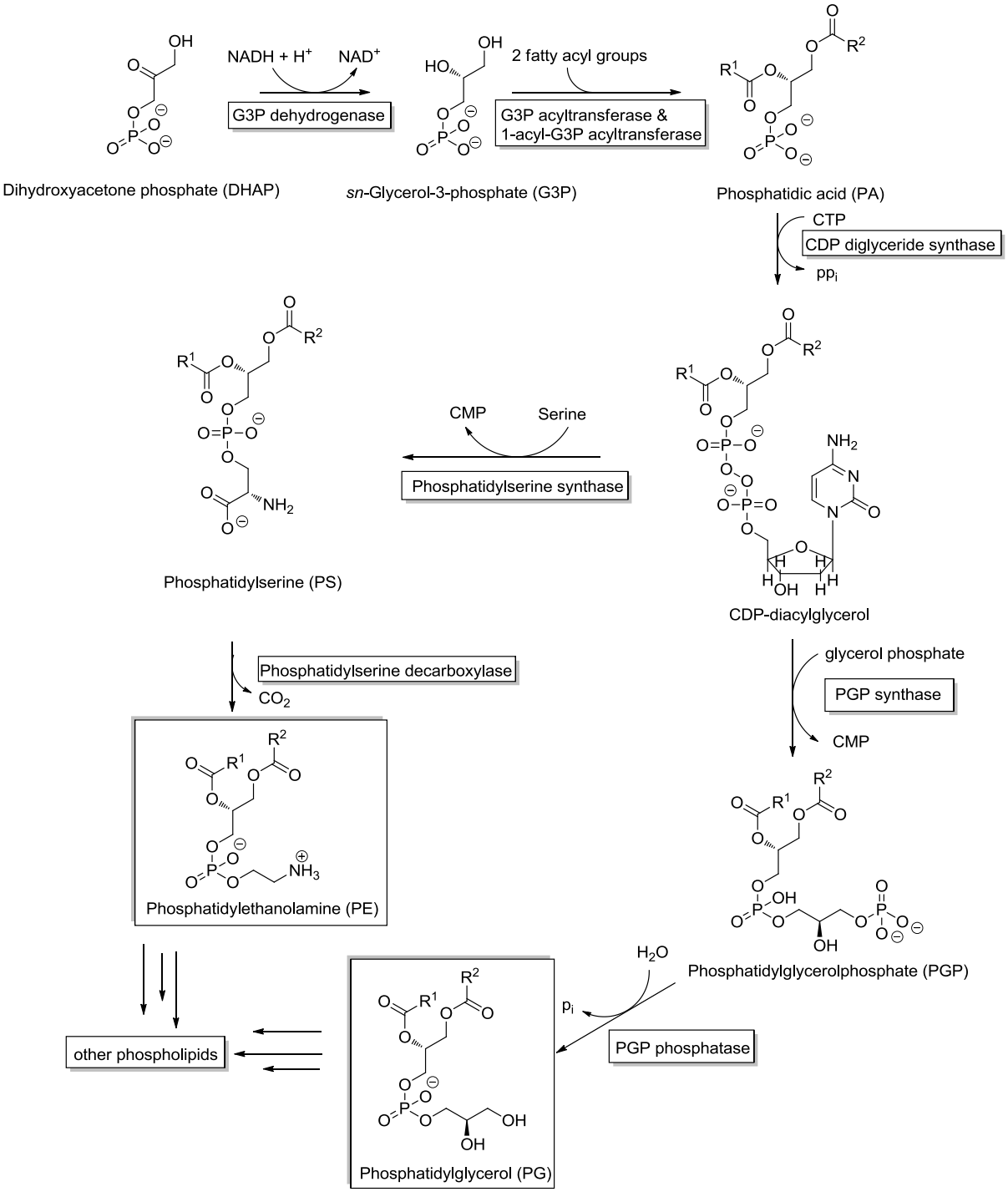
2 Theoretical background

2.1 General structure, function and diversity of phospholipids

Phospholipids (PLs) are phosphorous containing amphipathic molecules. Their structure derives from a polar phosphate group, which is esterified with an organic non-polar alcohol and they show a large molecular diversity. Most PLs consist of the essential phosphate group, a diglyceride and an additional alcohol such as choline. From this core structure, some structural differences are possible. Also the type, the count and the position of esterified FAs can differ. One of the most consequential differences is the number of attached glycerol units. All these mentioned structural differences can lead to unique physical and biochemical properties of different PLs.[7]

The most remarkable structural difference in the class of PLs has sphingomyelin, in which the glycerin moiety is exchanged by sphingosin, a C_{18} amino alcohol. All other PLs have at least one glycerol moiety attached to the phosphate and can be subdivided into monoglycerophospholipids and polyglycerophospholipids. The subclass of polyglycerophospholipids comprises molecules that have more than one esterified glycerol unit. The most famous representatives are the compounds phosphatidiylglycerol (PG), cardiolipin (CL) and bis(monoacylglycero)phosphate (BMP).[8]

This large structural diversity derives from the general biosynthetic pathways of PLs, which is outlined in Scheme 1. PL biosynthesis starts in all organisms with the stepwise esterification of *sn*-glycerol-3-phosphate to form phosphatidic acid (PA). These transformations are catalyzed by the enzymes G3P acyltransferase and 1-acyl-G3P acyltransferase. The formed PA is converted to CDP-diacylglycerol (CDP-DAG) by the enzyme CDP diglyceride synthase which catalyzes the ligation of a cytidine group. This liponucleotide is a central intermediate in PL biosynthesis and can act as a precursor either for the zwitterionic phosphatidylethanolamine (PE) or the negatively charged phosphatidylglycerol (PG). On the left side in Scheme 1 the formation of PE is depicted, which contains a substitution of CMP with serine, which is catalyzed by the enzyme phosphatidylserine synthase and a subsequent decarboxylation catalyzed by the enzyme phosphatidylserine decarboxylase. The formation of PG, also depicted in Scheme 1, contains a substitution of CMP by G3P catalyzed by the enzyme PGP synthase and the hydrolysis of the terminal phosphate group by the enzyme PGP phosphatase. PG itself is a precursor for other anionic PLs like CL.[9]



Scheme 1: Biosynthesis of phosphatidylethanolamine and phosphatidylglycerol[9]

Under different growth conditions, the head group composition of phospholipids seems relatively consistent. In *E. coli* for example 70-80% of all PLs are PE, 20-25% PG and 5% or less CL.[10] Another important attribute of PGs is their FA composition. Most PGs are esterified with a saturated FA at position 1 and with a monounsaturated FA at position 2. The FA composition can vary between different organisms, cell types, organelles, head groups and also

between different growth conditions. The ratio of saturated to unsaturated FAs for example in *E. coli* cells grown at 37°C is approximately 1:1. In cells, which were grown at 17°C, the FA composition changes to 1:2.[11]

In living organisms PLs act mainly as membrane lipids and are responsible for the formation of the for all cells very important lipid bilayer. This bilayer surrounds cells and organelles to prevent leakage of hydrophilic molecules. For such a barrier function, the before mentioned large structural diversity of PLs would not be absolutely necessary.[12]

2.2 Phospholipids in lipid bilayers

The bilayer defined by PL's has not only a barrier function, it contains also a lot of different membrane proteins in its interior. These proteins fulfill structural needs of the cell membrane and are also very important for different signaling, energy related and transport processes. PLs do not show catalytic activity, which enables them to realize the mentioned processes, but they can influence the structure and activities of all proteins, which are located in and around lipid bilayers. Due to their molecular building blocks (FAs, glycerin, other biorelevant alcohols and phosphate), they serve also as precursors or intermediates in many metabolic processes of the cell.[9]

Due to the grade of unsaturation, the FA tails can form a straight line, or have small kinks. Because the esterified FAs have also different lengths, PLs can differ in their ability to pack with each other. This affects the so called fluidity of the bilayer membrane. The membrane fluidity is an effect, which enables individual lipid molecules, to diffuse freely within lipid bilayers. This effect was first recognized around 1970, and is dependent of the composition of the PLs integrated in the membrane. With physical and chemical methods, the fluidity can be artificially increased, which leads to errors in certain membrane transport processes and enzyme activities. A synthetic bilayer made out of one single type of phospholipid for instance, can change its aggregate state, due to a change in temperature. If the temperature is low enough, it changes from a typical liquid state, with freely diffusing PLs, to a two-dimensional rigid crystalline state, with a rigid position of each PL and a characteristic freezing point. The temperature at which this so called phase transition occurs, is low with short FA chains and a high grade of unsaturation, because the membrane components cannot tightly pack together. Microorganisms which are not able to adjust their temperature independent from their environment, are able to adjust the fatty acid composition of the cytosol membrane by an

acceleration of unsaturated FA biosynthesis, if their temperature drops down. Due to that mechanism, the bilayer membrane fluidity stays the same at different temperatures.[13]

In the cytosol of a cell are not many lipids, although a rapid exchange of lipids between different membranes in bacterial cytoplasmic membranes is possible. These so called transmembrane movements are very interesting biochemical processes, which occur at rates about 1 µm/sec and allow membranes to communicate with each other.[14]

There are many different PLs in a membrane and one cannot investigate their function by simple assays. Due to their deficiency of catalytic activity, it is difficult to design experiments to reveal their specific role in living organisms. Most of the studies which deal with the function of specific PLs, derive from *in vitro* experiments. These kind of experiments can only give hints to the function of PLs in living organisms and can lead to misinterpreted data, which has no correlation to *in vivo* systems. For the validation of *in vitro* approaches, *in vivo* studies are absolutely required.[9]

2.3 Structure, properties and fatty acid composition of bis(monoacylglycero)phosphate

Bis(monoacylglycero)phosphate (BMP) is a very interesting PL, which was discovered in 1967 by Body and Grey.[3] They have isolated BMP together with its structural isomer PG from a lipid extract of pig lungs via column chromatography. After some chemical transformations (basic hydrolysis, periodate oxidation and acetolysis) they revealed, that this PL consists of two phosphate : glycerol : FA in the ratio 1:2:2, and that both FAs are attached to the same glycerol unit. This was a peculiar discovery, because such a polyglycerophospholipid was not known of that time. Despite their remarkable and brilliant conclusions to their experiments, Body and Grey have not predicted the structure of BMP completely right. They thought, that the two FAs are esterified with the primary hydroxyl groups of the glyceryl moiety, like in lysophosphatidic acid (LPA) and not with their secondary hydroxyl groups, which is the real constitution of BMP. This misinterpretation of the actual structure of BMP led to its first name lysobisphosphatidic acid (LBPA). Because the structure of BMP is not directly related to LPA, this led to some confusions and its old name should be avoided if possible. In Figure 2, you can see the two from Body and Grey isolated PLs (PG and BMP) and LPA with its not directly to BMP related structure.

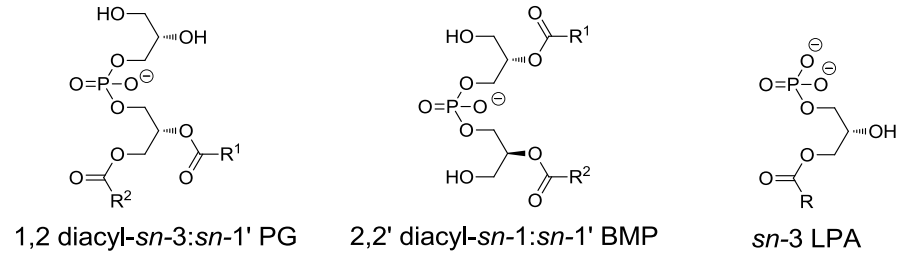


Figure 2: *sn*-3:*sn*-1'-phosphatidylglycerol (PG), *sn*-1:*sn*-1' bis(monoacylglycerophosphate (BMP) and *sn*-3 lyso phosphatidic acid (LPA)

The negatively charged PL BMP can be classified into the category of glycerophospholipids (GP), the class of glycerophosphoglycerols (GP04), and the subclass of monoacylglycerophosphomonoracylglycerols (GP0410), according to the LIPID MAPS structure database (LMSD).[1b, 15]

BMP has a very interesting constitution. The two FA chains are esterified to two different glycerin moieties at their so called *sn*-2, *sn*-2' positions. The abbreviation *sn* stands for stereospecific numbering and is a common stereo assignation for glycerols in biology. This IUPAC conform nomenclature derives from its absolute configuration. If you write down glycerol in its Fisher projection, with the hydroxyl group at carbon 2 on the left side, the top carbon is labelled with 1, the carbon in the middle with 2 and the bottom carbon with 3. So the esterified *sn*-2 and *sn*-2' positions are that ones in the middle as you can see in Figure 2.

This constitution pattern is very uncommon for polyglycerophospholipids and leads to remarkable structural and physical properties. One consequence of it is that BMP forms cone-like three dimensional shapes. These shapes lead to asymmetry in BMP rich membrane domains and favor membrane liquidity and the formation of internal vesicles in the interior of endosomes and lysosomes.[16]

A second consequence of BMP's outstanding structure is, that it has peculiar biophysical properties. With different physical experiments, the molecular packing, the intermolecular hydrogen bonding and the hydration degree of membrane incorporated BMP isomers, was analyzed. This study was done with respect to the stereoconfiguration of BMP and its FA composition.[17] The relatively strong intermolecular interactions found between different BMP molecules, can lead to the formation of BMP micro domains in the interior of bilayer membranes and even to the formation of small lamellar vesicles. The formation of these vesicles is strongly dependent from pH and the ionic strength.[18]

What is even more interesting than the constitution of BMP is its configuration, which seems to be unique in nature. Glycerophospholipids from mammalian cells occur normally in *sn*-3 configuration, but BMP has a *sn*-1:*sn*-1' stereoconfiguration, which was originally deduced after basic hydrolysis and stereospecific enzymatic analysis of the generated glycerol phosphate. This deduction was discussed controversial by some biologists for some time. However, BMP was also synthesized and its unique stereoconfiguration was confirmed by spectroscopic methods.[6] Due to this unique configuration, it seems to be resistant against hydrolysis by lipases, which makes it a perfect structure lipid for lipase rich late endosomes and lysosomes.

The FA composition of PLs has high influences on their biophysical and chemical behavior. BMP itself has mainly unsaturated FAs esterified with glycerol. Oleic acid (18:1n-9, OA) is the most frequent esterified FA in BMP. Another monounsaturated FA, which is present in BMP in a high amount, is palmitoleic acid (16:1n-7). Those two FAs have both a double bond at the same position and form a kink, which is responsible for the three dimensional structure of BMP, which is also related to its flexibility and increased membrane fluidity, or membrane fusion processes. With respect to polyunsaturated FAs, BMP is also rich in docosahexanoic acid (22:6n-3, DHA) and arachidonic acid (20:4n-6, AA).[4] These two polyunsaturated acids have also structural relevance for BMP, but what seems even more interesting, than their conformation is, that they are often involved in the formation of hormones like prostaglandins and other biorelevant molecules like leukotrienes. With a hydrolytic breakdown of the FA ester bond, DHA and AA can be released into different compartments of the cell and act as precursors for the before mentioned biorelevant compounds.

2.4 Structure and function of endosomes and lysosomes

Endosomes are vesicles in animal and plant cells, which originate from a process called endocytosis. They count to the organelles of a cell and are a kind of prelysosomal intermediate. Endocytosis itself is a membrane transport mechanism for small molecules, macromolecules, large nutrition particles and even small cells through a cell membrane. Therefore, the cell membrane is engulfing the material and forms a kind of notch. This notch is released from the membrane and becomes a part of the endomembrane system. Endocytosis, at which solid particles are incorporated is called phagocytosis and if dissolved material is incorporated, the process is called pinocytosis. Endosomes can be divided into early and late endosomes. Early endosomes are located to the cell periphery, while late endosomes are close to the nucleus. They are similar to lysosomes.[19]

The role of early endosomes is to sort the endocytosed material, which goes either back to the plasma membrane and is reused to initiate endocytosis again, or to specialized organelles. These specialized organelles either need this material for their own activity or function, or degrade the endocytosed material into small molecules.[20] In the pathway from early endosomes to late endosomes and lysosomes, different transport intermediates can occur. These intermediates originate from early endosomes and are called endosomal carrier vesicle (ECV) or multivesicular bodies (MVB). The differentiation between ECV, MVB and late endosomes is sometimes confusing. Late endosomes are sometimes also referred as MVB, which in fact transports molecules and enzymes to late endosomes. Therefore it moves to them and releases the transported material by fusion. Also the differentiation between late endosomes and lysosomes is not strictly regulated. Sometimes the term "late endosomes/lysosomes" is used if a closer differentiation is not necessary. These two organelles can also fuse together and make a dynamic exchange of enclosed material via a hybrid organelle existing of both, late endosomes and lysosomes.[21] Those large complexes may be the reason, why both organelles have a similar acidic interior and include many of the same proteins. They can form lysosomes again by the release of late endosomal components. Late endosomes have like lysosomes a lot of different hydrolases in their interior, which have due to their optimal (acidic) pH range high hydrolyzing activity, which makes late endosomes to degrading organelles as well. Even though lysosomes and late endosomes have some common enzymes and are difficult to distinct, their morphology and also their lipid and protein composition is very different. A large difference between late endosomes and lysosomes is their destined function. Late endosomes are supposed to be a dynamic organelle, which sorts endocytosed material, while lysosomes are a kind of end-point compartment in lipid and protein metabolism.[22]

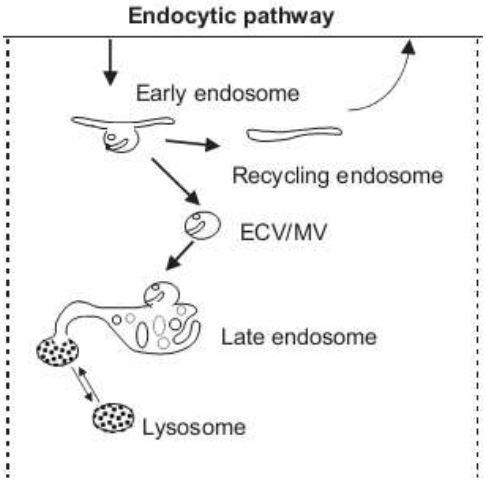


Figure 3: Schematic representation of the endocytic pathway. Picture was taken from Ref. [1b]

Lysosomes are the digesting organelles of animal cells, partly originating from the Golgi-apparatus. They have an acidic interior and contain a punch of different hydrolyzing enzymes. The function of lysosomes is to breakup biopolymers into their monomers. In plants, vacuoles fulfill the function of lysosomes. Lysosomes have an inner diameter of 0.1-1.1 µm. Beside lipases, they also contain proteases and nucleases. These enzymes hydrolyze proteins, polysaccharides, nucleic acids and lipids and work only in an acidic environment with a pH between 4.5 and 5.0. The reason for their

specific pH range activity is, to protect the cell from a possible breakup of the lysosomal membrane. In that case, the hydrolyzing enzymes would be in the pH neutral environment of the cytosol and therefore inactive. This pH dependence is an example for cellular compartments. The low pH value in lysosomes is generated by an ATP dependent proton pump. To protect the membrane proteins from hydrolysis, they are highly glycosylated at the inner side.[13]

2.5 Localization of BMP in the cell

After the discovery of BMP in 1967, the scientific community assumed that it is localized in lysosomes.[23] As mentioned before, the differentiation between late endosomes and lysosomes is very difficult and it has taken several years, to identify the right cell compartment, where BMP is localized. In 1998 Kobayashi and coworkers published the generation of the monoclonal BMP specific antibody 6C4, which was a breakthrough in BMP research.[24] To generate 6C4, they used baby hamster kidney (BHK) endosomal membranes as an antigen. In this paper they also showed that the so called internal membranes of late endosomes, which are membranes of vesicles in the lumen of late endosomes, contain BMP and that BMP is a specific antigen for the so called antiphospholipid syndrome. More evidence for the localization of BMP in internal membranes of late endosomes was gained by two studies, where suborganellar fractionation studies and immunogold labeling were used.[5, 25] In late endosomes, BMP accounts for about 15% of the PL mass.[1b] Internal membranes of late endosomes can be divided into different types. The two main types are the ones containing mainly phosphatidylcholine, or mainly BMP as the major PL. The opportunity of BMP to fuse with membranes and that also membranes being composed of BMP can easily fuse with other membranes, makes BMP an ideal lipid for the regulation of the dynamic properties of internal membranes.[5]

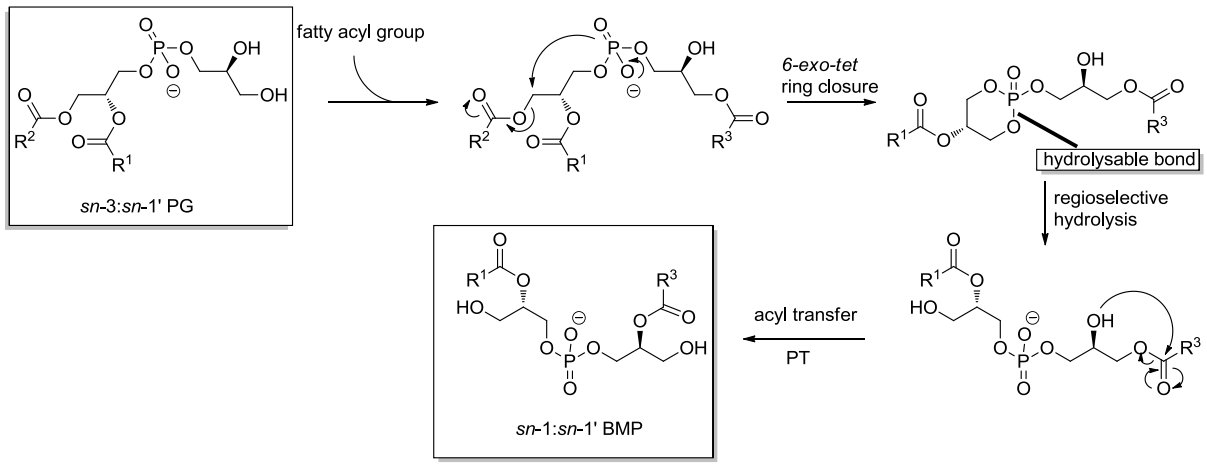
The suborganellar fractionation studies showed, that BMP accounts to the light lysosomal fractions. Domains, which have a high amount of BMP, can be identified by the BMP-specific antibody 6C4. It shows activity only from the luminal side of late endosomes and not from the cytosol, which is an important evidence for the localization of BMP in internal membranes of late endosomes. In these suborganellar compartments, it accounts for up to 70% of the total phospholipid mass.[1b, 26]

Although the localization of BMP in a living cell seems to be assured, the reason for that is not understood. It could be due to a controlled transport to late endosomes, or even to a regiospecific synthesis which is localized in late endosomes.

