Organic synthesis - applications in enzymatic studies, catalysis and surface modification

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Preface

This thesis presents the work carried out during my three years as a PhD student at the Technical University of Denmark under the supervision of Associate Professor Mads H. Clausen. The first chapter describes a short structure-activity study of the $sPLA_2$ enzyme on liposomes, the second chapter focuses on the synthesis and reactivity of functionalized liposomes, in the third chapter the synthesis of 2-mono(10,16-dihydroxyhexadecanoyl)glycerol for investigation of cutin biosynthesis in plants is described and the last chapter covers the synthesis and preliminary structural analysis of a new C_3-symmetrical phosphine oxide as potential ligand.

I would like to thank my supervisor, Mads H. Clausen for giving me the opportunity to work in his group, but also for always taking the time for discussions and for his support over the three years.

As any scientific project, this work could not have been so far without input from other people. Professor Thomas L. Andresen is thanked for giving me access to his MALDI-TOF, spectrofluorimeter, DLS and DSC equipment, as well as for his inputs in the lipid related projects. Associate Professor Günther H. Peters is thanked for his contribution with the MD simulations in the $sPLA_2$ project. Dr. Thomas Ruhland is thanked for his investment in the phosphine oxide project and Dr. Henrik Pedersen is acknowledged for the temperature NMR experiments.

Help from other members of DTU Chemistry have been crucial along my PhD, and I would especially like to thank Associate Professor Charlotte H. Gotfredsen both for her practical help with NMR experiments and structure elucidation, but also and more importantly for her personal support and for her immense kindness during the difficult times. Thank you for having been there for me. I would also like to thank Professor David Tanner for his good chemistry advice in desperate times.

I would like to thank the technicians, Anne, Janne, Tina and Brian for their practical help with NMR, HPLC and chemicals.

Former and current members of the Clausen group are also thanked for creating a good working atmosphere, and especially those whose friendship, love and support have been crucial for me, you have shown interest in my projects and participated in the advancements one way or the other: Dr. Kasper Worm-Leonhard, Dr. Palle Pedersen, Dr. Peter Hammershøj, Faranak Nami and Christine Kinnaert. I am looking forward to seeing you again very soon.

I would also like to thank Professor Richmond Sarpong from the University of California Berkeley for welcoming me in his group as a visiting graduate student during the last three months of my PhD, and for his understanding during my writing process. The work performed in his lab is still at an initial stage and will not be presented in this thesis. However, I am grateful for being able to join his awesome group, and would also like to thank all the members, especially my labmates Gary, Jess, Devon and Jack for the good times in the past months and for the many more to come. In relation to my external stay, I would like to thank the Lundbeck Foundation, Oticon Fonden, Otto Mønsteds Fond, Augustinus Fonden, Reinholdt W. Jorck og Hustrus Fond and Henry og Mary Skovs Fond for their generous and appreciated financial support.

Finally, I would like to thank my parents, Elodie and Alban, Matthieu, Marianne and Thierry for their love and support despite the distance, as well as my friends Pierre, Aurelo and Gen, Belou and Læti, Clem and Mag, Thibaut and in Copenhagen JC, Sidna, Pat, Marek, Marco and Juan for always having been there with an open ear and a party mood.

Hélène Viart

Berkeley, California, April 2013

Abstract

In a desire to explore various areas of synthetic organic chemistry, different projects have been carried out, and each of the four following chapters will describe the work carried out on each of them. The first three chapters are related in some extent and treat the synthesis and biochemical applications of (phospho)lipids, while the last chapter differs and deals with the synthesis and initial structural studies of a C_3 symmetric phosphine oxide.

In the first chapter, a series of phospholipids have been synthesised in order to perform a short structure-activity relationship study of an enzyme, secretory phospholipase A_2 ($sPLA_2$) capable of hydrolysing phospholipids in the *sn*-2 position specifically. This enzyme is over-expressed in several types of cancer and is under evaluation as a potential trigger for drug release from a new generation of liposomal drug delivery systems. However, little is known about the steric and electronic requirements in the vicinity of the *sn*-2 position for an effective hydrolysis catalysed by the enzyme. Based on previous observations and on MD experiments, we developed a theory to predict and/or explain the activity of the enzyme on engineered phospholipids. According to our theory, two aspects of the enzyme-substrate interactions are primordial for an effective hydrolysis to occur: the formation of a constructive Michaelis-Menten complex, and access of water to the hydrolysis site. In order to verify this theory, the synthesised phospholipids were formulated as liposomes and the enzymatic activity was studied. Hydrolysis (or absence of hydrolysis) was monitored by MALDI-TOF-MS. The results observed in these experiments are compared to MD predictions and confirm them.