2.6 Metabolism of BMP

The probably most outstanding property of BMP is its in nature unique *sn*-1:*sn*-1' stereoconfiguration, which makes both the anabolism as well as the catabolism for this special PL very interesting.

In the biosynthesis leading to this configuration, at least one unusual enzymatic transformation of the glycerol backbone is supposed to be involved, because all thinkable precursors for BMP have either a *sn*-3:*sn*-1' configuration like PG, or have a more complex structure like CL. Waite and coworkers showed in 1991, that PG can act as a precursor for BMP in *in vitro* and in *in vivo* studies.[27] They were also able to demonstrate, that BMP's stereospecific (or selective) synthesis does not involve an oxidation and subsequent reduction of the glycerol *sn*-2 carbon. With ^{14}C labeling, they further showed, that the fully esterified glycerol part of the PG precursor is probably reoriented by a stereospecific enzyme. This might be an explanation for the change of stereo configuration at this glycerol unit. The proposed mechanism for the change of stereochemistry is outlined in Scheme 2. It starts with PG, which is acylated with a FA at its 3' position. After a proposed 6-*exo*-tet ring closure, which probably is enzyme catalyzed, to a dioxaphosphinane and subsequent regioselective hydrolysis, an intermediate with changed stereochemistry is generated. This intermediate can now undergo an acyl transfer from its 3' position to its 2' position to form *sn*-1:*sn*-1' BMP.



Scheme 2: Mechanistic proposal of the biosynthesis of *sn*-1:*sn*-1' BMP with PG as an endogenous precursor.[27]

However, other studies showed, that CL can also act as a precursor for BMP in different *in vivo* and *in vitro* studies. In 2007, Kobayashi and coworkers have collected evidence that PG is the

endogenous precursor in BMP *de novo* biosynthesis of mammalian cells, with different mutation experiments involving enzymes in PG's and CL's biosynthesis.[28] The key experiment of this publication was the comparison of the BMP amount in phosphatidylglycerophosphate (PGP) synthase rich and deficient cells. Cells which were PGP synthase-deficient showed a decrease of 50% in BMP biosynthesis compared to the wild type cells. An overexpression of this mitochondrial enzyme, which catalyzes the transformation of CDP-diacylglycerol to PGP, induced a 2.5 fold increase in BMP synthesis. Another interesting observation in this experiment was, that the FA composition did not change significantly in the different mutants, compared to the wild type composition. That also indicates a connection between the *de novo* PG and BMP biosynthesis. Although, the location of BMP biosynthesis inside the cell is not known, it is postulated to be inside late endosomes/lysosomes.[29] It is astonishing, that in these organelles no PG and CL seems to be present. Therefore, a kind of continuous exchange between the membrane of late endosomes/lysosomes and other biomembranes seems to be required for BMP biosynthesis.[30]

What seems to be even more interesting than the biosynthesis of BMP is its biodegradation. A direct consequence of the unusual *sn*-1:*sn*-1' configuration is, that BMP seems to have a specific resistance to different phospholipases. That is also the reason, why it has a long lifetime in the acidic and hydrolase rich environment of the lumen of late endosomes/lysosomes.[31] Despite the long lifetime compared to other PLs, BMP seems to be a suitable substrate for a small set of phospholipases. Cochran and coworkers were able to show the BMP hydrolyzing activity of a putative lysosomal phospholipase A_1, through the release of arachidonate upon macrophage activation.[32] The same experimental setup showed no activity with pancreatic phospholipase A_2, which leads to the assumption, that these activated arachidonate BMP derivatives may be acylated at their terminal hydroxyl group. In another publication, Luquain and coworkers showed, that phospholipase C from *B. cereus*, phospholipase D from cabbage and phospholipase A_2 from porcine pancreas or *C. adamenteus* were not able to catalyze the release of arachidonate, or docosahexanoate from BMP isolated from UIII cells or alveolar macrophages, but that the phospholipase A_1 from *R. delmar* and *R. arrhizus*. had hydrolyzing activity.[33]

Although the biosynthetic and biodegradative pathways for BMP are not fully understood, several publications give hints to it and it seems to be a question of time, until both are established. Therefore, a lot of additional experiments have to be done, including enzymatic assays, mutation experiments and compound labeling.

2.7 Diseases related to BMP

Due to its structure and localization, BMP is related to two different diseases. On the one hand, it is like many other PL derivatives, an antigen in the so called anti-phospholipid syndrome (APS). And on the other hand it is accumulated in different lysosomal storage diseases (LSD) and drug-induced lipidosis.

2.7.1 Lysosomal disorders and drug-induced phospholipidosis

In healthy mammalian cells, the BMP amount is between 1-2% of the total phospholipid mass, but it is highly increased in different genetic caused LSDs and also in drug-induced lipidosis. LSD is a collective term for about 45 different diseases, which induce an accumulation of different endogenous or exogenous material in the interior of late endosomes or lysosomes. The reason for this accumulation is a deficiency of enzymatic degradation. The accumulated material is mostly the substrate of the missing or inactive enzyme. For example are in Gaucher and Fabry diseases glycolipids, in Niemann-Pick type A/B or C sphingomyelin or cholesterol, in Tay-Sachs/Sandhoff gangliosides, in mucopolysaccharidoses glycosaminoglycans and in neuronal ceroid lipofuscinosis lipopigments highly accumulated.[1b,34] The missing enzymatic activity, leading to these accumulations, can either be drug-induced, or a consequence of a monogenetic defect. Strictly, only the genetic diseases are called LSDs and the others are called drug-induced lipidosis.

The LSDs which have the best documented connection to BMP accumulation are the so called Niemann-Pick (NP) diseases. They can in turn be subdivided into three groups (NPA, NPB and NPC), from which NPC is the most common. It is both, a lysosomal and an endosomal disorder, which is reasonably well explored.[35] It is a confirmed fact, that the BMP content of NPC infected patients rises significantly in their liver and spleen during the disease. NP related diseases are not the only ones which lead to an accumulation of BMP. In different kinds of neuronal ceroid lipofuscinosis (NCL) various groups have shown, that the amount of BMP in brains is related to this disease. NCLs are a group of different neurodegenerative LSDs. However, it should to be mentioned at this point, that not all LSDs cause a lysosomal accumulation of BMP.

In contrast to genetically caused LSD, an uptake of drugs can also induce an accumulation of endogenous and exogenous material in late endosomes and lysosomes. If the accumulated material is a phospholipid, it is called drug-induced phospholipidosis. The molecules, which are responsible for that disease are cationic amphiphilic drugs (CADs) like the three tertiary

amines amiodarone, 4,4'-diethylaminoethoxyhexestrol and chloroquine. Their structure is presented in Figure 4. The affected body parts in drug-induced phospholipidosis are mainly the liver and spleen.[36] 4,4'-Diethylaminoethoxyhexetrol and chloroquine increase significantly the level of acidic PLs and therefore also of BMP in lysosomes and late endosomes. Hence, BMP might be used as a marker for drug-induced phospholipidosis.

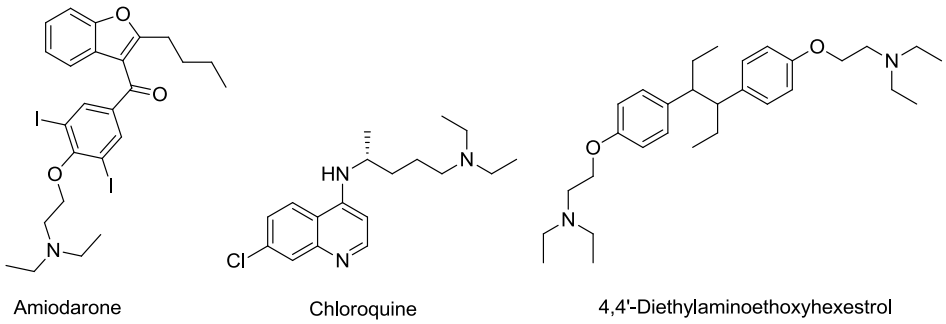


Figure 4: Structure of the cationic amphiphilic drugs amiodarone, chloroquine and 4,4'-diethylaminoethoxyhexestrol

Although many studies in this field have been done, the mechanism of BMP accumulation in LSD and drug-induced lipidosis, is yet not fully understood. The pathological influence of BMP is controversially discussed. While some studies provide evidence on its active participation in diseases, others argue, that the high BMP level can be explained with a kind of metabolic traffic jam and a following accumulation of different non related metabolites.

2.7.2 Anti-phospholipid syndrome

The other disease, which is related to BMP is the so called anti-phospholipid syndrome (APS). It is one of the most common autoimmune diseases and 2-5% of the human population (mostly women) are affected. It is also called Hughes-Stovin syndrome, cardiolipin-antibody syndrome, APA syndrome and lupus antikoagulans. The typical clinical symptoms, which give evidence of APS are mainly thrombosis and stillbirth. Instead of diagnoses which are based on clinical symptoms, APS can be detected with different anti-phospholipid antibodies. These antibodies are not specific for APS and can be also detected in combination with other diseases.

APS is caused by a group of antibodies, which recognize different PLs and phospholipid-bound proteins. Due to these so called antiphospholipid antibodies, blood clotting is favored and thrombosis can occur. APS itself can be subdivided into two different classes. Primary APS (PAPS) is independent from other diseases, while secondary APS (SAPS) is caused to other diseases, like lupus erythematosus, rheumatoid arthritis and the Sjörgen's syndrome.

Like mentioned before, it is not only one antibody, which is responsible for APS, but more a heterogeneous group of antibodies. The most relevant antigen for these antibodies is probably a phospholipid-bound glycoprotein (β_2-glycoprotein I). However, it was shown, that APS antibodies are also able to bind BMP, which causes a variation of sorting processes.[24] Different publications have also provided evidence, that BMP might be an endogenous antigen for APS antibodies.

2.8 Cellular function of BMP

2.8.1 Dynamic structural processes in endosomes

The function of BMP as a structural lipid seems to be more complex and important than that of other PLs. It is a well-known fact, that different biomolecules are responsible for the mediated regulation of membrane dynamics. Among these biomolecules BMP seems to be able to regulate the internal membrane dynamics of late endosomes. Kobayashi and coworkers have shown in 2002,[5] that the internal membranes of late endosomes consist of two different populations. One of them uses phosphatidylcholine as its major PL, the other one consists mainly of BMP. They argued, that this fact is an explanation for the so called microheterogeneity of internal membranes in late endosomes and also showed, that BMP is able to induce membrane fusion of liposomes at acidic pH values, which occur in the interior of late endosomes and lysosomes.

BMP constitution and the environmental conditions in late endosomes enable it to form a cone-shaped three dimensional amphiphilic structure, with a small polar head group and a large hydrophobic part. In membrane domains, which are rich in BMP, this structural feature, leads to a membrane imbalance or asymmetry. Asymmetry and imbalance in this particular case mean, that the membrane structure in these domains can change significantly, which further lead to invagination of the membrane and the formation of internal vesicles.[37] This was also shown for acidic liposomes in a publication by Matsuo and coworkers in 2004.[16] Due to the formation of internal vesicles in these acidic liposomes, a structure which is pretty similar to that of multivesicular late endosomes was generated. This process was strongly dependent on a pH gradient, which turned out to be the same pH gradient which exists in *in vivo* endosomal membranes. This pH gradient is *in vivo* controlled by a protein called Alix, which is related to the endosomal sorting complex required for transport (ESCRT) proteins. These proteins are the responsible proteins for invagination processes in MVB.[38] BMP and Alix together seem to be responsible for dynamic membrane processes. It is supposed to permit the back-fusion process of internal membranes to the limiting membrane of late endosomes. Together with its possibility

to induce asymmetry in the limiting membrane, BMP seems to be able to stabilize and destabilize limiting membranes of late endosomes at the same time. The process of invagination and vesicle formation weakens the limiting membrane, while the back-fusion process which is mediated by BMP and the protein Alix strengthens it. Alix seems to be also important in the *in vivo* biogenesis of BMP containing endosomes. Under particular pathological conditions like phospholipidosis of sphingolipids, the membrane structure of late endosomes and lysosomes changes from the described multivesicular to a more multilamellar structure. The resulting organelles are called membranous cytoplasmic bodies (MCB). BMP was also detected in exosomes of some cell types in small concentrations. Exosomes are vesicles, which are released into the extracellular matrix.[39] This observation gives also evidence to the role of BMP in dynamic membrane processes.

2.8.2 Mediation in degradation pathways

It is a known fact, that BMP can influence the activity of some enzymes. Among them are polysaccharide degrading enzymes like β-galactosidase and β-glucosidase.[40] The work of Harder and coworkers in 1984, which led to this insight, included an experiment, in which they added BMP to lysosomal fractions from rat liver. They observed a dropdown of β-galactosidase activity to only 20% of the original activity, but an increase of activity to 220% of β-glucosidase. They observed also a large affect (about 60% galactosidase activity and about 300 % glucosidase activity) when they applied liposomes to the lysosomal fractions which had a molar ratio of sphingomyelin/phosphatidylcholine/BMP = 1:1.5:0.5, which is very close to that found in human liver cells affected by the Niemann-Pick disease NPC. BMP cannot only influence the activity of polysaccharide degrading enzymes, it also stimulates the degradation of sphingolipids by influencing their degradative enzymes. The influence of BMP on hydrolyzing degrading enzymes is dependent on its FA composition. Dioleoyl-BMP, which is the main BMP derivative in normal healthy late endosomal/lysosomal membranes can bind to Saposin C. These Saposin C-BMP interactions are supposed to lead to MVBs and are probably the reason for the formation of multilamellar MVB membrane structures in shingolipidosis, because of Saposin C deficiency.[41]

Another enzyme activity which is mediated by BMP, is the lysosomal acid lipase (LAL). This enzyme is responsible for the degradation of cholesteryl esters and triacylglycerols. Both are brought to lysosomes by endocytosed low-density lipoproteins. In this case, BMP does not directly influence the enzyme, but is responsible for the availability of an inhibitor through the disorganization of BMP rich domains.[1b]

2009, Shayman and coworkers[42] investigated the influence of negatively charged PLs on the activity of lysosomal phospholipase A2 (LPLA2). They showed, that the adsorption of LPLA2 to negatively charged membrane lipids increases the hydrolytic activity towards insoluble substrates. In BMP deficient cells, LPLA2 stays in the inner part of the lysosomal lumen, because of its hydrophilicity. Its lipophilic substrates in contrast, stay close to the hydrophobic membrane and the enzyme and PLs cannot come into contact. Due to the attractive electrostatic forces between LPLA2 and the negatively charged BMP, it gets closer to the membrane and is capable for hydrolyzing its substrates.

2.8.3 Regulation of sorting functions in late endosomes

As mentioned before, the discovery of the BMP specific antibody 6C4 from Kobayashi and coworkers[24] was a key step in BMP research. When this antibody was endocytosed in BHK cells, it accumulated in late endosomes because it bound to BMP as its antigen. One consequence of the treatment of 6C4 to these cells was a redistribution of a receptor which binds insulin-like growth factor 2 and different ligands which have a mannose 6-phosphate moiety. Insulin-like growth factors are polypeptides with a similar amino acid sequence to insulin, which are responsible for the growth and differentiation of cells. The fact, that BMP can change the intracellular distribution of this multifunctional receptor, leads to the conclusion, that BMP can influence the protein-sorting function of endosomes.[1b]

BMP is also supposed to be able to mediate sorting of other molecules in endosomes. The sorting process might go via adsorption of these molecules at BMP rich membrane domains. Different properties of BMP rich domains can lead to the required attractive intermolecular interactions. BMP is a negatively charged molecule, which can build up an electrostatic force between polar molecules and cations. Another important property of BMP is its three dimensional structure, which leads to an asymmetry in the limiting membrane. Due to the asymmetry, the membrane develops space between its hydrophobic fatty acyl chains, and small hydrophobic molecules can be absorbed by the membrane. Also its capability to form internal vesicles can enclose molecules, which cannot be released from the endosomal lumen.

3 Aim of this thesis

As mentioned before, BMP has highly interesting biological properties due to its constitution and stereochemistry. It seems to have a unique biosynthetic pathway and also a very specific resistance to different phospholipases. To reveal its biodegradative pathway, enzymes which are capable for its degradation have to be found. If an enzyme would be found, it might either be able to hydrolyze one specific or different BMP derivatives. Due to the different functional groups of BMP, also the question of the site capable for hydrolysis arises. It could either be at the phosphate or at the carboxylic ester moieties.

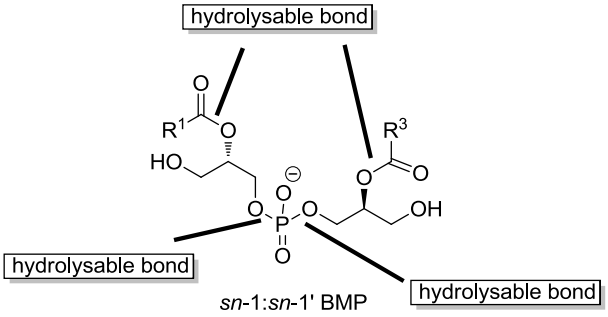


Figure 5: Possible sites of BMP hydrolysis

In both cases different degradation products would be formed and would be released into the lumen of late endosomes/lysosomes. These biodegradation products could be used for further biochemical processes, like their own biodegradation or even the biosynthesis of other metabolites. An identification of biodegradative products seems to be imperative for the elucidation of biological role of BMP in diseases, as well as in biomembrane formation and possible intermediate for other metabolites. Different BMP derivatives can be very important PLs in different biological processes.

One enzyme which is supposed to have BMP hydrolyzing activity is the hydrolase ABHD6, which stands for alpha-beta hydrolase domain-containing protein 6. It had been shown before, that it is able to hydrolyze the signaling lipid molecule 2-arachidonoylglycerol (2-AG).[43] The site of hydrolysis of 2-AG is its carboxylic ester moiety, which leads to the conclusion, that in the case of BMP hydrolysis, the free carboxylate of the respective FA would be released. In the case of enzymatic assays, which test the BMP hydrolyzing activity of ABHD6, the detection of this FA carboxylate can further be done by standard bioanalytical methods. BMP can also compete with other substrates of ABHD6 to change very sensitive metabolite concentrations and influence due to that very complex biochemical processes.

The specific aim of this thesis was to synthesize different BMP derivatives, which can be used in enzymatic assays for enzymes, which are supposed to have hydrolyzing activity, especially ABHD6. Because of the relatively high natural abundance of the oleic acid and arachidonic acid derivatives, those two seem to be the most reasonable to synthesize. The arachidonoyl group is also often present or involved in the formation of molecules with highly interesting biological activity like prostaglandins or 2-AG. The degradation products of the arachidonic acid derivative could also be metabolites with such outstanding properties.

The basic idea behind the development of a synthetic route for BMP was to find a central intermediate, which can be synthesized in few steps and is capable for the esterification with different FA acyl moieties to gain the possibility to make several BMP derivatives in short time.

4 Results and discussion

4.1 General comments to the synthetic route and retrosynthetic approach

In analogy to the synthesis published by Matile and coworkers of *S*,*S*-dioleoyl BMP (**17**)[6], we tried to find an short and effective synthetic route which is capable to make BMP derivatives with different fatty acyl residues. The most important feature of the desired route was to make a central intermediate, to which different fatty acids can be attached in an easy way.

Because BMP has additional to its fatty acid esters also two free primary hydroxyl groups, which are in esterification reactions more reactive than secondary hydroxyl groups, a protecting group strategy for the synthesis was absolutely necessary. The fact, that primary hydroxyl groups are more reactive to electrophiles is not only a drawback, but makes them capable for selective protection in the presence of secondary and tertiary hydroxyl groups. This advantage was also used in our synthetic approaches to protect the primary hydroxyl groups as silyl ethers.

Not only the free hydroxyl groups were required to be protected, also the free dialkyl phosphate oxygen would have made troubles during the synthesis due to its acidity and its resulting ionic structure. For phosphate protection we tried different approaches based on phosphoramidites and silylethyl esters. Different protecting groups for the free dialkyl phosphate were used because we tried to find a route to the mentioned central intermediate with inexpensive chemicals, to be able to make large amounts of this valuable compound. After it turned out that a quite sterically hindered silylethyl ester would lead to success, we decided to synthesize the needed rather expensive protecting group on our own, starting with cheap standard chemicals.

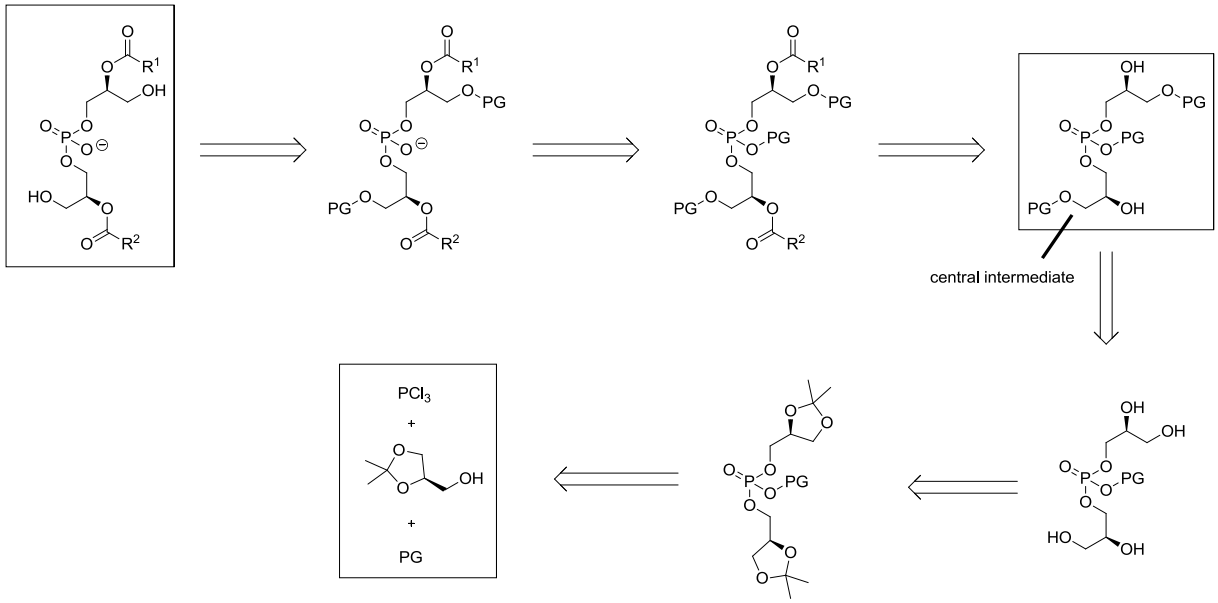
The glycerol backbone in naturally occurring BMP has a defined stereoconfiguration, so the synthetic route was also required to be enantioselective. To introduce this asymmetric glycerol unit we have used isopropylidene protected glycerol which has a defined stereoconfiguration and can be deprotected without racemization or stereoinversion.

There are two different possible approaches for the synthesis of protected alkyl phosphates, which are used in synthetic chemistry. At one approach a P^{III} intermediate is generated starting from phosphorous trichloride, which is subsequently oxidized to a P^{V} compound, while the second approach starts with P^{V}. We decided to use P^{III} chemistry with subsequent oxidation to build our desired protected dialkyl phosphate, because it gave us the opportunity to selectively form our compounds in a stoichiometric manner under mild conditions, in relatively few

reaction steps. P^{III} chemistry is commonly used in alkyl phosphate synthesis in different areas.[44]

To ensure that we were able to make different BMP derivatives in a quite short time, we had two requirements on our synthetic approach. First, the protected intermediate which is capable for the esterification reactions should be formed as late as possible in the synthetic route, and second, the synthesized fatty acid ester intermediate should be able to be deprotected in as few steps as possible. Because of the high price of some fatty acids, the deprotection steps were also required to have high yields.

The above mentioned requirements on the synthetic approach are summarized in the retrosynthetic approach which is pictured in Scheme 3:



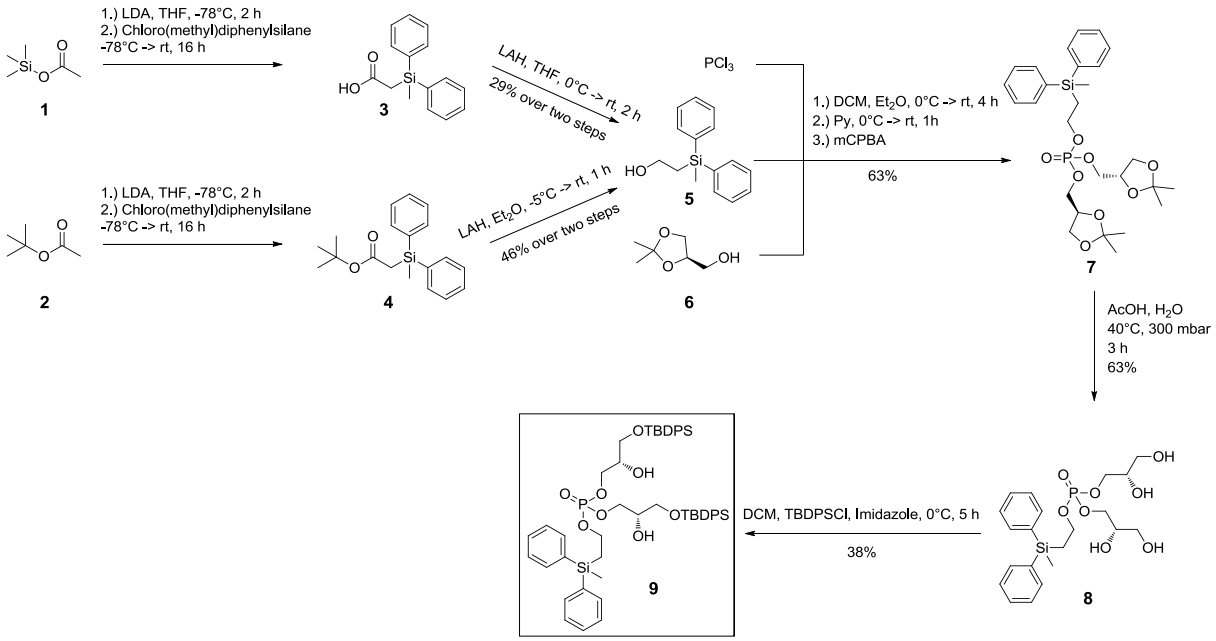
Scheme 3: Retrosynthetic approach for different BMP derivatives with a central intermediate capable for esterification with different FAs

A key step in this retrosynthetic approach was the deprotection of the isopropylidene protected glycerol backbone to the tetraol intermediate. This reaction is done under acid catalysis with oxygen nucleophiles, and requires high stability of the phosphate protecting group against low pH values and nucleophilic attack.

4.2 Synthesis of the 2-(methyldiphenylsilyl)ethyl protected central intermediate 9

Among other approaches, which are described later in the results and discussion section, the protection of the free dialkyl phosphate group with a 2-(methyldiphenylsilyl)ethyl (DPMSE) protecting group turned out to be the best choice. This protecting group includes the two

advantages that it is pretty easy to install via the addition of its free hydroxyl compound to phosphorous trichloride and has a moderate stability against nucleophiles in acidic environment. This stability is desperately required for the deprotection step of the isopropylidene protected backbone. But, it has the disadvantage, that it is expensive. After some attempts on small scale with commercially purchased DPMSE we knew, that this protecting group would lead to the desired intermediate **9** and decided to synthesize it on our own from inexpensive chemicals, to be able to make a large scale synthesis of intermediate **9**. In Scheme 4, the synthetic route to this intermediate is depicted.

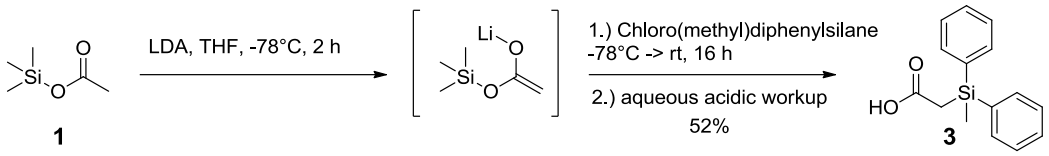


Scheme 4: Synthetic route to central intermediate **9**.[6, 45]

For the synthesis of the phosphate protecting group **5**, we tried two similar literature known procedures,[45] which are both described in detail in the following sections. They started with the silylation of the lithium enolate of an acetate and subsequent reduction to the hydroxyl compound. All following reactions to intermediate **9** are also described in detail in the following sections.

4.2.1 Synthesis of 2-(methyldiphenylsilyl)acetic acid (3)

To make the desired silyl carboxylic acid **3**, the lithium enolate of trimethylsilyl acetate (**1**) was generated with in situ prepared lithium diisopropylamide (LDA), stirred at -78°C for 2 h in THF and quenched with chloro(methyl)diphenylsilane at the same temperature. The title product was formed after acidic aqueous workup.



Scheme 5: Synthesis of 2-(methyldiphenylsilyl)acetic acid (**3**)[45a]

This reaction might seem surprising to most organic chemists, because the lithium enolate reacts at its nucleophilic carbon and not at its oxygen with a chlorosilane. The reaction of lithium enolates with chlorosilanes is one of many different ways for the synthesis of silylenol ethers, which are used as mild and selective nucleophiles in enol chemistry.[46]

A lithium enolate has two different nucleophilic sites, one at oxygen and one at its α-carbon. These two nucleophilic sites have a different reactivity due to the molecular orbital shape/energy of the lithium enolate and its electrostatic behavior. The carbon is the so called soft nucleophilic site of an enolate, while its oxygen is the so called hard nucleophilic side. This means, that the reactivity of the oxygen is mainly controlled by the electrostatic force, which is built between its negative charge and an approaching electrophile. While the oxygen atoms carries the greater part of the total negative charge, the α-carbon atom has a higher orbital coefficient of the HOMO. In contrast to the hard oxygen, the reactivity of the α-carbon is mainly controlled by the orbital interactions between the HOMO of an enolate and the LUMO of an approaching electrophile.[46]

A consequence of this reactivity differentiation of both nucleophilic sites is that enolates react at different sites with different electrophiles under different reaction conditions. If the electrophile has an energetically low LUMO, which has also a large diffuse orbital and a low or even no partial or total charge, it preferentially reacts at carbon, while positively charged or very polar electrophiles with energetically high LUMOs react rather at oxygen. Methyl mesylate and triethyloxonium tetrafluoroborate for example tend to react at oxygen, while alkyl halides (especially the very soft alkyl iodides) tend to react at carbon.[46]

Also the choice of solvent, the counterion and the temperature have a high influence on the outcome of the nucleophilic attack of an enolate. The reason for the influence of those three points is the separation of enolate anions from each other and or the counterion. Polar aprotic solvents as HMPA, DMSO or DMF coordinate the counterion, which seperates not only the ion pair, but also the enolate anions from each other. This strengthens the electrostatic attractive force between nucleophile and electrophile, which makes the nucleophile harder and promotes a reaction at oxygen. In contrast to that, ethereal solvents as THF and DME promote a reaction at carbon. The same effect can also be induced by the choice of the counter ion and the reaction

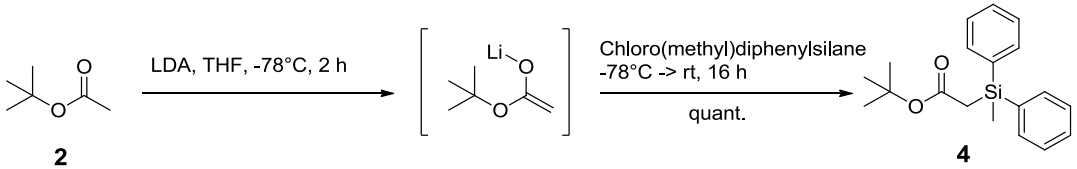
temperature. Enolates with large alkali metal cations as Cs or K have a less covalent character, which makes them more polar and harder, while Li enolates have a higher covalent and therefore softer character. Under low reaction temperatures, the anions stay also close to each other and to their counterions, which blocks the nucleophilic oxygen and makes them very soft.[46]

Becker and coworkers reported in 2010 that the treatment of trimethylsilyl acetate (**1**) with LDA and addition of $Ph_2MeSiCl$ at low temperatures led mainly to the C-silylated product.[45a] They explained the selectivity of the reaction with the aggregation state of the formed lithium enolate at low temperatures and with the softness of the chlorosilane electrophile. For our synthesis of compound **3** we also used their described method.

The only problems at this reaction occurred only at the isolation itself. The product was precipitated from ethyl acetate by the addition of cyclohexane at room temperature. At the first crystallization attempt, we isolated only about 25% of the theoretical yield because it turned out, that it is also quite well soluble in cyclohexane at room temperature, which was used to wash the precipitate. After several crystallization attempts from the concentrated mother liquor with cold cyclohexane we were able to isolate all together 52% of the theoretical yield, which is nearly the yield published in literature.[45a]

4.2.2 Synthesis of *tert*-butyl 2-(methyldiphenylsilyl)acetate (4)

As mentioned before, the β-silyl carboxylic ester **4** was also prepared by the formation of a lithium enolate and subsequent addition of chloro(methyl)diphenylsilane. The solvent, temperature and reaction times are again those described for compound **3**, but this time the reaction starts with the cheaper *tert*-butyl acetate (**2**) instead of its silicon derivate **1**.



Scheme 6: Synthesis of *tert*-butyl 2-(methyldiphenylsilyl)acetate (**4**) [45c]

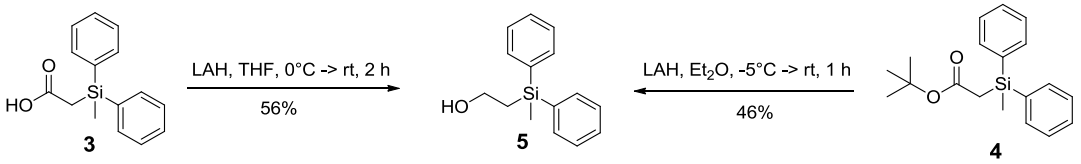
The regioselective addition of the chlorosilane electrophile to the enolate carbon can be explained by the same reasons as mentioned before for compound **3**. Again the solvent, temperature and enolate counterion define the order of aggregation of the enolates, which makes them more or less capable for the electrostatic attractive forces, which evolve at the approach of the electrophile (hardness of the nucleophile/electrophile). And also the resulting shape and

energy of the involved orbitals influences the regioselectivity of this reaction (softness of the nucleophile/electrophile). Again, the reaction proceeded under these reaction conditions mainly at the nucleophilic carbon of the enol equivalent reaction partner.

The big difference between the synthesis of compound **3** and compound **4** was, that in this case the ester was not hydrolyzed at the aqueous workup. The product of this reaction was isolated by simple extraction and evaporation of the solvent and was applied to reduction without any further purification. Nevertheless, the yield of this reaction seemed to be quantitative in high purity (judged via GC-MS). This makes the comparison between the synthesis of compounds **3** and **4** very difficult. However, it looks like the reaction of *tert*-butyl acetate (**2**) give better conversion and yield and it is also the cheaper starting material compared to trimethylsilyl acetate (**1**).

4.2.3 Synthesis of 2-(methyldiphenylsilyl)ethanol (5)

To obtain the desired silylethanol compound **5**, the carboxylic acid **3** and its *tert*-butyl ester **4** were reduced with lithium aluminum hydride (LAH).



Scheme 7: Synthesis of 2-(methyldiphenylsilyl)ethanol (**5**)[45b, 45c]

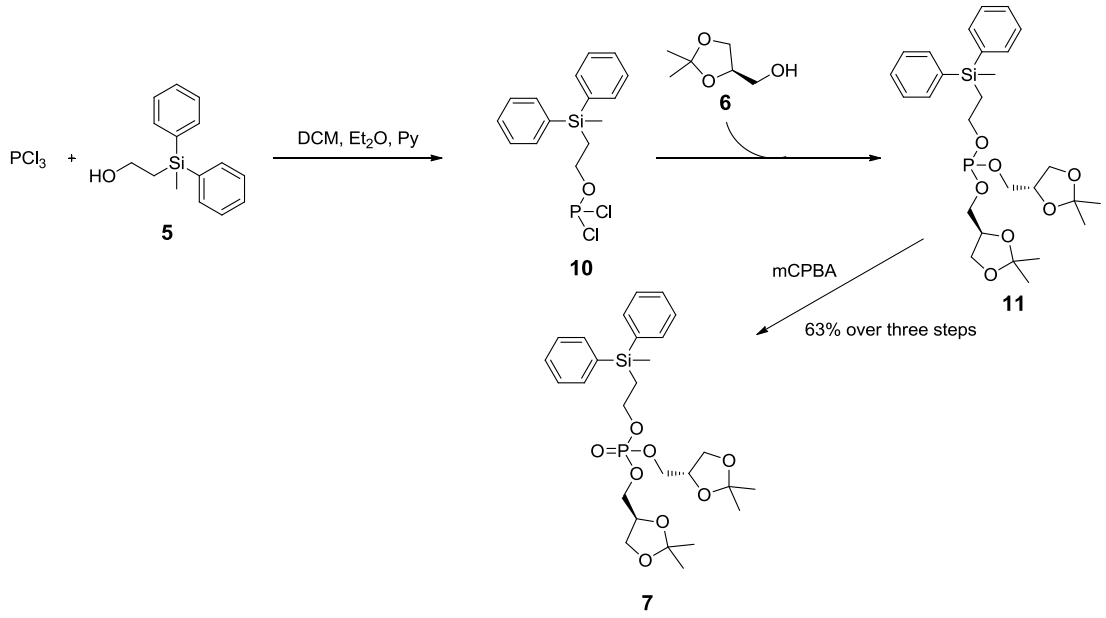
This reaction is done in aprotic solvents as THF or Et_2O, because otherwise the reagent would be quenched by protic hydrogen and form molecular hydrogen and a more stable aluminum species like an alkoxide or hydroxide. During the reaction the respective aldehyde is formed. This aldehyde is even more reactive than the corresponding carboxylic acid or ester educt and therefore rapidly reduced to the lithium salt of hydroxy-compound **5**, which was protonated during the aqueous workup of the reaction. For workup, the so called Fieser procedure was used. This is a generally known method for quenching an excess of LAH to form a granular precipitate which can easily be filtered off.

Though the reactions worked quite well, side products were generated which were seen via GC-MS analysis. Hence, the product had to be purified. We did this purification via vacuum distillation. We were able to isolate the silylethanol compound **5** in 29% of the theoretical yield via the trimethylsilyl acetate and in 46% via the *tert*-butyl acetate over two steps.

All together we synthesized about 20 g of this expensive compound, which enabled us to do the following reactions on quite large scale. As can be seen in the next section, the stoichiometric ratio of reactants for the formation of the phosphate backbone is very important, which was much easier to do on a large scale. Also the fact, that we introduced this protecting group at the first step of our multi-step synthesis, generated the demand for high amounts of it.

4.2.4 Synthesis of 2-(diphenylmethylsilyl)ethyl bis(2,3-*O*-isopropylidene-*sn*-glycero-1-)phosphate (7)

For the preparation of the isopropylidene and silylethanol protected P^V BMP scaffold **7**, we decided to use the published procedure from Matile and coworkers.[6] This reaction sequence included a one pot synthesis, which generated compound **7** over three steps without isolation of any intermediate. It started with the formation of the alkylphosphorodichloridite **10**, which was subsequently transformed to the trialkyl phosphite **11** by the addition of isopropylidene protected glycerol. Up to this compound the oxidation state of the central phosphorus atom stayed at III. To generate the desired P^V center, the phosphorus center was oxidized with 3-chloroperbenzoic acid (mCPBA).



Scheme 8: Synthesis of the isopropylidene and silylethanol protected P^V BMP scaffold **7**

This reaction was done about twenty times in different scales varying between 100 mg and 20 g and also with enatiopure and racemic starting materials. In our first attempts, which we performed only on a small scale, the yield was fluctuating between trace amounts in a mixture of compounds, which was not possible to separate and about 70% isolated yield. The reason

was, that the stoichiometric ratios especially for the addition of the two different alcohols **5** and **6** and the reaction conditions (concentration and reaction temperature) have to be very accurate.

In a multi component one pot synthesis like in this case, the proportions of reactants are difficult to control on a small scale and different side reactions can occur. The main observed side products were those, which had either two silylethanol protecting groups and one isopropylidene glycerol unit, or even three isopropylidene glycerol units attached to the phosphorus atom.

The main problem seemed to be, that the ratio of phosphorous trichloride and alcohol **5** seemed to be wrong. To avoid this problem, PCl_3 was freshly distilled prior use and serial dilution for the alcohol was tried, but again, the yield of this synthesis varied. Only when we did this reaction on multi gram scale, we achieved reproducible yields of about 65% after column chromatography which is in the same range as the yield published by Matile and coworkers (63%).[6]

4.2.5 Synthesis of bis((*S*)-2,3-dihydroxypropyl) (2-(methyldiphenylsilyl)ethyl) phosphate (8)

For the deprotection of compound **7** we initially tried the same conditions, which were described by Matile and coworkers,[6] namely 80% aqueous AcOH and a reaction temperature of 60°C in an open reaction apparatus. The reaction temperature of 60°C is in this case important for both, the rate of the reaction and the influence on the equilibrium between educts and products. As a side product of this acetal hydrolyzing reaction, acetone is formed, which itself can undergo acid catalyzed acetal formation to build again the initial educt of the reaction. At the reaction temperature of 60°C acetone is removed by distilling it out from the reaction mixture, which shifts the equilibrium to the desired product side.

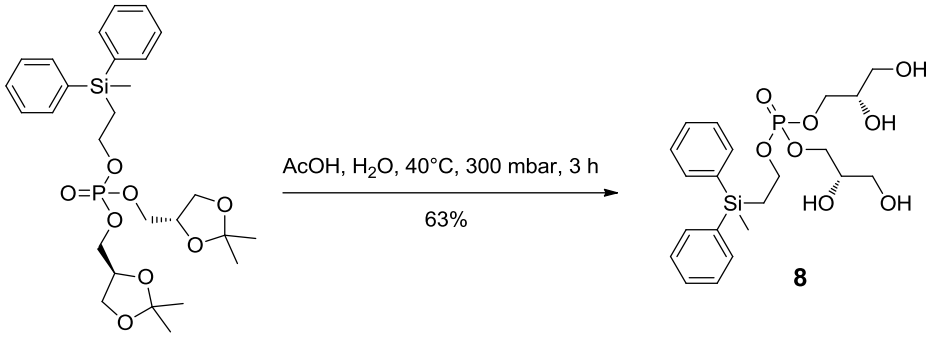
However in these initial attempts the outcome of the reaction was not reproducible and we always got some undesired side products, which were difficult to separate from tetraol **8**. We reasoned , that under these conditions, the acid catalyzed cleavage of the DPMSE phosphate protecting group also takes place. Because we wanted to synthesize large amounts of intermediate **9**, we needed a reproducible outcome of this reaction, which should allow us to do it on a large scale, without loss of valuable starting material.

After these initial attempts, we tried to find alternative ways for phosphate protection and milder reaction conditions for the selective isopropylidene deprotection of our DPMSE protected

compound **7**. The alternative attempts for phosphate protection failed and are described later in this chapter.

To find milder reaction conditions for the preparation of **8**, we tried out the cleavage of the dioxolane ring with MeOH instead of H_2O and PPTS as the acidic catalyst. This methanolysis is a transketalisation, which uses MeOH as nucleophile and forms 2,2-dimethoxymethane instead of acetone. The reaction equilibrium is now shifted with the large excess of methanol, which is commonly used as solvent in these reactions. This reaction gave reasonable and more reproducible yields (50%-70%) and was applied for several times and different scales of that reaction.

Although this method gave reproducible outcome and reasonable yields, we had also another idea, to avoid the cleavage of the DPMSE protecting group. The main problem in the AcOH catalyzed hydrolysis seemed to be the reaction temperature of 60°C which was required to shift the equilibrium to the desired side. If one would be able to remove the side product acetone at lower reaction temperatures, the DPMSE might be stable under the deprotection conditions. Based on this idea, we decided to do the reaction at 300 mbar and 40°C in 80% aqueous AcOH at a rotary evaporator.



7 **8**

Scheme 9: Synthesis of the deprotected tetraol BMP scaffold **8**

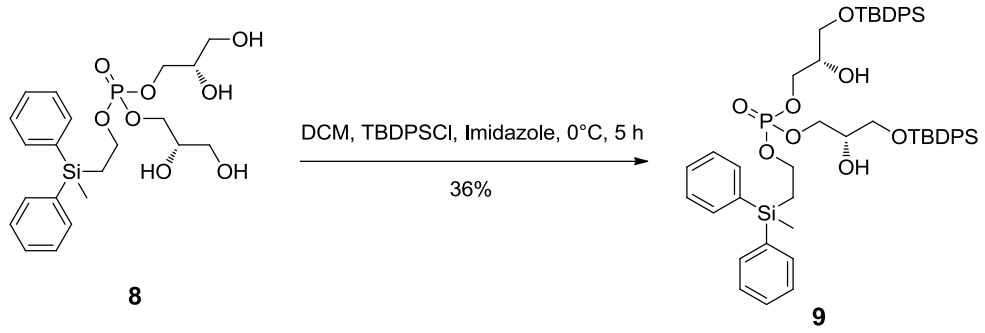
This reaction setup worked pretty well and we were able to isolate the product in 63% after column chromatography.