The second chapter deals with surface functionalization of liposomes. The copper mediated [3+2] azide-alkyne cycloaddition has been successfully applied for this purpose by different groups, but no general optimization has been developed for the reaction on functionalised liposomes. Since the reaction generally takes place between one functionality on the surface of the liposomes membrane and a functionality covalently linked to a coupling partner (such as small molecule, peptide, etc.), we investigated the efficiency of the reaction depending on the position of the functional groups (whether on the liposome or on the coupling partner). Our results indicate that the reaction is most efficient when the liposome carries the alkyne functionality rather than the azide. We also investigated and developed a novel selective method for functionalizing liposomes, which has not yet been reported in the literature, based on the reaction between propargyl-amine decorated liposomes and isothiocyanate derived coupling partners that results in a coupling *via* formation of an iminothiazolidine.

In the third chapter, the synthesis of *sn*-2 glyceryl 10,16-dihydroxyhexadecanoate is reported, in the context of the identification of the process of formation of the cutin polymer, one of the primary protective components of the epidermis of land plants. The enzyme responsible for the polymerization (CD1), as well as its substrate, has been identified, and the role of the enzyme has been demonstrated by its activity on the synthetic dihydroxyacylglycerol.

Finally, the last chapter differs greatly from the first three by its focus: a C_3 symmetric phosphine oxide has been synthesised, which we intend to test, after reduction to the phosphin, as a ligand in organometallic catalysed reactions. The ultimate goal is to obtain enantioselectivity, introduced by the organization of aryl substituents around phosphorous in our ligand.

Resumé

Ud fra et ønske om at udforske forskellige grene af syntetisk organisk kemi er forskellige projekter blevet udført. Hvert af de følgende kapitler vil beskrive det arbejde, som er blevet udført på disse projekter. De første tre kapitler er til en vis grad relaterede og omhandler syntese og biokemisk anvendelse af (phospho)lipider. Det fjerde kapitel er noget forskelligt, da det beskriver syntese og foreløbige strukturelle studier af et C_3 symmetrisk phosphinoxid.

I det første kapitel beskrives syntesen af en række phospholipider, med det formål at undersøge sammenhængen mellem struktur og aktivitet af substrater for enzymet sekretorisk phospholipase A_2 ($sPLA_2$), som selektivt kan hydrolysere phospholipider i *sn*-2 positionen. Dette enzym er overudtrykt i adskillige cancertyper og bliver undersøgt i forbindelse med udvikling af en ny generation af lægemiddelfremføringssystemer baseret på kontrolleret frigivelse i tumorvæv. Imidlertid er der begrænset viden om de steriske og elektroniske forudsætninger for enzymets hydrolyse i *sn*-2 positionen, hvorfor vi, baseret på tidligere observationer og MD simuleringer, har udviklet en teori til at forklare og forudsige enzymaktiviteten overfor nye typer phospholipider. Ifølge denne teori er der to aspekter af enzym-substrat interaktionen som influerer effektiv hydrolyse: Dannelse af et produktivt Michaelis-Menten kompleks og tilgang af vandmolekyler til det aktive site. For at verificere denne teori blev de syntetiserede phospholipider formuleret som liposomer og enzymaktiviteten på disse substrater blev undersøgt. Hydrolyse, eller mangel på samme, blev fulgt med MALDI-TOF-MS og resultaterne blev sammenlignet med MD simuleringer. Resultaterne viste god overensstemmelse mellem eksperimenter MD simuleringer.

Det andet kapitel handler om overfladefunktionalisering af liposomer. Den kobberkatalyserede [3+2] azid-alkyn cykloaddition har været anvendt med succes til dette formål af andre grupper, men en generel optimering af reaktionen på funktionaliserede liposomer har ikke været foretaget. Reaktionen finder generelt sted mellem en funktionalitet på overfladen af liposommembranen og en anden kovalent bundet til en koblingspartner, såsom et lille molekyle eller et peptid. Vi har undersøgt betydningen af placeringen af de to funktionelle grupper, dvs. hvorvidt de er placeret på overfladen eller i opløsning. Resultaterne indikerer at reaktionen er mest effektiv, når liposomet bærer alkynfunktionaliteten fremfor azidet. Vi har også undersøgt og udviklet en ny selektiv metode til at funktionalisere liposomer. Reaktionen har ikke tidligere været beskrevet i litteraturen til dette formål og er baseret på reaktion mellem en propargylamin på liposomoverfladen med et isothiocyanat i opløsning og fører til dannelse af en iminothiazolidin