In conclusion both applied methods for deprotection gave similar yields and used cheap standard chemicals, but the reaction setup under reduced pressure is much more convenient. In that case, the reaction workup is just to set the pressure at the rotary evaporator as low that AcOH and H_2O can be distilled off.

4.2.6 Synthesis of bis((*S*)-3-((*tert*-butyldiphenylsilyl)oxy)-2-hydroxypropyl) (methyldiphenylsilyl)ethyl) phosphate (9)

As mentioned before, the basic idea behind our retrosynthetic approach was to synthesize a central intermediate, which is capable for esterification reactions with different fatty acyl moieties. To invert the intrinsic reactivity of the primary and secondary hydroxyl groups in these esterification reactions, the primary ones had to be protected. We decided to use the literature known procedure from Matile and coworkers[6] and varied slightly the reaction conditions to get the desired central intermediate **9**.

Therefore we used the *tert*-butyl(chloro)diphenylsilyl (TBDPS) ether as a sterically very hindered hydroxyl protecting group. To introduce it, we applied it as its chloride to the free tetraol **8** and used imidazole as a nucleophilic catalyst and as a base to remove the formed hydrogen chloride. To obtain regioselectivity, only a slight excess (2.10 equivalents) of the silylchloride was used and the reaction was performed at low temperature (0°C).



8 **9**

Scheme 10: Synthesis of the central intermediate **9**

TBDPS is only one of many commonly used silyl ether protecting groups. Silyl ethers themselves are among the most common used protecting groups for hydroxyl groups, including alcohols and phenol functionalities.[47] They are mainly introduced via their silyl chloride and can be cleaved off via acidic or basic hydrolysis and by fluoride ions. The reason, why they are used in diverse applications, is that their reactivity can be easily modulated by the substitution pattern on the silicon atom. This fact is valid for both, its formation and its stability and is based on electronic and steric effects. Electron withdrawing substituents like phenyl for example increase their stability against acidic deprotection, while they increase their capability towards basic hydrolysis. The reason is on the one hand the basicity of the silyl ether oxygen, which is increased by electron rich silyl groups and decreased by electron deficient ones. This effect can strongly influence the reactivity against acidic deprotection. Electron deficient silyl ethers are on the other hand not only less basic at oxygen than electron rich ones, but are also more

electrophilic at the silicon atom. This effect makes them easier to deprotect via basic hydrolysis.[47]

What is even more important and probably easier to understand than the influence of electronic effects to silyl ether stability is the influence of steric hindrance to it. This steric hindrance mediates also the regioselectivity in their formation process.[47] In our synthetic problem, we needed a silyl ether protecting group with large substituents as *tert*-butyl, which induces selectivity in its formation with respect to sterically not hindered hydroxyl groups. Therefore we used TBDPS, which is a particularly stable protecting group against acid hydrolysis and moderately stable against basic hydrolysis.[47] It shows high selectivity in the differentiation between secondary and primary alcohol functionalities.

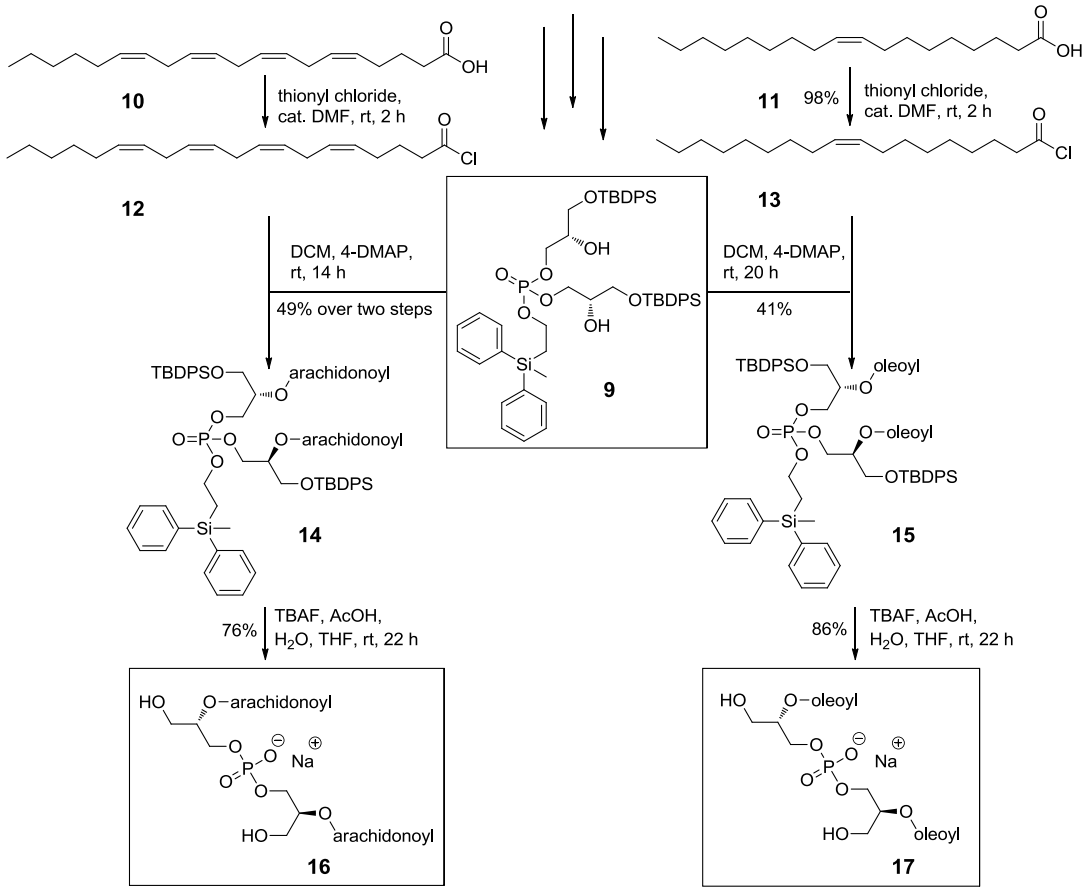
After some attempts with different solvents, reaction temperatures and stoichiometric ratios of base and silyl chloride, the conditions which worked best for our substrate were 5.0 equivalents of imidazole as base, 2.1 equivalents of *tert*-butyl(chloro)diphenylsilane (TBDPSCl) in dichloromethane (DCM) as a solvent and at a reaction temperature of 0°C. Despite the carefully chosen conditions, we got some side products and needed to purify our central intermediate **9** via column chromatography and isolated it in only 38% of the theoretical yield.

4.3 Esterification and deprotection of central intermediate 9 to different BMP derivatives

The above described synthesis of compound **9**, which was the central intermediate in our synthetic route, enabled us to synthesize different protected BMP derivatives, which could be deprotected following the same methodology. For the esterification of **9** with fatty acids, we decided to try a variation of the synthetic route published by Matile and coworkers.[6] They synthesized the oleic acid BMP derivative via an esterification of the *sn*-2 and *sn*-2' hydroxyl group with oleic anhydride, pyridine as a solvent and stoichiometric base and catalytic amounts of 4-DMAP as a nucleophilic catalyst. This method has the drawback, that one has to prepare the fatty acid anhydride first and that one equivalent of acid is lost in the esterification step. Instead of this procedure, we decided to try the esterification with the acid chloride of the required fatty acid. This method saved one synthetic step and also one equivalent of fatty acid per esterified hydroxyl group.

At the end of our synthetic route, we had to deprotect both, the phosphate and the hydroxyl protecting groups. Fortunately, both protecting groups included a labile silicon center and are

labile against nucleophilic attack from fluoride anions. Therefore, the deprotection of the esterified intermediates was done with buffered tetrabutylammonium fluoride (TBAF), as described by Matile and coworkers.[6] The final esterification and deprotection steps are summarized in Scheme 11.

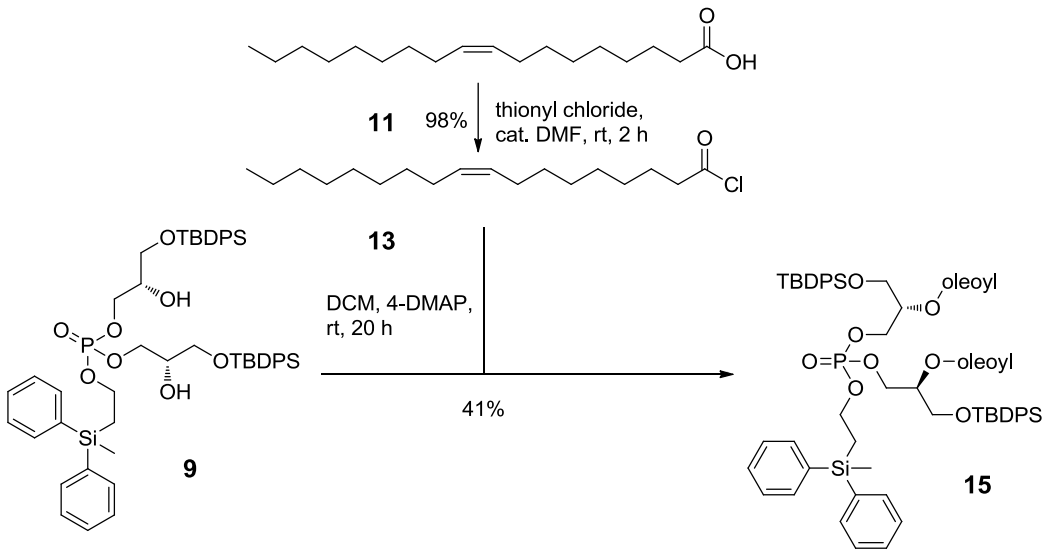


Scheme 11: Synthetic route to the sodium salt of the arachidonic acid derivative **16** and the oleic acid derivative **17** of *S*,*S*-BMP

We were able to synthesize the sodium salts of the BMP derivatives of oleic acid and arachidonic acid from diol intermediate **9** and thereby showed, that our approach to different derivatives was successful and that other fatty acyl chains can easily be introduced via this method.

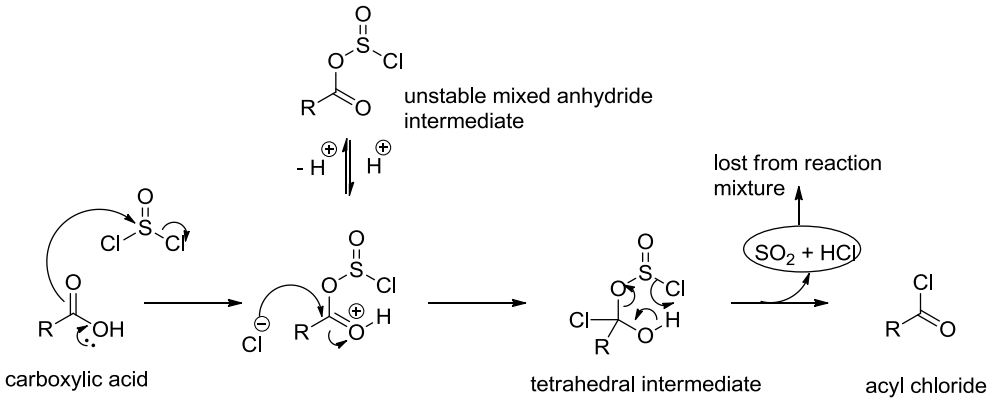
4.3.1 Synthesis of 2-(methyldiphenylsilyl)ethyl bis(3-*tert*-butyldiphenylsilyl-2-oleoyl-*sn*-glycero-1-) phosphate (15)

The esterified and protected oleoyl intermediate **15** was prepared from compound **9** via an esterification reaction with oleoyl chloride (**13**) (Scheme 12).



Scheme 12: Synthesis of the esterified protected oleoyl intermediate **15**

For the preparation of acyl chlorides from carboxylic acids exist different methods in organic synthesis, including chlorinating reagents such as thionyl chloride, phosphorus pentachloride and oxalyl chloride. These methods have in common, that they first form an activated carboxylic acid derivative which is further protonated at the carbonyl oxygen and undergoes a nucleophilic substitution with chloride ions at the protonated carbonyl group. In the cases of phosphorous pentachloride or thionyl chloride, this activated intermediate is a mixed anhydride containing the carboxylic acid group and an inorganic acid group.[46] The mechanism for the reaction with thionyl chloride is outlined in Scheme 13.



Scheme 13: Mechanism for the preparation of an acyl chloride with thionyl chloride.[46]

The acyl chloride formation with thionyl chloride is normally done neat (without any solvent) at ca. 50°C in an open apparatus.[48] This temperature enables relatively high reaction rates, but is below the boiling point of thionyl chloride (75°C). We have chosen to perform the reaction at room temperature but used catalytic amounts of dimethyl formamide (DMF) to accelerate the reaction.

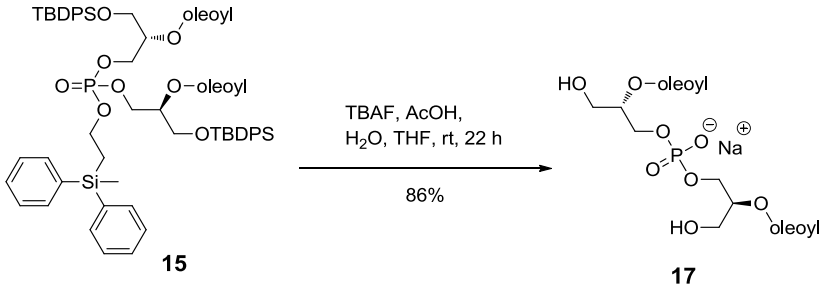
Oleic acid (**11**) itself is quite stable against oxidation and isomerization reactions and the higher reaction temperatures would have done no harm to the fatty acyl chain. But in contrast to it, polyunsaturated fatty acids like arachidonic acid (**10**), are much more sensitive to oxidation and isomerization reactions and because we tried to find reaction conditions, which are suitable for as many different fatty acids as possible, we have decided to do the reaction at lower temperatures but with DMF as a catalyst. After complete conversion (the evolution of gaseous SO_2 and HCl stopped), the excess of thionyl chloride was removed under reduced pressure and the crude product was pure enough to be used without further purification.

The esterification itself was done in DCM with 4-DMAP as a nucleophilic catalyst and stoichiometric base at the same time. 4-DMAP is a commonly used catalyst for esterification reactions of alcohols with carboxylic anhydrides or acyl chlorides and for other acyl transfer reactions.[49]

Despite an excess of oleoyl chloride (**13**), we observed always the monoesterified compound as a side product and needed to purify our product via column chromatography. We were able to isolate the pure product in 41% of the theoretical yield.

4.3.2 Deprotection of protected *S,S*-dioleoyl BMP (15) to the sodium salt of *S,S*-dioleoyl BMP (17)

After the formation of the oleoyl ester **15**, the final synthetic transformation in our synthetic route was the deprotection of both the hydroxyl protecting group as well as the phosphate protecting group. Because both are cleavable with fluoride ions, we were able to do this in one step with TBAF as reagent in a buffered system (Scheme 14).



Scheme 14: Deprotection of protected *S,S*-dioleoyl BMP (**15**) to the sodium salt of *S,S*-dioleoyl BMP (**17**)

In our first attempts, were not able to obtain the desired product. Deprotection of silyl protecting groups with TBAF and water in the presence of carboxylic ester moieties is not a simple task and can lead to saponification products.[50] The cleavage of the ester bond can be easily observed in the ^{1}H-NMR spectra of the reaction products. The hydrogen atoms which are

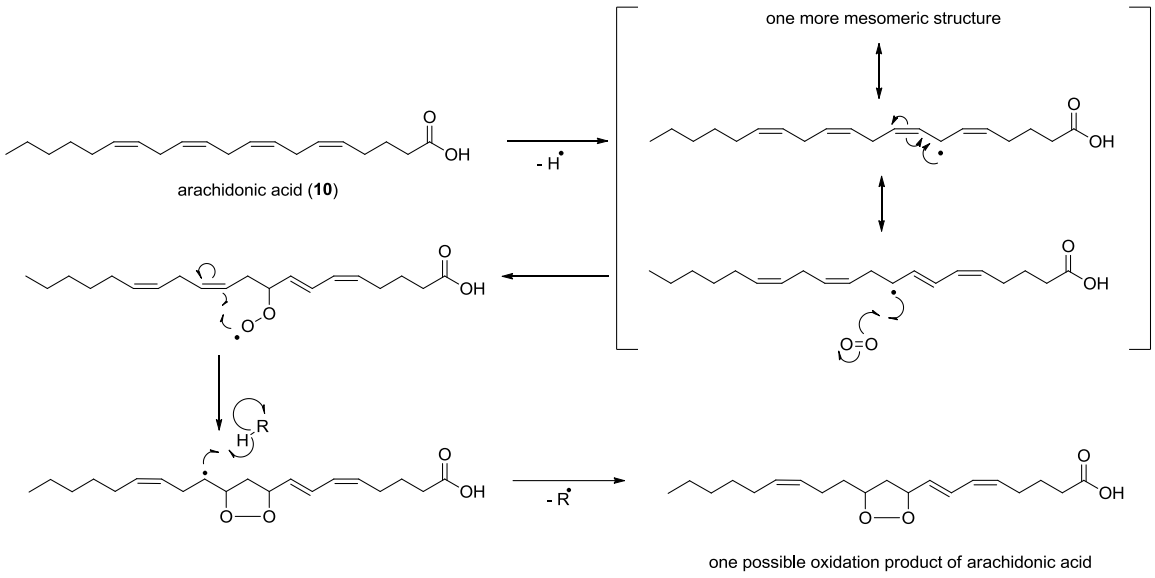
attached at the *sn*-2 and *sn*-2' carbons have a characteristic shift of ca. 5.0 ppm (5.08 ppm for compound **15** and 4.97 ppm for compound **17**). Every time we tried to deprotect diester **15** with TBAF and no additional additives, this characteristic signal disappeared. A simple way to avoid this problem is to use an acidic buffered system,[6,50] and with addition of 2 equivalents of AcOH (respective to TBAF) the ester moiety remained untouched.

For the separation of the nonionic side products and our desired acidic phospholipid we used an anionic ion exchange column. As described by Matile and coworkers[6] a diethyl amino ethyl (DEAE) exchanger on a Sephadex™ support (dextran based polymer) worked quite well and we were able to isolate *S,S*-dioleoyl BMP (**17**) in 86% of the theoretical yield.

4.3.3 Esterification and deprotection to synthesize the sodium salt of *S,S*-diarachidonoyl BMP (16)

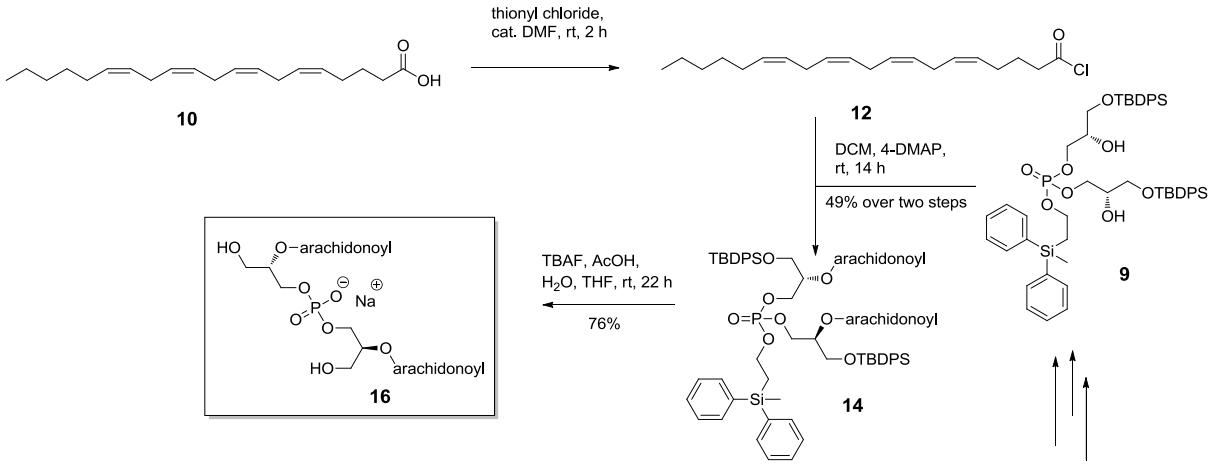
Arachidonic acid (**10**, AA) and oleic acid (**11**, OA) have one major difference. OA has only one isolated double bond and belongs to the monounsaturated fatty acids, while AA has four double bonds and belongs to the class of poly unsaturated fatty acids. Polyunsaturated fatty acids can easily undergo autoxidation processes.[51] The most important among these autoxidation processes is probably the formation of peroxide products. The four double bonds of AA have all *cis* configuration and are separated by methylene groups. These methylene groups are double allylic and hydrogen radicals can easily be abstracted to form stable radicals. These radicals can easily react with triplet oxygen to form a peroxy radical. This peroxy radical can either cyclize to form a 1,2-dixolane ring or undergo other reactions (most frequently hydrogen radical abstraction) to form other oxidized species.[51] In Scheme 15, the mechanism of one possible pathway of AA autoxidation is outlined.

The formation of the initial radicals is promoted by electromagnetic radiation, which makes arachidonic acid derivatives light sensitive (especially in the presence of molecular oxygen). Due to that special property of poly unsaturated fatty acids, the synthesis of the arachidonoyl BMP derivative **16** was performed under strict light and oxygen exclusion.



Scheme 15: Mechanism of the formation of a cyclized peroxy species of arachidonic acid[51]

For the synthesis of *S,S*-diarachidonoyl BMP (**16**) the same synthetic transformations were used as for the synthesis of *S,S*-dioleoyl BMP (**17**) but all used solvents or reagents were freshly distilled under an argon atmosphere or purged with argon to make them oxygen free. The used apparatuses were also covered with aluminum foil and the electric lighting in the lab was reduced to a minimum for that time, what made work for me and my colleagues to an exciting experience.



Scheme 16: Esterification and deprotection to synthesize the sodium salt of *S,S*-diarachidonoyl BMP (**16**)

The challenging part of this synthesis was obviously to work under inert conditions and light exclusion over all three steps. These three steps included also two preparative column chromatographies and several washing steps, which were all done under an argon atmosphere, in with aluminum foil covered apparatuses. The formation of arachidonoyl chloride (**12**) with thionyl chloride was again done at room temperature, with catalytic amounts of DMF and

worked quantitatively. Also the esterification reaction with 4-DMAP in DCM worked with 49% of the theoretical yield, which was slightly better than all attempts with oleoyl chloride done before. After deprotection with AcOH buffered TBAF in THF/H_2O, we purified the desired product again by the separation from nonionic side products via anionic ion exchange chromatography and isolated **16** in 76% of the theoretical possible yield for that step.

In conclusion, the synthetic transformations for the oleoyl BMP derivative (**17**) and its arachidonoyl counterpart (**16**) work similar well and give comparable yields, but the synthesis of **16** needs about three times longer than the synthesis of **17** because of the need to work completely inert and under light exclusion.

4.4 Alternative attempts for phosphate protection

In our first deprotection attempts of the isopropylidene protected glycerol moiety in compound **7**, we were not able to isolate the desired product **8** in acceptable yield, but got a complex mixture of different polar compounds. Even after column chromatography, we were not able to identify all generated products.

As mentioned before, we first tried to do this reaction in an open apparatus and higher reaction temperature under acid catalysis, to enable the acetone byproduct to evaporate from the reaction mixture. To get rid of the formed acetone, is in this acid catalyzed acetal hydrolysis important, because the reverse reaction (formation of an acetal) is also favored by acid catalysis and higher reaction temperature, which makes it an equilibrated reaction. The acetone evaporation should therefore shift the reaction equilibrium to the desired product side.

These first attempts with an open system and higher reaction temperatures resulted in undesired complex mixtures of different polar compounds. Because we did not know, if one of the phosphate ester bonds (either the glycerol ester or the ester bond to the DPMSE protecting group) was hydrolyzed, or any unexpected side reactions occurred, we decided to synthesize two other protected phosphate species, which could fit into our retrosynthetic approach (Scheme 3) and should help us to understand the reactivity of our substrate.

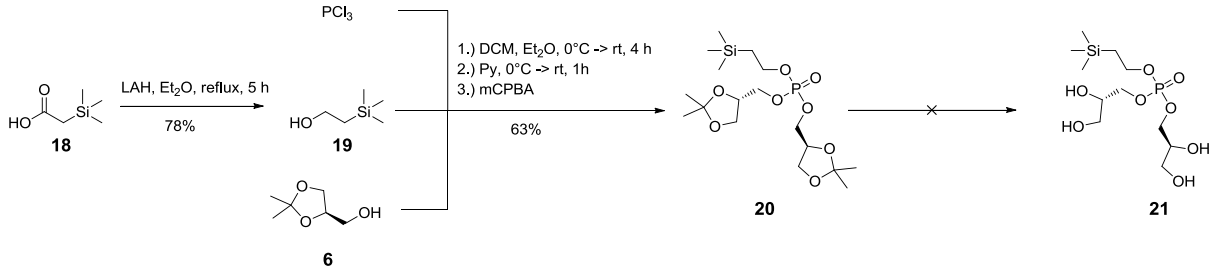
Another reason, why we tried alternative protecting groups for the synthesis of different BMP derivatives was the price of 2-(methyldiphenylsilyl)ethanol (**5**). It costs ca. 50 € per gram (227 € / 5 g, Sigma-Aldrich, December 4, 2013) and has a relatively high mass for a protecting group (242.39 g/mol), which is equivalent to a price per mole of about 11.000 €. Because the protecting group had to be attached in the first of 5 synthetic steps, which still had to be

optimized, and we wanted to make a large amount of the central intermediate in our retrosynthetic approach, we assumed that we would need large quantities of it.

4.4.1 Trimethylsilylethanol protected phosphate

2-(Trimethylsilyl)ethanol (TMSE) and 2-(Diphenylmethylsilyl)ethanol (DPMSE) have along with other trialkylsilylethanol phosphate PGs similar properties.[47] Most of these protecting groups have the drawback, that they are not stable for long time at room temperature, which makes their storage quite difficult. Nevertheless, TMSE was used in the synthesis of an interesting nucleoside sugar conjugate, where it was present during an oxidation of P^{III} to P^{V} and selectively cleaved off with TBAF.[52] TMSE is usually cleaved off with different common bases like pyridine, ammonium hydroxide or methylamine, or with different kinds of fluoride containing reagents like hydrogen fluoride (HF) or TBAF.[47] Rarely, also acidic conditions are used to cleave off a TMSE group. These involve rather hard Lewis acids like zinc bromide, or rather strong Brønsted acids like trifluoroacetic acid (TFA). We hoped, that we would be able to deprotect the isopropylidene PG in the presence of TMSE, but it turned out, that it is less stable to acid catalyzed hydrolysis than the dioxolane ring, even with weak Brønsted acids like AcOH or pyridinium *p*-toluenesulfonate (PPTS).

We synthesized the isopropylidene and TMSE protected BMP backbone **20** in analogy to the synthesis of compound **7** and tried to hydrolyze the dioxolane ring. We prepared the required protecting group **19**, via LAH reduction in diethyl ether. The educt for that reaction itself was prepared by Felix Anderl via a literature known procedure.[53] Both, the reduction and the formation of compound **20** worked in reasonable yields and we were able to isolate **20** in 34% of the theoretical yield over two steps.



Scheme 17: Synthesis of isopropylidene and TMSE protected BMP backbone (**20**) and attempt to deprotect the hydroxyl groups

For the deprotection of compound **20** we first tried the same reaction conditions as at our initial attempts, for the deprotection of the similar compound **7**, namely dissolving the educt in 80% aqueous AcOH and heating the reaction mixture to 60°C for about 30 min. The AcOH and H_2O

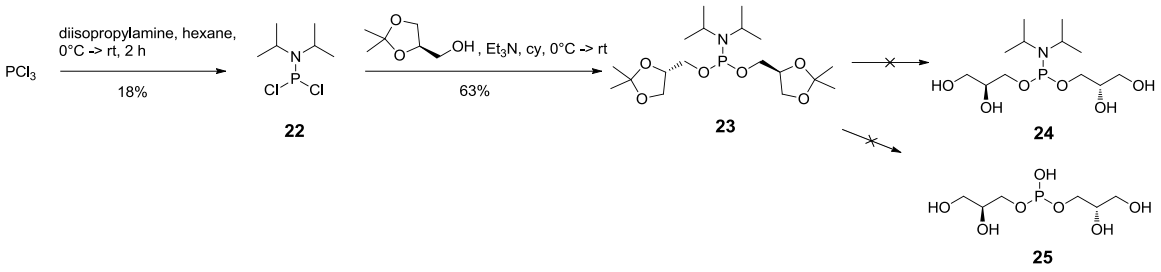
were removed under reduced pressure and the crude product was analyzed via ^{1}H-NMR. The resulting spectrum showed that the TMSE group was cleaved off at these conditions and that the acetal group of the dioxolane ring was just partially hydrolyzed.

We further tried a transketalisation with PPTS as the acid catalyst and MeOH as nucleophile and solvent. The side product of this methanolysis is 2,2-dimethoxypropane. To monitor the process of deprotection/decomposition, we used ^{1}H-NMR. Therefore, we dried deuterated methanol with magnesium sulfate and filled it into an inert NMR tube. The educt of our reaction was dissolved in the methanol in an argon counterflow and PPTS was added. The reaction progress was directly measured from the reaction mixture via ^{1}H-NMR analysis after 10 min and 30 min at room temperature. These two recorded spectra showed no conversion. So we decided to heat the reaction mixture to 45°C. After 30 min at this temperature, we recorded again a ^{1}H-NMR spectrum of the reaction mixture, which showed some changes to the initial spectra. After workup and further analysis, it turned out, that both the TMSE and the isopropylidene protecting groups were cleaved off. Due to the results of these experiments, we came to the conclusion that TMSE as a phosphate protecting group would not lead to success in our retrosynthetic approach.

4.4.2 Phosphoramidite attempt

Another synthetic attempt we tried, was via a phosphoramidite. Phosphoramidites are widely used in oligonucleotide synthesis.[54] They are quite stable under basic or neutral conditions, but are prone to nucleophilic attack under acidic conditions. Even mild acids as 1*H*-tetrazole can provoke an activation of the phosphoramidite group for nucleophilic substitution at the phosphorous atom.[54] Although the commonly used activators for phosphoramidites show very good efficiency, the mechanism of these substitution reactions is not fully understood and seems to be very complicated.[55]

In our approach we used the dichloride **22** to block one site of the trivalent phosphorous atom to enable the selective introduction of two isopropylidene protected glycerol groups. After the formation of this phosphoramidite compound **23**, we tried to hydrolyze the dioxolane acetale either with or without hydrolysis of the P-N bond. After some attempts with AcOH as an activator, we always got a mixture of different polar compounds and were not able to isolate one of the desired products of this reaction (either **24** or **25**).



Scheme 18: Phosphoramidite approach

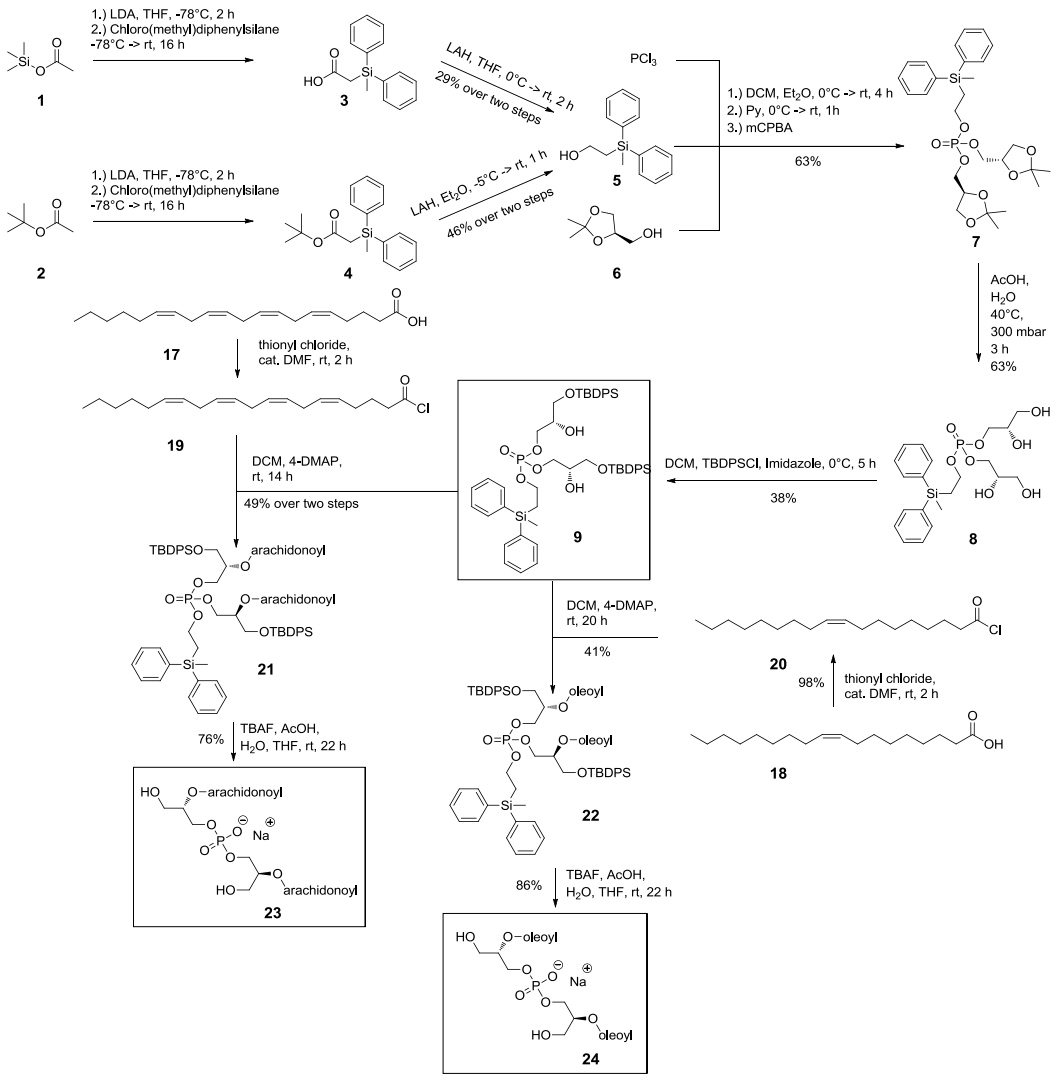
As we had optimized the isopropylidene deprotection of our initial route in the meantime (described earlier in this chapter), we did not further try to optimize the hydrolyzing reaction of compound **23**.

5 Summary and outlook

5.1.1 Summary

The aim of this thesis was the synthesis of different BMP derivatives, including the synthesis of a central intermediate, which is capable for the esterification with different FAs. In this thesis, an established retrosynthetic approach and synthetic route for *S,S*-dioleoyl BMP[6] was used to synthesize the desired central intermediate with small variations of this published work. The central intermediate was applied to esterification reactions with fatty acids.

We showed, that monounsaturated as well as polyunsaturated FAs can be used in this synthetic approach and that the yield for different FAs seems to be consistent. For the suitability of polyunsaturated FAs in this synthetic approach, special techniques for light and oxygen sensitive products were applied and adjusted for our purpose.

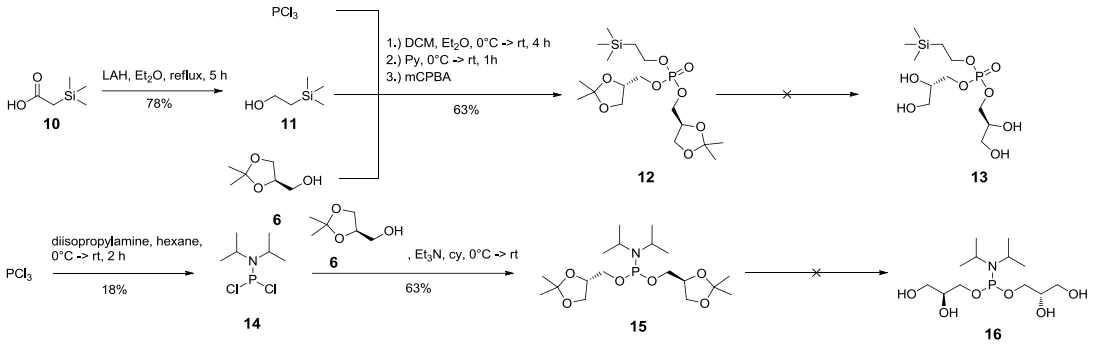


Scheme 19: Synthetic scheme to central Intermediate **9**, *S,S*-dioleoyl BMP (**24**) and *S,S*-diarachidonoyl BMP (**23**)

We were able to produce the sodium salts of *S,S*-dioleoyl BMP and *S,S*-diarachidonoyl BMP in good purity, as probes for catabolic processes. The synthesized products will be used in enzymatic assays including hydrolyzing enzymes, to test the BMP hydrolyzing activity of these enzymes with various BMP derivatives and identify the metabolized products.

The quite expensive protecting group 2-(methyldiphenylsilyl)ethanol was synthesized, to enable a large scale synthesis of the central intermediate. The synthesis was done via two different ways and their convenience was compared.

Two additional alternative attempts for phosphate protection, within the same retrosynthetic approach, were tried. Both of them failed at the hydrolyzing step of the dioxolane ring, due to their bad stability against acid catalyzed hydrolysis.



Scheme 20: Alternative attempts for phosphate protection

5.1.2 Outlook

After the successful synthesis of *S,S*-dioleoyl BMP and *S,S*-diarachidonoyl BMP, many other BMP derivatives are accessible via this established synthetic route. Among all possible derivatives, the docosahexaenoic acid (DHA) derivative seems to have interesting structural as well as biological properties. Starting from central intermediate **9**, the synthesis of different BMP derivatives includes only an esterification step and the final deprotection of both the primary hydroxyl group as well as the phosphate group. This means, that depending on the sensitivity of the desired FA, a new BMP derivative can be synthesized in relatively short time.

Another interesting and important molecule would be labeled *S,S*-dioleoyl BMP. The enzymatic assays, which test the BMP hydrolyzing activity of different enzymes are based on the detection of liberated free fatty acid (FFA). In some experimental setups (including for example whole cells), it is not easy to distinguish, where the FFAs come from. They could theoretically arise from every FA containing molecule in a cell. To distinguish, if the detected FFAs come really from the provided substrate, it would be helpful to label the substrate with some kind of tag. An

easy method to attach a label on *S,S*-dioleoyl BMP would be to make the same reaction sequence including the esterification and final deprotection with ^{13}C labeled OA instead of naturally occurring OA. This simple method does not change anything in the chemistry of its synthesis, which guarantees a high chance of success and also assures, that the enzymatic reaction itself is not inhibited in any kind.

6 Experimental section

6.1 General aspects, materials and methods

6.1.1 General

All reactions were carried out under air, unless noted otherwise. Inert reactions were carried out under protective gas atmosphere, namely nitrogen or argon with standard Schlenk techniques and anhydrous solvents. Anhydrous solvents were prepared by standard procedures, which are mentioned below and stored over molecular sieves under argon. The used apparatuses for inert reactions were dried in oil pump vacuum by heating with a heat-gun, cooled to room temperature and flushed with nitrogen or argon. Solvents and reagents were added to the inert apparatuses using Schlenk technique with protective gas counterflow. Highly viscous substances were either transferred to the dry apparatuses dissolved in an appropriate solvent followed by evaporation of the solvent and drying in oil pump vacuum, or directly dissolved into the dry solvent from a spatula. All given temperatures refer to the water bath, oil bath or cooling bath temperature. Solvents were degassed by one of the following procedures.

For degassing via ultrasonic bath, the Schlenk flask was closed with a septum. Thereupon, a long cannula was pushed through the septum and a balloon filled with argon was attached at the end of the cannula. An additional short cannula was pushed through the septum to ensure an argon flow through the flask. Via this argon flow and ultrasonication of at least 20 min, all remaining gases were removed from the solvent.

The second method for the preparation of degassed solvents comprised to freeze out the solvent in liquid nitrogen. Therefore the used Schlenk flask and the desired solvent was frozen in liquid nitrogen and oil pump vacuum was applied until the pressure dropped to 10^{-2} mbar. After the removal of the Schlenk flask out of the liquid nitrogen, the solvent was allowed to warm up to room temperature and oil pump vacuum was applied, until the pressure raised recognizable. This procedure was repeated several times until the solvent was supposably free from oxygen. This procedure is only suitable for high boiling solvents.

6.1.2 Reagents

2-(Trimethylsilyl)acetic acid was prepared by Felix Anderl using a literature known procedure.[53] All of the used chemicals and reagents were purchased from ABCR, ACROS

Organics, Alfa Aesar, Aldrich, Fisher Scientific, Fluka, Merck, Sigma-Aldrich and VWR. If not otherwise mentioned, chemicals were used without further purification.

6.1.3 Solvents and washing solutions

For the preparation of dry solvents 4 Å molecular sieves (Sigma Aldrich, beads, 8-12 mesh) or respectively 3 Å molecular sieves (Sigma Aldrich, beads, 8-12 mesh) were activated by filling a 500 mL round-bottomed flask to the half of its volume and heating it to about 150°C under oil pump vacuum for 3 d followed by cooling to room temperature and filling it with argon atmosphere**.**

Chloroform ($CHCl_3$): Already anhydrous chloroform (stabilized with 1-pentene) was bought from Acros Organics. It was stored over 4 Å activated molecular sieves (specified humidity: < 50 ppm).

Dichloromethane (DCM): Already with P_4O_{10} predried DCM was heated for three days over CaH_2 under reflux. Afterwards, the dry DCM was distilled over a 20 cm Vigreux column and stored in a dry brown Schlenk bottle over 4 Å activated molecular sieves.

Diethylether (Et_2O): Already distilled Et_2O was heated several hours over sodium in an argon atmosphere under reflux. As soon as benzophenone as indicator turned blue due the dryness of Et_2O, it was distilled over a 20 cm Vigreux column into a dry Schlenk tube and used within the next days for the related reactions.

***N*,*N*-Dimethylformamide (DMF):** Anhydrous DMF was bought from Acros Organics and transferred into a dry brown Schlenk bottle over 3 Å activated molecular sieves (specified humidity: < 50 ppm).

***n*-Hexane:** Anhydrous n-hexane was bought from Sigma-Aldrich and transferred into a dry brown Schlenk bottle over 4 Å molecular sieves (specified humidity: < 50 ppm).

Methanol (MeOH): Methanol was heated over magnesium and iodine until hydrogen starts to evolve. After this violent reaction, it was heated for 2 h in an argon atmosphere under reflux and distilled into a dry brown Schlenk bottle over activated 3 Å molecular sieves.

Tetrahydrofurane (THF): Prior distilled inhibitor free THF was heated several hours over sodium in an argon atmosphere under reflux. As soon as benzophenone as indicator turned blue due the dryness of THF, it was distilled over a 20 cm Vigreux column into a dry Schlenk bottle over activated 4 Å molecular sieves.

The following solvents were used for reactions and workups, which were done under a normal air atmosphere: acetone, acetonitrile (ACN), cyclohexane, dichloromethane (DCM), diethylether (Et_2O), ethanol (EtOH), ethyl acetate (EtOAc), methanol (MeOH), tetrahydrofurane (THF). These solvents were bought from either, Fisher Scientific, Roth or Riedel-de-Haën. Chloroform, Et_2O and THF were distilled carefully at the rotary evaporator to remove the respective stabilizer. Chloroform was stored in a dark bottle. Et_2O and THF were stored over KOH in a dark bottle.

The following five aqueous solutions were used as washing solutions in workups. They were prepared via the following procedures:

Saturated NaCl aqueous solution: sodium chloride was dissolved in deionised H_2O under vigorous stirring until a precipitate remained.

Saturated $NaHCO_3$ aqueous solution: sodium bicarbonate was dissolved in deionised H_2O under vigorous stirring until a precipitate remained.

Saturated Na_2CO_3 aqueous solution: sodium carbonate was dissolved in deionised H_2O under vigorous stirring until a precipitate remained.

Saturated NH_4Cl aqueous solution: ammonium chloride was dissolved in deionised H_2O under vigorous stirring until a precipitate remained.

0.8 M NaOAc aqueous solution: 68.0 g sodium acetate trihydrate were dissolved under vigorous stirring in 500 mL deionised H_2O.