I det tredje kapitel rapporterer vi syntesen af *sn*-2 glyceryl 10,16-dihydroxyhexadecanoat med det formål at identificere en proces involveret i dannelse af cutin, en polymer som udgør en af de primære beskyttende komponenter i planters epidermis. Enzymet, som er ansvarlig for polymerisering (CD1) samt substratet blev identificeret og enzymets funktion og rolle i cutin syntese blev verificeret ud fra dets aktivitet overfor det syntetiske dihydroxyacylglycerolmolekyle.

Det fjerde og sidste kapitel adskiller sig væsentligt fra de tre foregående ved at fokusere på fremstilling af et nyt C_3 symmetrisk phosphinoxid. Vi ønsker at teste stoffet, efter reduktion til den tilsvarende phosphin, som ligand i metalkatalyserede reaktioner, med det det endelige mål at opnå enantioselektivitet, induceret af organisationen af tre aryl substituenter bundet til phosphor.

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1. Structure-activity relationship study of potential substrates for $sPLA_2$

1.1. Introduction

1.1.1. Liposomes are valuable drug delivery systems

Despite advances in the research for cancer treatment, the main issues inherent to anti-cancer drugs remain their high toxicity towards healthy cells resulting in severe side effects. The potential of liposomes as drug carriers was first introduced in 1974,[1] about ten years after the discovery of those structures by Alec Bangham,[2,3] and intensive research lead to the effective use of liposomes as drug delivery systems (DDS) for antitumour drugs. Liposomes can be designed for specific purposes, by altering their biophysical properties through the use of diverse formulations. By doing so, pharmacological and therapeutic properties of parenterally administered drugs have been improved. Indeed, the use of liposomes as DDS resulted in enhanced activity of the encapsulated drug and reduced toxicity towards healthy tissues.[4]

Among the drug properties that have been improved and/or issues that have been addressed through encapsulation of free drugs in liposomal DDS, we can mention the poor solubility of hydrophobic drugs in aqueous media. Free drug solubility is enhanced by the use of liposomes, which provide both hydrophilic and hydrophobic environments (this property of liposomes is explained in more details in section 1.1.2.). Another example is the protection provided by encapsulation of the drug into the liposomes, which prevents premature degradation of the drug in vivo when the drug is unstable at physiological pH for instance. This protection also alters the drug pharmacokinetics, by preventing degradation of the drug via metabolic pathways in the blood stream and by reducing renal clearance of small molecules. Finally, the use of liposomes as DDS reduces toxicity toward healthy tissues by improving selectivity toward target tissues. This can be achieved by taking advantage of the enhanced permeability and retention effect and/or via ligand-targeting (passive and active targeting respectively), these last notions will be described in detail in section 2.1.

Liposomes are especially suited for carrying small molecules, of which they can encapsulate up to tens of thousands of drug molecules,[5] and liposomal formulation of anticancer small molecules have reached the market. As an example, the pegylated liposomal doxorubicin marketed as Doxil/Caelyx,[6] is reported to encapsulate 15,000 molecules of doxorubicin per liposome, the latter displaying an average diameter of 80-90 nm.[7]

1.1.2. Liposomes structures and nomenclature

Liposomes are spherical structures, formed by aggregation of amphiphilic molecules such as phospholipids in aqueous media. They can consist of one or several concentric spheres of lipid bilayer(s) (unilamellar vesicles or multilamellar vesicles, respectively), in the case of multilamellar vesicles, lipid bilayers are separated by aqueous compartments.[11] When dispersed in a polar