6.1.4 Analytical Methods

6.1.4.1 Thin-layer chromatography

For analytical thin layer chromatography, TLC-plates were purchased from Merck (TLC aluminium foil, silica gel 60 F_{254}, 20 x 20 cm). After processing, the analytes were detected using a UV lamp with a wavelength of 254 nm (fluorescence quenching) and/or a stain reagent and developing in a stream of hot air. The used eluent/detection system and the R_f values are mentioned in the appropriate procedure.

The following two stain reagent systems were used for analyte detection:

CAM: 5.0 g phosphomolybdic acid were dissolved in a solution of 16 mL concentrated sulphuric acid and 200 mL deionised H_2O. 2.0 g solid cerium(IV) sulphate were slowly and carefully added to this yellow solution. The formed suspension was stirred until every solid dissolved to form a pale yellow solution.

$KMnO_4$: 3.0 g potassium permanganate, and 20.0 g potassium carbonate were dissolved in a 1.5% NaOH aqueous solution to form an intense purple solution.

6.1.4.2 Flash chromatography

Preparative column chromatography was performed using silica gel 60 from Acros Organics with a bead size between 35 µm and 70 µm. To ensure a continuous flow of eluent gentle pressure was applied using a hand pump. The amount of silica gel was adapted to the separation problem (depending on the different R_f-values, between 30- to 100- fold (w/w) mass of the dry crude product). To ensure an efficient separation, a pad of silica between 10 cm and 30 cm height was chosen. For air sensitive substances, the solvents used as an eluent were degassed prior use.

6.1.4.3 Gas chromatography with mass sensitive detector

The used system for analytical gas chromatography included an "Agilent Technologies 7890A GC system" with an "Agilent Technologies 7683B Series injector" and an "Agilent Technologies 7683 Series autosampler". Injection was done in split mode. The analytes were separated via polarity and boiling point over a polar HP-5MS capillary column (length: 30 m, diameter: 0.25 mm, coating: 0.25 µm). Helium 5.0 was used as a carrier gas. After ionisation via electron impact cell with a potential of 70 eV, the formed cations were detected by an "Agilent Technologies 5975C inert MSD with Triple-Axis detector". The following temperature program was used for analysis:

Method-A: 50°C 1 min, ramp 40°C/min linear to 300°C, 5 min

For quantification (concerning yield and conversion), the correlating areas under the chromatogram-curve were used. The measured data shows only relative values and is not corrected by an internal standard. The retention times t_R and the masses of main fragments with their relative intensity normalized by the basis peak are stated in the corresponding experimental procedure.

6.1.4.4 High performance liquid chromatography

The analytical HPLC measurements were performed with a "Shimadzu Nexera Liquid Chromatograph" with tempered column oven. The analytes were separated via polarity and adsorption over a C-18 reversed phase column type "Poroshell® 120 SB-C18, 3.0 x 100 mm, 2.7 µm" from "Agilent Technologies". The analytes were detected with a "Shimadzu SPD-M20A Prominence Diode Array detector" at a wavelength of λ =210 nm, or with the mass selective detector "Shimadzu LCMS-2020 Liquid Chromatograph Mass Spectrometer" in the modes ESI positive or ESI negative. The following elution programs were used for analysis:

Method-A: 0.0-0.5 min 70% H_2O + 0.01% HCOOH/30% ACN, 0.5 – 5.0 min linear to 100% CAN, 5.0 – 5.7 min 100% ACN, 5.7 -5.8 min linear to 70% H_2O + 0.01% HCOOH/30% ACN, 5.8 – 8.0 70% H_2O + 0.01% HCOOH/30% ACN; 0.70 mL/min; 40°C

Method-B: 0.0-0.5 min 30% H_2O + 0.01% HCOOH/70% ACN, 0.5 – 5.0 min linear to 100% CAN, 5.0 – 7.5 min 100% ACN, 7.5 -8.0 min linear to 30% H_2O + 0.01% HCOOH/70% ACN, 8.0 – 9.0 30% H_2O + 0.01% HCOOH/70% ACN; 0.70 mL/min; 40°C

The retention times and masses of the found ions are stated in the corresponding experimental procedure.

6.1.4.5 Micro scale workups

For the monitoring of some reaction progresses, the following micro workups were done:

Micro workup A: Ca. 10 µL of the reaction mixture were taken, poured into 500 µL EtOAc, and washed with 500 µL 1 M HCl. The organic layer was used for TLC analysis.

Micro workup B: Ca. 10 µL of the reaction mixture were taken, poured into 500 µL EtOAc and washed with 500 µL sat. NH_4Cl aqueous solution. The organic layer was dried over $MgSO_4$ and filtered before it was injected into the GC-MS.

Micro workup C: Ca. 30 µL of the reaction mixture were taken, poured into 500 µL EtOAc and washed with 500 µL 10 % NaOH aqueous solution. The organic layer was dried over $MgSO_4$ and filtered before it was injected into the GC-MS.

Micro workup D: Ca. 40 µL of the reaction mixture were taken and poured into 500 µL EtOAc. 100 µL MeOH were added and the organic layer was washed with 500 µL sat. NH_4Cl aqueous solution, dried over $MgSO_4$ and filtered before it was injected into the GC-MS.

6.1.4.6 Nuclear magnetic resonance spectroscopy

All described nuclear magnetic resonance (NMR) spectra were recorded at one of the two following machines:

Bruker AVANCE III with autosampler: 300.36 MHz- ^{1}H-NMR, 75.53 MHz- ^{13}C-NMR

Varian Unity Inova: 499.91 MHz- ^{1}H-NMR

For recording the ^{1}H-NMR and ^{13}C-NMR spectra, the residual resonance signal of protons from the deuterated solvents were used and all ^{13}C-NMR spectra are ^{1}H-decoupled. To distinguish between similar carbon atoms, APT-spectra were recorded for some compounds. If the number of hydrogen atoms attached to a carbon is determined, the carbon atoms are labelled as C_q (quaternary carbon), CH (carbon with one attached hydrogen), CH_2 (carbon with two attached hydrogen atoms) and CH_3 (carbon with three attached hydrogen atoms). To distinguish between similar hydrogen atoms, HH-COSY and HSQC spectra were recorded. Signal multiplicities *J* are abbreviated as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), p (pentet), h (heptuplett) and m (multiplet). Furthermore, the chemical shift δ in ppm (parts per million), coupling constants *J* in Hz (Hertz) and integrals of single signals are noted in the corresponding experimental procedure. Deuterated chloroform ($CDCl_3$) purchased from VWR and deuterated methanol (MeOH-d_4) purchased from Euriso top were used as solvents. $CDCl_3$ was additionally filtrated through basic Alox (aluminium oxide activated, basic Typ 5016A, 58 Å, bead size: 150 mesh, Brockmann grade I) from Acros Organics to remove residual amount of formed deuterated hydrogen chloride (DCl).

6.1.4.7 Optical rotation

Optical rotation was measured with a "Polarimeter 331" from Perkin Elmer with integrated sodium lamp as light source. The measurements were done using the D-line of sodium (λ = 589 nm) at a temperature of 20°C. Chloroform was used as a solvent ("Analytical reagent grade Chloroform" by Fisher Scientific). The concentration of the analyzed solutions was between 0.45 g/100 mL and 1.15 g/100 mL.

6.1.4.8 Titration of stock solutions

For determination of the accurate concentration of stock solutions, these reagents were titrated prior to use. Because of the instability against hydrolysis this was done using inert Schlenk tubes. All measurements were done at least three times to make a significant arithmetic average. The titrated stock solution was used directly after this analysis.

Titration of *n*-butyl lithium (in *n*-hexane):[56] A 10 mL Schlenk tube equipped with a magnetic stirring bar and a stopper, was flame dried under vacuum, flushed with argon and charged with 455 mg (2.14 mmol, 1.0 eq) diphenylacetic acid and 1.0 mL anhydrous THF. *n*-Butyllithium solution was added via a syringe and septum in argon counterflow under vigorous stirring, until a yellow suspension was formed. The volume of used butyl lithium solution was noted and the concentration was recalculated.

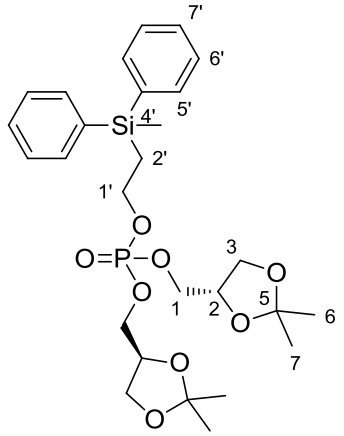
6.2 Experimental procedures and analytical data

6.2.1 Synthesis of oleic *S,S*-bis(monoacylglycero)phosphate (17)

All reactions in this chapter were done several times and on different scales. The syntheses were also performed with *rac*-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol as a starting material, to give a diastereomeric mixture of compounds **7**, **8**, **9**, **15** and **17** whereupon all chiral diastereomers appeared as a racemate.

The following procedures describe examples of the syntheses which are picked because of their good preparative convenience and average yield starting with (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol as an enantiomerically pure starting material.

6.2.1.1 2-(Methyldiphenylsilyl)ethyl bis(2,3-*O*-isopropylidene-*sn*-glycero 1-)phosphate (7)[6]



A 500 mL round-bottomed flask equipped with a magnetic stirring bar, Schlenk adapter and a stopper, was flame dried under vacuum, flushed with nitrogen and charged with 50 mL anhydrous diethyl ether, 20 mL anhydrous dichloromethane and 3.27 mL (37.5 mmol, 1.01 eq) phosphorus trichloride. The resulting colourless solution was cooled to 0°C whereupon a solution of 8.51 mL (37.3 mmol, 1.00 eq) 2-(methyldiphenylsilyl)ethanol (**5**) in 50 mL dry diethyl ether was added dropwise over a period of 10 min via a syringe and a septum in nitrogen counter flow under vigorous stirring. The colourless reaction mixture was warmed up to RT and stirred for 4 h. Then it was cooled again to 0°C whereupon 9.44 mL (117.0 mmol, 3.14 eq) anhydrous pyridine was added dropwise over a period of 7 min via a syringe and a septum in argon counter flow under vigorous stirring. After the addition of pyridine, the reaction mixture turned into a suspension with colourless liquid and a white chunky solid. To ensure further homogenic mixing, the solid was crushed in an ultrasonic bath for 5 min. Then it was warmed up to RT over 10 min, stirred for 10 min and cooled to 0°C again, whereupon a solution of 9.38 mL (75.7 mmol, 2.03 eq) (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol in 50 mL dry

diethyl ether was added dropwise over a period of 7 min via a syringe and a septum in argon counter flow under vigorous stirring. The resulting white suspension was again crushed in an ultrasonic bath and stirred at RT. After 1 h, it was again cooled to 0°C and 10.36 g (42.0 mmol, 1.13 eq) 3-chloroperbenzoic acid (70% with H_2O) dissolved in 100 mL dichloromethane was added dropwise. The reaction mixture turned into a bright yellow emulsion. This emulsion was further stirred for 1 h. Ethylacetate (250 mL) was added and the solution was washed successively with 1 M HCl aqueous solution (100 mL), brine (100 mL), saturated $NaHCO_3$ aqueous solution (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4, filtrated and the solvent was removed by rotary evaporation to give a viscous, yellow oil as a crude product. The product was purified via flash-chromatography (500 g silca gel, filling height = 28 cm, dichloromethane/acetone = 9:1, fraction size: 150 mL, fractions 16 to 27 pooled) and dried under oil pump vacuum.

$C_{27}H_{39}O_8PSi$ [550.65]

yield: 12.97 g (23.6 mmol, 63%), colourless viscous liquid

R_f = 0.53 (dichloromethane/acetone = 7:1 (v/v), 254 nm, CAM, $KMnO_4$)

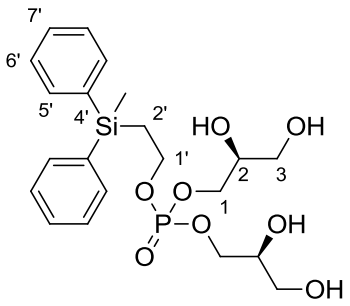
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.52-7.47 (m, 4H, arom-CH), 7.41-7.31 (m, 6H, arom-CH), 4.31-4.15 (m, 4H, H-1), 4.06-3.90 (m, 6H), 3.78 (dd, *J* = 8.6, 5.5 Hz, 2H), 1.73-1.62 (m, 2H, H-2'), 1.39 (s, 6H, H-6 orH-7), 1.34 (s, 6H, H-6 or H-7), 0.60 (s, 3H, SiMe-H).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 135.7 (C_q, C-4'), 134.5 (CH), 129.8 (CH), 128.2 (CH), 110.0 (C_q, C-5), 74.1 (d, $J_{PC'}$ = 8.0 Hz, CH, C-2), 67.8-67.31 (m, CH_2), 66.5 (d, J_{PC} = 6.2 Hz, CH_2), 66.3 (CH_2, C-3), 26.9 (CH_3, C-6 or C-7), 25.4 (CH_3, C-6 or C-7), 17.7 (CH_2, C-2'), -3.93 (CH_3, C-MeSi)

$[\alpha]_D^{20}$ = +3.93°(c = 1.15, $CHCl_3$)

HPLC-MS (Method-A): t_R = 4.83 min

6.2.1.2 Bis((*S*)-2,3-dihydroxypropyl) (2-(methyldiphenylsilyl)ethyl) phosphate (8)[6]



A 100 mL round-bottomed flask was charged with 12.78 g (23.2 mmol, 1.00 eq) **7**, 60 mL glacial acetic acid and 15 mL H_2O. The colorless solution was heated up to 40°C at a rotary evaporator, which was adjusted to a rotation speed of 150 rpm and a pressure of 300 mbar. For

TLC reaction control, the apparatus was filled with air, the rotation was stopped and the reaction mixture was directly spotted on a TLC plate. After 3 h, the reaction control showed full conversion and the pressure in the apparatus was reduced to evaporate the excess of water and acetic acid (down to 10 mbar over 20 min and 10 mbar for 1 h). The colourless crude product was further dried under oil pump vacuum for 1 h and purified by flash-chromatography (500 g silica gel, filling height = 25 cm, dichloromethane/methanol = 8:1, fraction size: 150 mL, fractions 17 to 38 pooled) and dried under oil pump vacuum.

$C_{21}H_{31}O_8PSi$ [470.53]

yield: 6.96 g (14.8 mmol, 63%), colourless viscous oil

R_f = 0.44 (dichloromethane/methanol = 7:1 (v/v), 254 nm, CAM, $KMnO_4$)

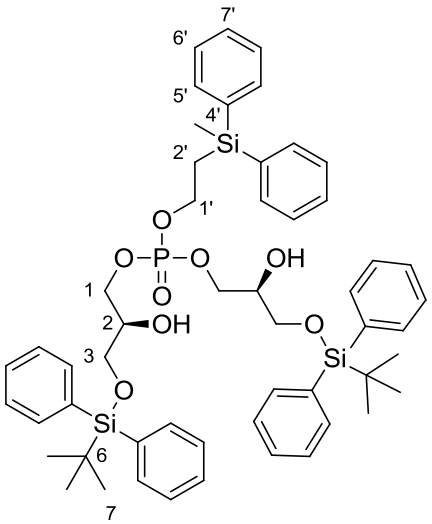
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.53-7.43 (m, 4H, arom-CH), 7.40-7.31 (m, 6H arom-CH), 4.66 (bs, 2H, protic OH), 4.18 (dd, *J* = 16.6, 7.8 Hz, 2H), 4.12-3.89 (m, 6H), 3.88-3.87 (m, 2H), 3.70-3.49 (m, 4H), 1.71-1.57 (m, 2H, H-2'), 0.59 (s, 3H, SiMe-H).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 135.6 (C_q, C-4'), 134.5 (CH), 129.8 (CH), 128.2 (CH), 71.1-70.4 (m, CH, C-2), 68.9 (d, *J* = 13.2 Hz, CH_2), 66.8 (d, *J* = 6.3 Hz, CH_2), 62.8 (CH_2, C-3), 17.6 (CH_2, C-2'), -4.0 (CH_3, C-MeSi)

$[\alpha]_D^{20}$ = 0.00°(c = 0.96, $CHCl_3$)

HPLC-MS (Method-A): t_R = 2.75 min

6.2.1.3 Bis((*S*)-3-((*tert*-butyldiphenylsilyl)oxy)-2-hydroxypropyl) (2-(methyldiphenylsilyl)ethyl)phosphate (9)[6]



A 100 mL round-bottomed flask equipped with a magnetic stirring bar was charged with 1.33 g (2.83 mmol, 1.00 eq) **8** and 50 mL dichloromethane. The resulting colourless solution was cooled to 0°C, and 970 mg (14.25 mmol, 5.03 eq) imidazole was added at once. A solution of 1.63 g (5.95 mmol, 2.10 eq) *tert*-butylchlorodiphenylsilane in 20 mL dichloromethane was

added dropwise via a syringe over a period of 30 min. After complete addition, a white solid began to precipitate and the suspension was stirred at 0°C. The reaction progress was monitored via TLC and the reaction was quenched after 5 h by addition to 50 mL saturated $NaHCO_3$ aqueous solution. Additional 30 mL dichloromethane were added. After phase separation, the organic phase was washed with brine (30 mL) and dried over Na_2SO_4. The drying agent was removed by filtration and the solvent was removed by rotary evaporation to give a yellowish viscous oil as a crude product. The product was further purified via flash-chromatography (90 g silica gel, filling height = 12 cm, cyclohexane/ethyl acetate = 5:2, fraction size: 50 mL, fractions 8 to 13 pooled) and dried under oil pump vacuum.

$C_{53}H_{67}O_8PSi_3$ [947.32]

yield: 1.03 g (1.09 mmol, 38%), colourless viscous oil

R_f = 0.66 (cyclohexane/ethyl acetate = 1:1 (v/v), 254 nm, CAM, $KMnO_4$)

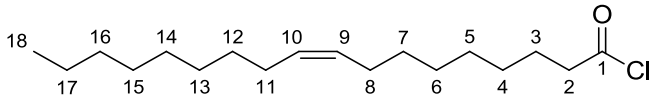
^{1}H-NMR (300.36 MHz, $CDCl_3$):) δ 7.67-7.59 (m, 8H, arom-CH), 7.51-7.44 (m, 4H, arom-CH), 7.44–7.29 (m, 18H, arom-CH), 4.27–4.02 (m, 6H), 3.95–3.80 (m, 2H), 3.73–3.57 (m, 4H), 1.71–1.61 (m, 2H, H-2'), 1.05 (s, 18H, H-7), 0.57 (s, 3H, SiMe-H).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ 135.7 (CH), 134.5 (CH), 133.0 (C_q), 130.0 (CH), 129.8 (CH), 128.2 (CH), 128.0 (CH) 70.9-70.7 (m, CH, C-2), 69.2-69.0 (m, CH_2), 66.7-66.5 (m, CH_2), 64.1 (CH_2, C-3), 27.0 (CH_3, C-7), 19.4 (CH_2, C-2'), -4.0 (CH_3, C-MeSi).

$[\alpha]_D^{20}$ = +17.5°(c = 0.45, $CHCl_3$)

HPLC-MS (Method-B): t_R = 6.16 min

6.2.1.4 Oleoyl chloride (13) [48]



A 10 mL Schlenk tube equipped with a magnetic stirring bar and a stopper, was flame dried under vacuum, flushed with nitrogen and charged with 2 mL (27.6 mmol, 3.51 eq) freshly distilled thionylchloride. 2.22 g (7.86 mmol, 1.00 eq) oleic acid were added via a syringe and a septum in argon counter flow under vigorous stirring. After addition, the septum was replaced with a bubbler. The reaction mixture starts to bubble and turns yellow. After 45 min of stirring at room temperature, the gas evolution has stopped. One drop of *N*,*N*-dimethylformamide was added to the reaction mixture to ensure complete conversion. More gas evolved for two more hours. After complete conversion the excess of thionylchloride was removed under reduced pressure and collected in a cooling trap. The residual product was pure enough to be used in the next step without further purification.

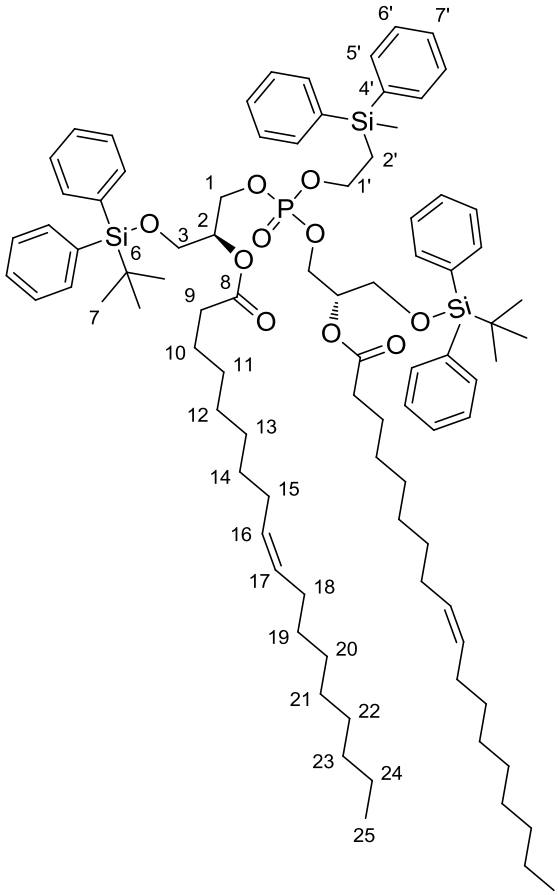
$C18H_{33}ClO$ [300.91]

yield: 2.32 g (7.71 mmol, 98%), brown liquid

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ 5.42-5.47 (m, 2H, H-9 and H-10), 2.88 (t, *J* = 7.3 Hz, 2H, H-2), 2.07-1.95 (m, 4H, H-8 and H-11), 1.71 (p, *J* = 7.2 Hz, 2H, H-3), 1.39-1.23 (m, 20H, aliphatic Hs), 0.88 (t, *J* = 6.5 Hz, 3H, H-18).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ 174.0 (C_q, C-1), 130.3 (CH, C-9 or C-10), 129.8 (CH, C-9 or C-10), 47.3 (CH_2, C-2), 32.1 (CH_2), 30.0 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 28.5 (CH_2), 27.4 (CH_2), 27.3 (CH_2), 25.2 (CH_2, C-17), 22.8 (CH_3, C-18).

6.2.1.5 2-(Methyldiphenylsilyl)ethyl bis(3-*tert*-butyldiphenylsilyl-2-oleoyl-*sn*-glycero-1-) phosphate (15)[6]



A 10 mL Schlenk tube equipped with a magnetic stirring bar and a stopper, was flame dried under vacuum, flushed with argon and charged with 5 mL dry dichloromethane. 576 mg (0.60 mmol, 1.00 eq) **9** were directly added and dissolved from a spatula under argon counter flow. To the resulting colourless solution, 541 mg (1.80 mmol, 3.0 eq) oleoyl chloride and 235 mg (1.92 mmol, 3.2 eq) 4-DMAP were added under argon counter flow. The reaction progress was monitored via TLC analysis. Because of low conversion further 459 mg (1.53 mmol, 2.54 eq) oleoyl chloride were added after 3 h and 300 mg (2.45 mmol, 4.09 eq) DMAP were added after 4 h. After further stirring at RT for 20 h (overnight) a white solid had

precipitated and TLC analysis (micro workup A) showed good conversion. 25 mL ethyl acetate were added and the reaction mixture was washed with 5% HCl aqueous solution (25 mL). After phase separation, the aqueous phase was extracted with ethyl acetate (2 x 25 mL) and the combined organic layers were washed with saturated $NaHCO_3$ aqueous solution (25 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4, filtrated and the solvent was removed by rotary evaporation to give a viscous, colourless oil as a crude product. The crude product was dissolved in dichloromethane, suspended with 2 g silica gel and the solvent was removed using a rotary evaporator. The residual solid was used for flash-chromatography (130 g silca gel, filling height = 28 cm, cyclohexane/ethyl acetate/triethylamine = 8.5:1:0.002, fraction size: 20 mL) and the product was dried under oil pump vacuum.

$C_{89}H_{131}O_{10}PSi_3$ [1476.22]

yield: 359 mg (0.24 mmol, 41%), colourless viscous oil

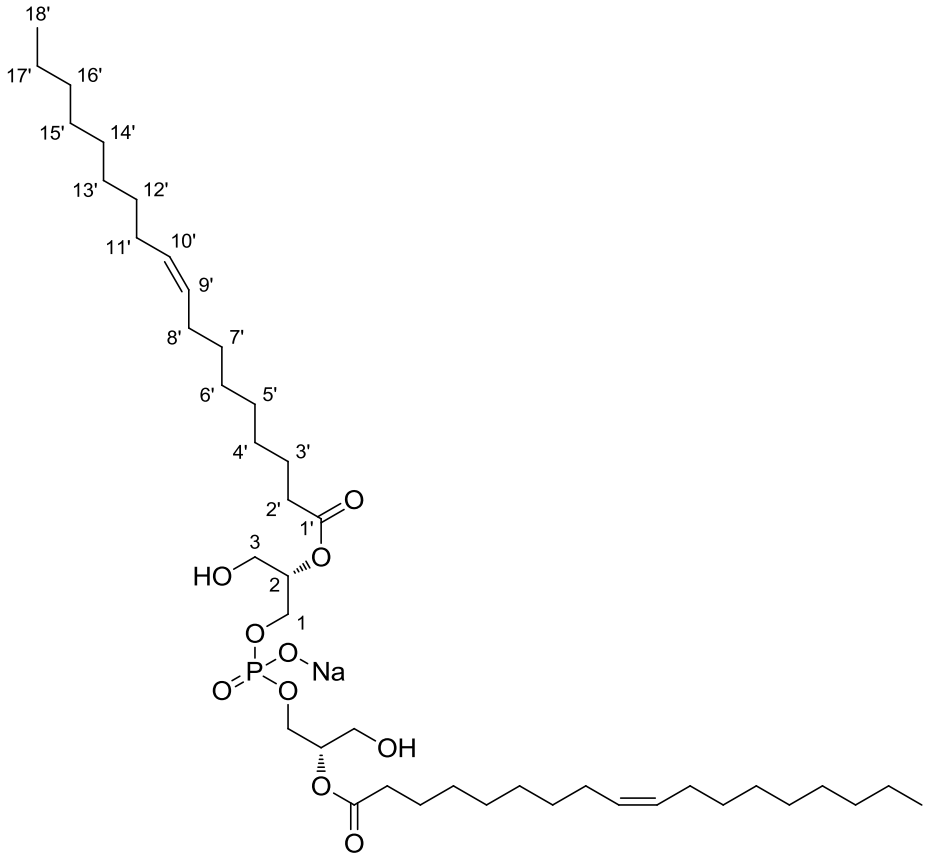
R_f = 0.60 (cyclohexane/ethyl acetate = 6:1 (v/v), 254 nm, CAM, $KMnO_4$)

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ 7.68–7.54 (m, 8H, arom-CH), 7.51–7.41 (m, 4H, arom-CH), 7.41– 7.28(m, 18H, arom-CH), 5.40–5.28 (m, 4H, H-16 and H-17), 5.13–5.02 (m, 2H, H-2), 4.26–4.07 (m, 6H), 3.78–3.66 (m, 4H), 2.28–2.12 (m, 4H), 2.08–1.91 (m, 8H), 1.67–1.50 (m, 6H), 1.37–1.18 (m, 40H, aliphatic Hs), 1.02 (s, 18H, H-7), 0.87 (t, J = 6.5 Hz, 6H, H-25), 0.55 (s, 3H, SiMe-H).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ 173.0 (Cq, C-8), 135.7 (CH), 135.6 (CH), 134.5 (CH), 133.1 (CH), 133.1 (CH), 130.1 (CH), 130.0 (CH), 129.9 (CH), 129.7 (CH), 128.2 (CH), 127.9 (CH), 127.9 (CH), 72.2 (d, J_{PC} = 7.3 Hz, CH-C-2), 66.4 (d, J_{PC} = 6.4 Hz, CH_2), 65.9–65.6 (m, CH_2), 62.0 (CH_2), 34.3 (CH_2), 32.1 (CH_2), 29.9 (CH_2), 29.9 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 27.4 (CH_2), 27.4 (CH_2), 27.1 (CH_2), 26.9 (CH_3, C-7), 24.9 (CH_2), 22.8 (CH_2), 19.4 (CH_2), 17.7 (CH_2), 14.3 (CH_2), -4.0 (CH_3, C-MeSi).

$[\alpha]_D^{20}$ = +4.5°(c = 0.50, $CHCl_3$)

6.2.1.6 Sodium bis((*S*)-3-hydroxy-2-(oleoyloxy)propyl)phosphate (17)[6]



A 25 mL round-bottomed flask equipped with a magnetic stirring bar, Schlenk adapter and a stopper, was flame dried under vacuum, flushed with argon and charged with 5 mL anhydrous THF. 240 mg (160 µmol, 1.00 eq) **15** were directly added and dissolved with a spatula under argon counter flow. After 30 min, to the colourless solution 30 µL (1.66 mmol, 10.38 eq) H_2O and 118 µL (2.06 mmol, 12.88 eq) AcOH were added under argon counter flow. After 20 min 1.00 mL (1.00 mmol, 6.25 eq) of a TBAF solution (1.00 M in THF) was added via a syringe and septum under argon counter flow. The reaction progress was monitored via TLC analysis. After 22 h (overnight), the reaction mixture turned yellowish and was transferred to a separatory funnel, where $CHCl_3$ (30 mL), MeOH (30 mL) and H_2O (30 mL) were added. After mixing, the lower layer was concentrated under reduced pressure with a rotary evaporator. The yellowish crude product was dissolved in 2 mL $CHCl_3$/MeOH/H_2O = 30:60:8 and applied to a DEAE-Sephadex ion exchange column (A-25, acetate form, 2 g) equilibrated with the same mixture of solvents ($CHCl_3$/MeOH/H_2O = 30:60:8). The column was carefully washed with the same solvent mixture (3 x 20 mL) and the eluted fractions were analysed by TLC analysis (dichloromethane/methanol = 8:2, 254 nm, $KMnO_4$). As soon as no more detectable side product were seen by TLC, the solvent mixture was changed to $CHCl_3$/MeOH/0.8 M NaOAc = 30:60:8 and the title compound was eluted (11 x 2 mL). The fractions containing the product

(detected by TLC analysis, dichloromethane/methanol = 8:2, $KMnO_4$) were combined, transferred to a separatory funnel and $CHCl_3$ and H_2O were added to give the ratio of $CHCl_3$/MeOH/H_2O = 1:1:1. After mixing and phase separation the lower layer was separated, dried over $MgSO_4$ and concentrated under reduced pressure with a rotary evaporator. The product was dried under oil pump vacuum.

$C_{42}H_{78}NaO_{10}P$ [797.03]

yield: 110 mg (0.14 mmol, 86%), colourless viscous oil

R_f = 0.70 (dichloromethane/methanol = 8:2 (v/v), CAM, $KMnO_4$)

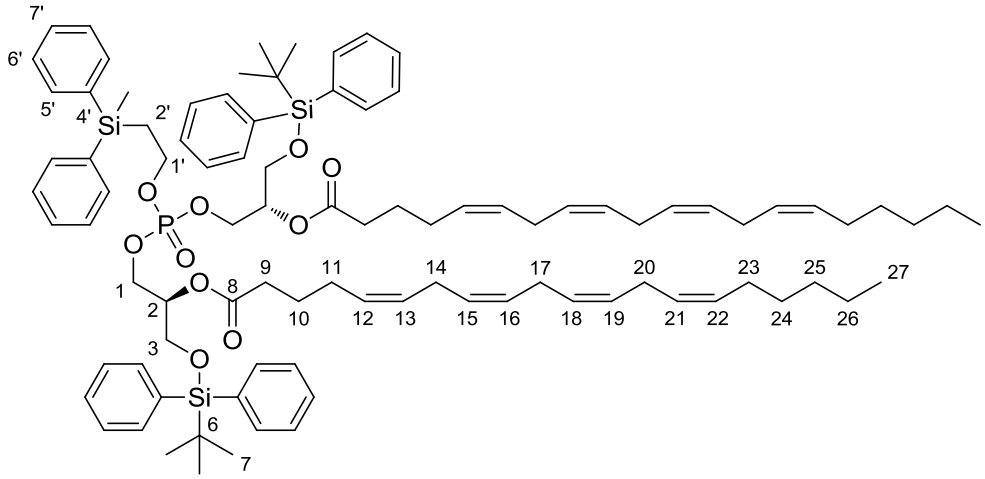
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ 5.47–5.10 (m, 4H, H-9 and H-10), 5.10 – 4.80 (m, 2H, H-2), 4.16–3.85 (m, 4H), 3.85–3.57 (m, 4H), 2.63 (bs, 2H, protic-OH), 2.30 (t, J = 7.3 Hz, 4H, H-2'), 2.09–1.90 (m, 8H), 1.68–1.47 (m, 4H), 1.4 –1.13 (m, 40H), 0.88 (t, J = 6.3 Hz, 6H, H-18').

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ 173.7, 130.2, 129.8, 72.9, 63.8, 60.4, 34.4, 32.1, 29.9, 29.7, 29.5, 29.5, 29.4, 29.4, 27.4, 25.0, 22.8, 14.23.

$[\alpha]_D^{20}$ = -1.9°(c = 0.95, $CHCl_3$)

6.2.2 Synthesis of arachidonic *S,S*-bis(monoacylglycero)phosphate (16)

6.2.2.1 2-(Methyldiphenylsilyl)ethyl bis(3-*tert*-butyldiphenylsilyl-2-arachidonoyl-*sn*-glycero-1-) phosphate (14)[6, 48]



A 10 mL Schlenk tube equipped with a magnetic stirring bar and a stopper, was flame dried under oil pump vacuum, flushed with argon and charged with 2 mL (27.57 mmol, 22.79 eq) freshly distilled thionylchloride. The apparatus was covered with aluminium foil, to protect the reaction mixture from sunlight and ~ 400 µL (1.21 mmol, 1.00 eq) arachidonic acid were added directly from an inert ampule. To the resulting colourless solution two drops of dry DMF were added under vigorous stirring and argon counter flow. After the addition, the stopper was replaced by a bubbler, the reaction mixture started to bubble and turned yellow. It was further stirred under light exclusion for 3 h at RT. After no more gas evolution was recognized, the

bubbler was replaced by a stopper and the excess of thionylchloride was removed under reduced pressure and collected in a cooling trap. The brown liquid residual product was dissolved in 3 mL dichlorometane and 600 mg (0.63 mmol, 0.52 eq) **9** and 250 mg (2.04 mmol, 1.69 eq) 4-DMAP were added under argon counterflow. The reaction mixture turned immediately into a red solution. The reaction progress was monitored via TLC analysis (micro workup A). After 14 h of stirring under light exclusion and argon, TLC analysis showed moderate conversion and additional 1.00 g (8.16 mmol, 6.76 eq) 4-DMAP and 400 mg (0.42 mmol, 0.35 eq) **9** were added. After one more hour, further 200 mg (0.21 mmol, 0.17 eq) **9** was added. After two more hours of stirring, the reaction mixture was transferred to a with aluminium foil covered and with argon flushed separatory funnel, diluted with 50 mL degassed ethyl acetate and washed successively with 1 M HCl aqueous solution (3 x 50 mL), saturated $NaHCO_3$ aqueous solution (50 mL) and degassed brine (50 mL) under light and oxygen exclusion. The organic layer was concentrated under reduced pressure, using a nitrogen flushed and with aluminium foil covered rotary evaporator. The resulting crude product was purified by oxygen free flash chromatography (30 g silica gel, filling height = 18 cm, cyclohexane/ethyl acetate/triethylamine = 8.5:1:0.002, fraction size: 15 mL). Therefore the column was connected to a Schlenk line and flushed with nitrogen for 1 h. The eluent was degassed before using it and the fractions were collected in Schlenk tubes, which had been dried under oil pump vacuum, flushed with nitrogen and covered with aluminium foil. Fractions 6 to 9 were pooled and the product was dried under oil pump vacuum.

$C_{93}H_{127}O_{10}PSi_3$ [1520.23]

yield: 455 mg (0.30 mmol, 49%), yellow viscous oil

R_f = 0.61 (cyclohexane/ethyl acetate = 6:1 (v/v), 254 nm, CAM, $KMnO_4$)

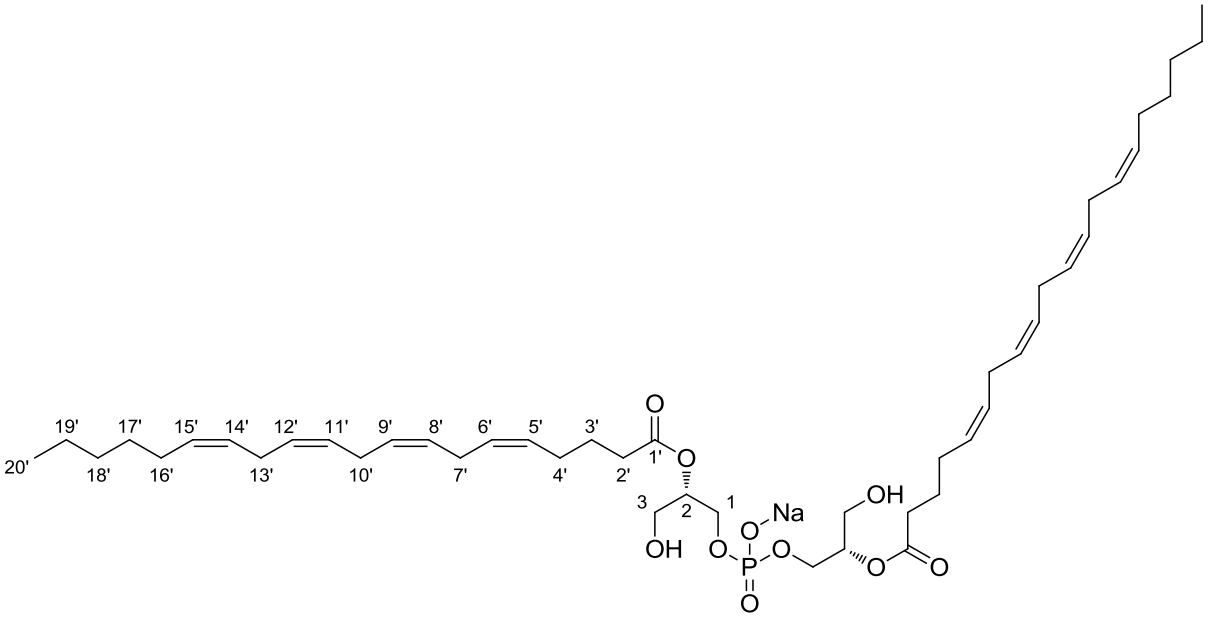
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ 7.65–7.58 (m, 8H, arom-CH), 7.49–7.42 (m, 4H, arom-CH), 7.42–7.28 (m, 18H, arom-CH), 5.44–5.25 (m, 16H, olefinic Hs), 5.08 (s, 2H, H-2), 4.25–4.07 (m, 6H, H-1 and H-1'), 3.76–3.68 (m, 4H, H-3), 2.86–2.70 (m, 12H, H-14, H-17 and H-20), 2.29–2.16 (m, 4H, H-9), 2.09 – 1.97 (m, 8H), 1.72–1.53 (m, 6H), 1.38–1.20 (m, 12H), 1.01 (s, 18H, H-7), 0.88 (t, *J* = 6.6 Hz, 6H, H-27), 0.55 (s, 3H, H-MeSi).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ 172.7 (C_q, C-8), 151.8 (CH), 135.7 (CH), 135.6 (CH), 134.5 (CH), 133.1 (CH), 133.1 (CH), 130.7 (CH), 130.0 (CH), 129.7 (CH), 129.1 (CH), 129.0 (CH), 128.8 (CH), 128.4 (CH), 128.3 (CH), 128.2 (CH), 127.9 (CH), 127.9 (CH), 127.7 (CH), 33.8 (CH_2), 31.7 (CH_2), 29.5 (CH_2), 27.8 (CH_3), 27.4 (CH_2), 27.1 (CH_2), 26.9 (CH_3), 26.7 (CH_2), 25.8 (CH_2), 24.8 (CH_2), 22.7 (CH_2), 19.4 (CH_2), 14.2 (CH_3).

$[\alpha]_D^{20}$ = +4.5°(c = 0.70, $CHCl_3$)

Comment: missing signals in ^{13}C-NMR

6.2.2.2 Sodium bis((*S*)-3-hydroxy-2-((5*Z*,8*Z*,11*Z*,14*Z*)-icosa-5,8,11,14-tetraenoyloxa)propyl)phosphate (16)[6]



A 25 mL round-bottomed flask equipped with a magnetic stirring bar, Schlenk adapter and a stopper, was flame dried under vacuum, flushed with argon and charged with 5 mL anhydrous THF. The apparatus was covered with aluminium foil for light exclusion. 30 µL (1.66 mmol, 13.60 eq) degassed H_2O and 118 µL (2.06 mmol, 16.89 eq) degassed AcOH were added under argon counter flow. 185 mg (122 µmol, 1.00 eq) **14** were directly added and dissolved from a spatula under argon counter flow. To the resulting yellow solution 1.00 mL (1.00 mmol, 8.20 eq) TBAF solution (1.00 M in THF) was added via a syringe and septum under nitrogen counter flow. The reaction progress was monitored via TLC analysis. After 22 h (overnight), the reaction mixture was transferred to a with aluminium foil covered and with nitrogen flushed separatory funnel, where degassed $CHCl_3$ (30 mL), degassed MeOH (30 mL) and degassed H_2O (30 mL) were added under nitrogen counter flow. After mixing and phase separation, the lower layer was concentrated under reduced pressure by an aluminium foil covered and nitrogen flushed rotary evaporator, to give 250 mg of a yellow oil. The crude product was dissolved in 2 mL degassed $CHCl_3$/MeOH/H_2O = 30:60:8 and applied to an inert DEAE-Sephadex ion exchange column (A-25, acetate form, 2 g) equilibrated with the same mixture of solvents (degassed $CHCl_3$/MeOH/H_2O = 30:60:8). Therefore, prior to equilibration the column had been dried under oil pump vacuum and flushed with nitrogen for 30 min. All fractions were collected in vacuum dried and nitrogen flushed Schlenk tubes. The column was carefully washed with the same solvent mixture (3 x 20 mL) under light and oxygen exclusion and the eluted fractions

were analysed by TLC analysis (dichloromethane/methanol = 8:2,254 nm, $KMnO_4$). As soon no more detectable side product was seen by TLC, the solvent mixture was changed to degassed $CHCl_3$/MeOH/AcOH = 30:60:8 and the residue of free fatty acids was eluted (2 x 4 mL). Then the eluent was changed to degassed $CHCl_3$/MeOH/0.8 M NaOAc = 30:60:8 and the title compound was eluted (13 x 2 mL). The fractions containing the product (detected by TLC analysis, dichloromethane/methanol = 8:2, $KMnO_4$) were combined, transferred to an aluminium foil covered and nitrogen flushed separatory funnel and degassed $CHCl_3$ and degassed H_2O were added to give a ratio of $CHCl_3$/MeOH/H_2O = 1:1:1. After mixing, NaCl in nitrogen counter flow was added, to separate the layers. After separation, the lower layer was dried over $MgSO_4$, filtered and the solvent was removed by an aluminium foil covered and nitrogen flushed rotary evaporator. The product was dried under oil pump vacuum.

$C_{46}H_{74}NaO_{10}P$ [841.04]

yield: 78 mg (93 µmol, 76%), yellowish soid

R_f = 0.70 (dichloromethane/methanol = 8:2 (v/v), CAM, $KMnO_4$)

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ 5.47–5.24 (m, 16H, olefinic Hs), 5.07–4.91 (m, 2H, H-2), 4.13–3.92 (m, 4H, H-1 or H-3),3.80– 3.65(m, 4H, H-1 or H3), 2.90–2.69 (m, 12H, H-7', H-10' and H-13'), 2.33 (t, *J* = 7.5 Hz, 4H, H-2'), 2.14–1.98 (m, 8H, H-4' and H-16'), 1.67 (p, *J* = 7.0 Hz, 4H, H-3'), 1.39–1.22 (m, 12H, H-17', H-18' and H-19'), 0.87 (t, *J* = 6.9 Hz, 6H, H-20').

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ 173.3 (C-1'), 130.6 (olefinic C), 129.1 (olefinic C), 129.0 (olefinic C), 128.8 (olefinic C), 128.5 (olefinic C), 128.2 (olefinic C), 128.0 (olefinic C), 127.7 (olefinic C), 72.5, 63.1, 59.8, 33.8, 31.7, 29.9, 29.5, 27.4, 26.9, 26.7, 25.8, 24.9, 22.7 (C-19'), 14.2 (C-20').