aqueous medium, the polar head-groups of the amphiphilic molecules interact through hydrogen bonding and solvation, and the hydrophobic tails aggregate by virtue of the hydrophobic effect, forming bilayer structures that "protect" the hydrophobic tails from water. Therefore, liposomes consist of one or a series of closed bilayer(s), the space inside the bilayers being hydrophobic, while the compartments between the bilayers and core of the sphere are polar and filled with the buffered acqueous medium. Spontaneously formed liposomes are multilamellar vesicles as depicted in Figure 1, having an average diameter above 500 nm and these were the structures originally observed by Bangham.[2,3] However, multilamellar vesicles can be forced into unilamellar vesicles by sonication[8,11] or extrusion.[9,11] Liposomes used as DDS are the large unilamellar vesicles (typically around 100 nm in diameter). As a consequence of the structural properties of liposomes (as unilamellar vesicles) hydrophilic drugs can readily be entrapped in the aqueous core of the liposomes, but neutral drugs, or drugs with a poor to intermediate water solubility tend to be released rapidly, especially in the presence of other membranes or proteins.[10] Alternatively, apolar drugs can be contained within the lipid bilayer.[11] Phospholipids with a glycerol backbone are the major constituent of biological membranes and are very commonly used for liposomes formulations. A schematic description of the assembly of glycerophospholipids into liposomes as multilamellar vesicles and as unilamellar vesicles is shown in Figure 1. A phosphodiester linked to the third position of the glycerol backbone and to various organic alcohols such as glycerol, choline, ethanolamine, serine and inositol can make up the polar head group. Similarly, a variety of either saturated or unsaturated alkyl chains can be attached to the first two positions of the glycerol backbone via ether or ester functionalities to afford the aliphatic tails.[11]

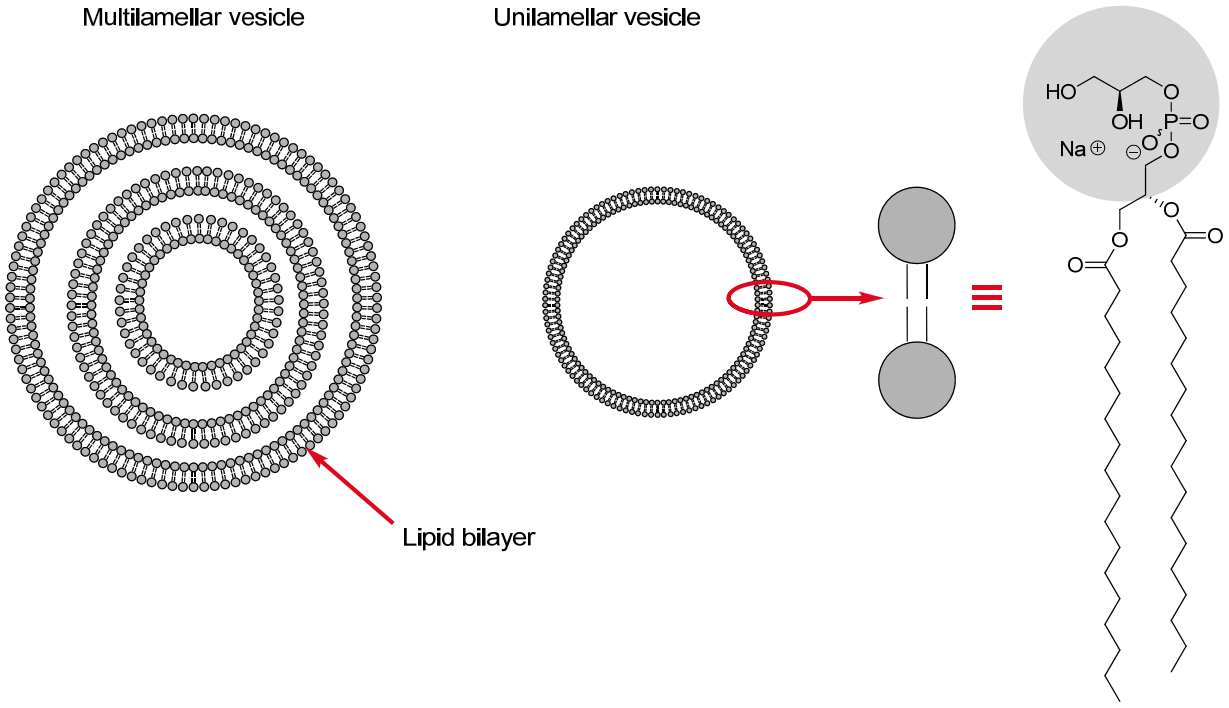


Figure 1: Liposomes are structures made by the spontaneous assembly of amphiphilic compounds such as phospholipids (here DSPG) and can be multilamellar vesicles or unilamellar vesicles. Adapted from ref. 11.

A widely applicable and generally used nomenclature for phospholipids is the *sn*-nomenclature (stereospecific numbering) introduced by Hirshman,[12] which relies on a stereospecific numbering of the carbon atoms of the glycerol backbone, and associates a number (*sn*-1, *sn*-2 and *sn*-3) to the carbons atoms of the backbone independently of the substituents on those atoms. The *sn*-1 position refers to the carbon at the top of a Fischer projection of the glycerol, having the hydroxyl group in the second position (*sn*-2) on the left, and the bottom carbon of the chain in the Fischer projection is the *sn*-3 carbon. An example is given in Figure 2.