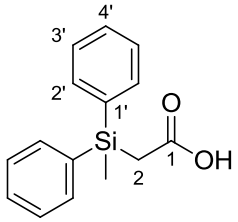
HR-MS: $[M+Na]^+$ calc. 863.4815, found 863.479

$[\alpha]_D^{20}$ = -3.7°(c = 1.15, $CHCl_3$)

6.2.3 Synthesis of 2-(methyldiphenylsilyl)ethanol (5)

5 was synthesized in two different ways. The experimental procedure for both ways is elucidated on the next pages.

6.2.3.1 2-(Methyldiphenylsilyl)acetic acid (3)[45a]



A 250 mL round-bottomed flask equipped with a magnetic stirring bar, Schlenk adapter and a stopper, was flame dried under vacuum, flushed with argon and charged with 40 mL anhydrous THF and 3.40 mL (24.2 mmol, 1.14 eq) anhydrous diisopropylamine. The resulting solution was cooled to -78°C in a dry ice/acetone cooling bath before 11.20 mL (24.1 mmol, 1.15 eq) *n*-butyllithium (2.15 M in hexanes) were added dropwise via a syringe and septum under argon counter flow over a period of 15 min under vigorous stirring. The resulting solution is allowed to warm up to RT, stirred for 5 min and again cooled to -78°C. At this temperature 3.20 mL (21.3 mmol, 1.00 eq) trimethylsilyl acetate were added dropwise via syringe and septum under argon counter flow over a period of 8 min. The reaction mixture was stirred for 2 h at – 78°C before 5.00 mL (23.8 mmol, 1.13 eq) chloro(methyl)diphenylsilane in dry THF (5 mL) was added dropwise via syringe and septum under argon counter flow over a period of 10 min. After additional 3 h, the cooling bath was removed and the reaction mixture was allowed to warm up to RT overnight under further stirring. After 16 h (overnight) reaction time at RT, the reaction mixture turned yellow and brine (30 mL) was added. Due to the addition, a suspension with a white solid and a colourless liquid was formed. This suspension was transferred to a separatory funnel and carefully acidified by addition of 1 M HCl aqueous solution to a pH value of 1 whereupon the suspension turned into a solution. This solution was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with H_2O (2 x 20 mL), dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The resulting yellow oil was treated with cyclohexane (50 mL) whereupon a white solid began to precipitate. This precipitate was filtrated and washed with cyclohexane. The product was dried under oil pump vacuum.

$C_{15}H_{16}O_2Si$ [256.37]

yield: 2.79 g (10.9 mmol, 52%), white crystals

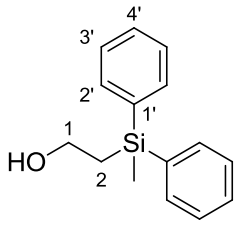
R_f = 0.62 (cyclohexane/ethyl acetate = 1:1 (v/v), 254 nm, CAM, $KMnO_4$)

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ 7.54 (d, *J* = 7.5 Hz, 4H, H-2'), 7.44–7.31 (m, 6H, H-3' and H-4'), 2.43 (s, 2H, H-2), 0.71 (s, 3H, H-MeSi).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ 179.1 (C_q, C-1), 134.8 (C_q, C-1'), 134.6 (CH), 130.0 (CH), 128.2 (CH), 25.3 (CH_2, C-2), -3.9 (CH_3, C-MeSi).

GC-MS (Method-A): t_R = 6.24 min; *m/z* = 213 (6%), 199 (100%, BP), 137 (9%).

6.2.3.2 2-(Methyldiphenylsilyl)ethanol (5) procedure A[45b]



A 25 mL round-bottomed flask equipped with a magnetic stirring bar, Schlenk adapter and a stopper, was flame dried under vacuum, flushed with argon and charged with 209 mg (5.50 mmol, 1.01 eq) LAH and 1 mL anhydrous THF. The resulting grey suspension was cooled to 0°C and 1.40 g (5.46 mmol, 1.00 eq) **3** in dry THF (6 mL) were added dropwise via a syringe and septum under argon counter flow over a period of 10 min. After complete addition, the cooling bath was removed and the reaction was stirred at RT. The reaction progress was monitored by GC-MS analysis (micro workup B, Method-A). After 2 h the GC-MS analysis showed full conversion and the reaction was quenched at 0°C by successive addition of 250 µL H_2O, 250 µL 10% NaOH aqueous solution and 750 µL H_2O. Due to the addition a fine grey foam formed. THF (10 mL) was added to ensure good mixing and the formed grey suspension was stirred for 30 min at RT. The suspension turned white, was filtered through a pad of Celite® and the residue was washed with diethyl ether (3 x 15 mL). The filtrate was successively washed with sat. $NaHCO_3$ (20 mL) aqueous solution, sat. NH_4Cl (20 mL) aqueous solution and brine (20 mL). The organic layer was dried over Na_2SO_4, filtrated, the solvent was removed under reduced pressure and the crude product was dried under oil pump vacuum. The product was further purified by Kugelrohr distillation (130°C, 0.17 mbar)

$C_{15}H_{18}OSi$ [242.39]

yield: 684 mg (3.00 mmol, 56%), colourless viscous liquid

R_f = 0.37 (cyclohexane/ethyl acetate = 5:2 (v/v), 254 nm, CAM, $KMnO_4$)

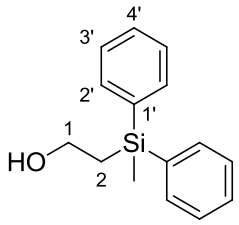
bp: 130°C, 0.17 mbar)

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ 7.56–7.49 (m, 4H, H-2‘), 7.41–7.32 (m, 6H, H-3‘ and H-4‘), 3.80 (t, *J* = 8.2 Hz, 2H, H-1), 1.53 (t, *J* = 8.2 Hz, 2H, H-2), 0.59 (s, 3H, H-MeSi).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ 136.7 (C_q, C-1’), 134.6 (CH), 129.4 (CH), 128.0 (CH), 60.10 (CH_2, C-1), 19.9 (CH_2, C-2), -3.95 (CH_3, C-MeSi).

GC-MS (Method-A): t_R = 6.95 min; *m/z* = 199 (100%, BP), 197 (41%), 181 (7%), 137 (23%), 105 (10%), 77 (8%).

6.2.3.3 2-(Methyldiphenylsilyl)ethanol (5) procedure B[45c]



A 250 mL round-bottomed flask equipped with a magnetic stirring bar, Schlenk adapter and a stopper was flame dried under vacuum, flushed with nitrogen and charged with anhydrous THF (30 mL) and 21.0 mL (150 mmol, 1.00 eq) anhydrous diisopropylamine. The resulting solution is cooled to -78°C in a dry ice/acetone cooling bath and 71.1 mL (150 mmol, 1.00 eq) *n*-butyllithium (2.11 M in hexanes) was added dropwise via a syringe and septum under nitrogen counter flow over a period of 10 min. The resulting solution was allowed to warm up to RT and turned yellow. After 15 min at RT, the solution was cooled again to -78°C in a cooling bath and 20.2 mL (150 mmol, 1.00 eq) *t*-butyl acetate in THF (30 mL) was added dropwise via a syringe and septum under nitrogen counter flow over a period of 30 min. The reaction mixture was stirred for 2 h at -78°C and a solution of 32.5 mL (150 mmol, 1.00 eq) chloromethyldiphenylsilane in THF (30 mL) was added via a syringe and septum under nitrogen counter flow over a period of 30 min. The reaction mixture was allowed to warm up to RT overnight. The reaction progress was monitored by GC-MS analysis (micro workup B, Method-A). After 19 h the formed suspension of a yellow precipitate and an orange solution was transferred to a separatory funnel and carefully quenched with saturated NH_4Cl aqueous solution (100 mL). After phase separation, the aqueous phase was extracted with diethyl ether (2 x 100 mL), the combined organic layers were washed with saturated $NaHCO_3$ aqueous solution (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4, filtrated and the solvent was removed under reduced pressure to obtain the intermediate **4** as an orange oil (44.66 g, 143 mmol). A 500 mL round-bottomed flask equipped with a magnetic stirring bar, Schlenk adapter and a stopper was flame dried under vacuum, flushed with argon and charged with intermediate **4** (44.66 g, 143 mmol) and anhydrous diethyl ether (200 mL). The resulting solution was cooled down to -10°C with a cooling bath and 5.69 g (150 mmol, 1.00 eq) LAH was carefully added in small portions under nitrogen counter flow over a period of 15 min. The reaction progress was monitored by GC-MS analysis (micro workup B, Method-A) and it was allowed to warm up to RT. After complete conversion the reaction mixture was cooled to -5°C and EtOAc (20 mL), H_2O (6 mL), 10% NaOH aqueous solution (6 mL) and $MgSO_4$ (100 mg) were added successively. The newly formed white suspension was filtered and washed with

diethyl ether (2 x 100 mL). The solvent was removed under reduced pressure and the product was purified by distillation (140°C, 0.13 mbar).

$C_{15}H_{18}OSi$ [242.39]

yield: 16.69 g (68.9 mmol, 46%), yellowish viscous liquid

R_f = 0.37 (cyclohexane/ethyl acetate = 5:2 (v/v), 254 nm, CAM, $KMnO_4$)

bp: 140°C, 0.13 mbar)

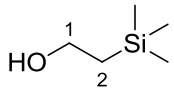
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ 7.56–7.49 (m, 4H, H-2‘), 7.41–7.32 (m, 6H, H-3‘ and H-4‘), 3.80 (t, *J* = 8.2 Hz, 2H, H-1), 1.53 (t, *J* = 8.2 Hz, 2H, H-2), 0.59 (s, 3H, H-MeSi).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ 136.7 (C_q, C-1’), 134.6 (CH), 129.4 (CH), 128.0 (CH), 60.10 (CH_2, C-1), 19.9 (CH_2, C-2), -3.95 (CH_3, C-MeSi).

GC-MS (Method-A): t_R = 6.95 min; *m/z* = 199 (100%, BP), 197 (41%), 181 (7%), 137 (23%), 105 (10%), 77 (8%).

6.2.4 Synthesis of 2-(trimethylsilyl)ethanol protected phosphate (20)

6.2.4.1 2-(Trimethylsilyl)ethanol (19)[57]



A 100 mL two necked round-bottomed flask equipped with a magnetic stirring bar, Schlenk adapter, water cooler and a stopper, was flame dried under vacuum, flushed with argon and charged with 40 mL anhydrous diethyl ether and 1.83 mL (10.0 mmol, 1.00 eq) 2-(trimethylsilyl)acetic acid. The resulting solution was cooled to 0°C and 910 mg (24.0 mmol, 2.40 eq) LAH were added in small portions in argon counterflow over a period of 20 min. The reaction mixture turned into a grey suspension, was heated under reflux (35°C). After 5 h, the reaction showed full conversion and was cooled again to 0°C before 1 mL H_2O, 1 mL 10% NaOH aqueous solution and 3 mL H_2O were added successively. Diethyl ether (20 mL) were added and the resulting white suspension was filtered through a pad of Celite®. The pad was washed with THF (3 x 15 mL) and the combined organic layers were concentrated under reduced pressure. The product was dried under oil pump vacuum.

$C_5H_{14}OSi$ [118.25]

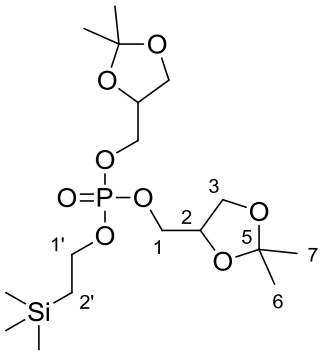
yield: 927 mg(7.84 mmol, 78%), colourless liquid

R_f = 0.62 (cyclohexane/ethyl acetate = 1:1 (v/v), CAM, $KMnO_4$)

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ 3.74 (t, *J* = 8.3 Hz, 2H, H-1), 0.96 (t, *J* = 8.3 Hz, 2H, H-2), 0.02 (s, 3H, H-MeSi).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ 60.4 (CH_2, C-1), 22.4 (CH_2, C-2), -1.2 (CH_3, C-MeSi).

6.2.4.2 Bis((2,2-dimethyl-1,3-dioxolan-4-yl)methyl) (2-(trimethylsilyl)ethyl) phosphate (20)[6]



A 100 mL round-bottomed flask equipped with a magnetic stirring bar, Schlenk adapter and a stopper was flame dried under vacuum, flushed with nitrogen and charged with 10 mL anhydrous dichlromethane and 443 µL (5.07 mmol, 1.00 eq) phosphorus trichloride. The resulting colourless solution was cooled to 0°C whereupon a solution of 727 µL (5.07 mmol, 1.00 eq) (trimethylsilyl)ethanol (**19**) in 10 mL anhydrous dichloromethane was added dropwise over a period of 20 min via a syringe and a septum in nitrogen counterflow. The colourless reaction mixture was warmed up to RT and stirred for 2 h. Then it was cooled again to 0°C whereupon 1.50 mL (17.00 mmol, 3.35 eq) anhydrous pyridine was added dropwise via a syringe and a septum in a nitrogen counterflow over a period of 10 min. The colour of the reaction turned intense pink during addition and slightly pink after the addition. The reaction was allowed to warm up to RT and stirred again for 5 min. After cooling down to 0°C again, a solution of 1.39 mL (11.15 mmol, 2.20 eq) (2,2-dimethyl-1,3 dioxolan-4-yl)methanol in dichloromethane (10 mL) was added dropwise over a period of 15 min via a syringe and a septum in nitrogen counterflow. The reaction mixture turned into a suspension with a white solid in an orange solution. After warming up to RT, 1.26 g (5.10 mmol, 1.01 eq) 3-chloroperbenzoic acid (70% with H_2O) dissolved in 10 mL dichloromethane were added dropwise. The reaction mixture turned into a bright yellow solution. The oxidation progress was monitored via TLC analysis and after 1 h, ethyl acetate (100 mL) was added and the resulting solution was washed successively with 1 M HCl aqueous solution (50 mL), brine (50 mL) and saturated $NaHCO_3$ aqueous solution (50 mL). The organic layer was dried with Na_2SO_4, filtrated and the solvent was removed by rotary evaporation. The product was purified via flash-chromatography (170 g silica gel, cyclohexane/ethyl acetate = 1:1, fraction size: 60 mL, fractions 17 to 43 pooled) and dried under oil pump vacuum.

$C_{17}H_{35}O_8PSi$ [426.51]

yield: 947 mg (2.22 mmol, 43%), yellowish viscous liquid

R_f = 0.34 (cyclohexane/ ethyl acetate = 1:1 (v/v), CAM, $KMnO_4$)

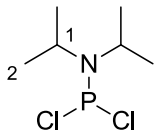
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ 4.30 (p, *J* = 7.8 Hz, 2H, H-2), 4.17 (dd, *J* = 17.1, 7.6 Hz, 2H, H-3a), 4.10–3.93 (m, 6H, H-1 and H-1'), 3.81 (dd, *J* = 8.5, 6.5 Hz, 2H, H-3b), 1.40 (s, 6H, H-6 or H-7), 1.34 (s, 6H, H-6 or H-7), 1.10 (t, *J* = 8.6 Hz, 2H H-2'), 0.02 (s, 9H, H-MeSi).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ 110.1 (C_q, C-5), 74.3 (CH, d, J_{PC} = 8.3 Hz, C-2), 67.7 (CH_2, t, J_{PC} = 5.2 Hz), 67.2 (CH_2, d, J_{PC} = 6.3 Hz), 66.4 (CH_2, C-3), 27.0 (CH_3, C-6 or C-7), 25.5 (CH_3, C-6 or C-7), 19.8 (CH_2, C-2'), -1.30 (CH_3, C-MeSi).

HPLC-MS (Method-A): t_R = 4.12 min

6.2.5 Synthesis of (dioxolanylmethyl) phosphoramidite (23)

6.2.5.1 1,1-Dichloro-*N*,*N*-diisopropylphosphinamine (22)[58]



A 500 mL round-bottomed two necked flask equipped with a magnetic stirring bar, Schlenk adapter and a dropping funnel was flame dried under vacuum, flushed with nitrogen and charged with anhydrous *n*-hexane (350 mL) and 13.1 mL (150 mmol, 1.00 eq) freshly distilled phosphorous trichloride. The resulting solution was cooled to 0°C, whereupon 39.3 mL (280 mmol, 1.87 eq) anhydrous diisopropylamine were added dropwise over the droping funnel over a period of 15 min. The droping funnel was not tight and some base was lost. The resulting white suspension was stirred at RT for 2 h before the solid was filtered off with an inert frit and washed with cyclohexane (3 x 40 mL). The filtrate was collected and the solvent was removed in vacuum and the product was purified by distillation (0.14 mbar, 42°C).

$C_6H_{14}Cl_2NP$ [202.06]

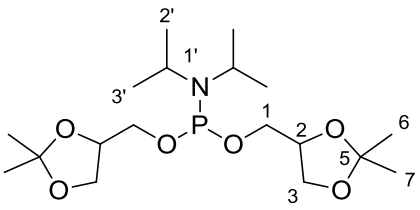
yield: 5.17 g (25.6 mmol, 18%), colourless liquid

bp: 42°C, 0.14 mbar

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ 3.91 (h, *J* = 6.8 Hz, 2H, H-1), 1.26 (d, *J* = 6.8 Hz, 12H, H-2).

^{13}C-NMR (75.53 MHz, CDCl3): δ 48.4 (d, J_{PC} = 14.0 Hz, C-1), 23.7 (d, J_{PC} = 8.4 Hz, C-2).

6.2.5.2 Bis((2,2-dimethyl-1,3-dioxolan-4-yl)methyl) diisopropylphosphoramidite(23)[58]



A 50 mL round-bottomed flask equipped with a magnetic stirring bar and Schlenk adapter was flame dried under vacuum, flushed with nitrogen and charged with cyclohexane (10 mL) and

1.00 mL (5.48 mmol, 1.00 eq) **22**. The resulting solution was cooled to 0°C, whereupon a solution of 1.52 mL (10.96 mmol, 2.00 eq) Et_3N and 1.36 mL (10.96 mmol, 1.00 eq) (2,2-dimethyl-1,3 dioxolan-4-yl)methanol in cyclohexane (5 mL) was added dropwise over a period of 10 min via a syringe and a septum in nitrogen counter flow. The reaction mixture turned into a white suspension. After stirring the reaction for 1 h, it was warmed up to RT. The reaction progress was monitored via TLC and GC-MS analysis (micro workup D, Method-A). After further 6 h, the white precipitate was filtered off and washed with ethyl acetate (2 x 5 mL). The filtrate was washed successively with sat. $NaHCO_3$ aqueous solution (10 mL), brine (10 mL), sat. NH_4Cl aqueous solution (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation. The product was dried under oil pump vacuum.

$C_{18}H_{36}NO_6P$ [393.46]

yield: 2.00 g (5.08 mmol, 93%), colourless liquid

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ 4.29–4.18 (m, 2H), 4.10–3.99 (m, 2H), 3.89–3.80 (m, 2H), 3.77–3.47 (m, 6H), 1.41 (s, 6H, H-6 or H-7), 1.35 (s, 6H, H-6 or H-7), 1.17 (d, *J* = 6.8 Hz, 12H, H-2’ and H-3’).

GC-MS (Method-A): t_R = 7.26 min; *m/z* = 280 (33%), 262 (16%), 222 (100%, BP), 179 (36%), 115 (41%), 86 (57%), 57 (42%).

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8 Abbreviations

2-AG	2-arachidonoylglycerol
4-DMAP	4-dimethylaminopyridine
6C4	BMP specific antibody
AA	arachidonic acid
ABHD6	alpha beta domain containing protein 6
AcOH	acetic acid
ACN	acetonitrile
APA	synonym for anti-phospholipid syndrome
APS	anti-phospholipid syndrome
ATP	adenosine triphosphate
BHK	baby hamster kidney
BMP	Bis(monoacylglycero) phosphate
CAD	cationic amphiphilic drug
CAM	cerium-ammonium-molybdate
CDP	cytidin diphosphate
CL	cardiolipin
CMP	cytosine monophosphate
CDP	cytosine diphosphate
CTP	cytosine triphosphate
d	doublet
DAG	diacylglycerol
DCM	dichloromethane
dd	doublet of doublets
DEAE	diethylaminoethyl
DHA	docosahexanoic acid

DHAP	dihydroxyacetone phosphate
DME	dimethoxyethane
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DPMSE	2-(methyldiphenylsilyl)ethyl
ECV	endosomal carrier vesicle
ESCRT	endosomal sorting complex required for transport
ESI	electron spray ionization
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
FA	fatty acid
G3P	glyceraldehyde 3-phosphate
GC	gas chromatography
GP	glycerophospholipid
HH-COSY	proton-proton correlation spectroscopy
HMPA	hexamethylphosphoramide
HOMO	highest occupied molecular orbital
HSQC	heteronuclar single quantum coherence
HPLC	high performance liquid chromatography
IUPAC	international union of pure and applied chemistry
LAH	lithium aluminum hydride
LAL	lysosomal acid lipase
LBPA	lysobisphosphatidic acid
LDA	lithium diisopropylamide
LMSD	LIPID MAPS structure database

LPA	lysophosphatidic acid
LPL	lysosomal phospholipase
LSD	lysosomal disorder
LUMO	lowest unoccupied molecular orbital
m	multiplet
MCB	membraneous cytoplasmic bodies
mCPBA	3-chloroperoxybenzoic acid
MeOH	methanol
MS	mass spectrometry
MVB	multivesicular bodies
NAD	nicotinamide adenine dinucleotide
NCL	neuronal ceroid liposuscinoses
NMR	nuclear magnetic resonance
NP	Niemann-Pick disease
NPA	Niemann-Pick disease A
NPB	Niemann-Pick disease B
NPC	Niemann-Pick disease C
OA	oleic acid
p	pentet
p_i	phosphate
PA	phosphatidic acid
PAPS	primary anti-phospholipid syndrome
PE	phosphatidylethanolamine
PG	phosphatidyl glycerol
PGP	phosphatdiyl glycerophosphate
PL	phospholipid

pp_i	pyrophosphate
ppTs	pyridinium *p*-toluenesolfonate
PS	phosphatidylserine
R_f	retardation factor
s	singlet
SAPS	secondary anti-phospholipid syndrome
sn	stereospecific numbering
t	triplet
TAG	triacylglycerol
TBAF	*tert*-butylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
THF	tetrahydrofurane
TLC	thin layer chromatography
TMSE	trimethylsilylethyl
q	quartet

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Beschluss der Curricula-Kommission für Bachelor-, Master- und Diplomstudien vom 10.11.2008 Genehmigung des Senates am 1.12.2008

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