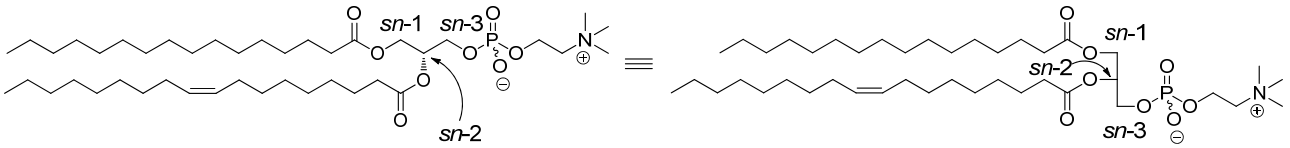


Figure 2: Use of the *sn*-nomenclature with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC).

1.1.3. Triggering drug release

If the shielding offered by a DDS and the effective encapsulation of the drug in liposomes are an advantage during their transit in the blood stream, this characteristic becomes a drawback when the liposomes have reached the targeted tissue, since the drug remains inactive when encapsulated in the liposome. Thus too slow release of the drug after accumulation of the liposomes at the tumour results in poor therapeutic effect.[13] Therefore, release of the drug from the liposome is a prerequisite for the efficacy of the drug. Leakage of the drug from the liposome is generally achieved passively upon destabilization of the liposomes with time. However, intensive research has focused on designing liposomes that can be destabilized under specific conditions and can deliver their content at controllable rates. For that purpose, different potential triggers have been explored, and pH-,[14,15,16,17,18] temperature-[19,20,21] and light-sensitive liposomes, [22,23,24,25] have been engineered, to take advantage of tumour physiological properties and/or external stimuli to trigger the drug release from the liposomes when and where desired. However, despite encouraging results, these strategies have yet to translate into marketed DDSs and the clinic.

1.1.4. $sPLA_2$ triggered drug release

Another potential trigger considered for release of the active compound in tumour tissues was envisioned to be the enzyme secretory phospholipase A_2 ($sPLA_2$). This enzyme is over-expressed in various types of cancers and its serum concentration increases in advanced stages of the disease.[26] The enzyme is secreted by the cells to act extracellularly, thus it is found and operates in the interstitial space.[26] Moreover, $sPLA_2$ specifically hydrolyses the *sn*-2-acyl position of phospholipids,[27] and has shown increased activity toward aggregated lipids substrates,[28] as is the organization found in the bilayers of liposomes. Those characteristics make the enzyme an ideal candidate as trigger for drug release from liposomes in the treatment of cancers.

$sPLA_2$ has attracted great interest, its mode of action has been studied extensively and a mechanism has been proposed for the enzyme-catalysed hydrolysis of phospholipids, which is illustrated in Figure 3. The enzyme is Ca^{2+}-dependant and the important interactions between the enzyme, its substrate and the required Ca^{2+} ion have been determined by high-resolution x-ray crystallography. The x-ray structure illustrates the role of the Ca^{2+} ion both in the binding of the enzyme to the substrate and in the hydrolysis reaction. Indeed, initially, the ion ensures a close interaction between the enzyme and the substrate and helps stabilize the $sPLA_2$-substrate Michaelis-Menten complex by interacting with two residues of the active site of the enzyme (Gly 29 and Asp 48), the phosphate oxygen and the carbonyl oxygen in the *sn*-2 position of the phospholipid (marked as O22 in Figure 3A), this increases the electrophilicity of the latter and facilitates nucleophilic attack by the water molecule. As a result, Ca^{2+} also stabilizes the transition state obtained after nucleophilic attack by water (shown in Figure 3B).[29] The hydrolysis is initiated by deprotonation of an incoming water molecule by the imidazole moiety of His 47 in the active site of the enzyme and subsequent nucleophilic attack on the ester carbonyl in the *sn*-2 position of the substrate, leading to the intermediate shown in Figure 3B. This intermediate collapses to afford the carboxylic acid, and the glycerol's *sn*-2 hydroxide abstracts the proton from the previously protonated His 47 to regenerate the active enzyme. Thus after departure of the carboxylic acid and lysolipid, the enzyme can initiate a new catalytic cycle for substrate hydrolysis.[30,31]

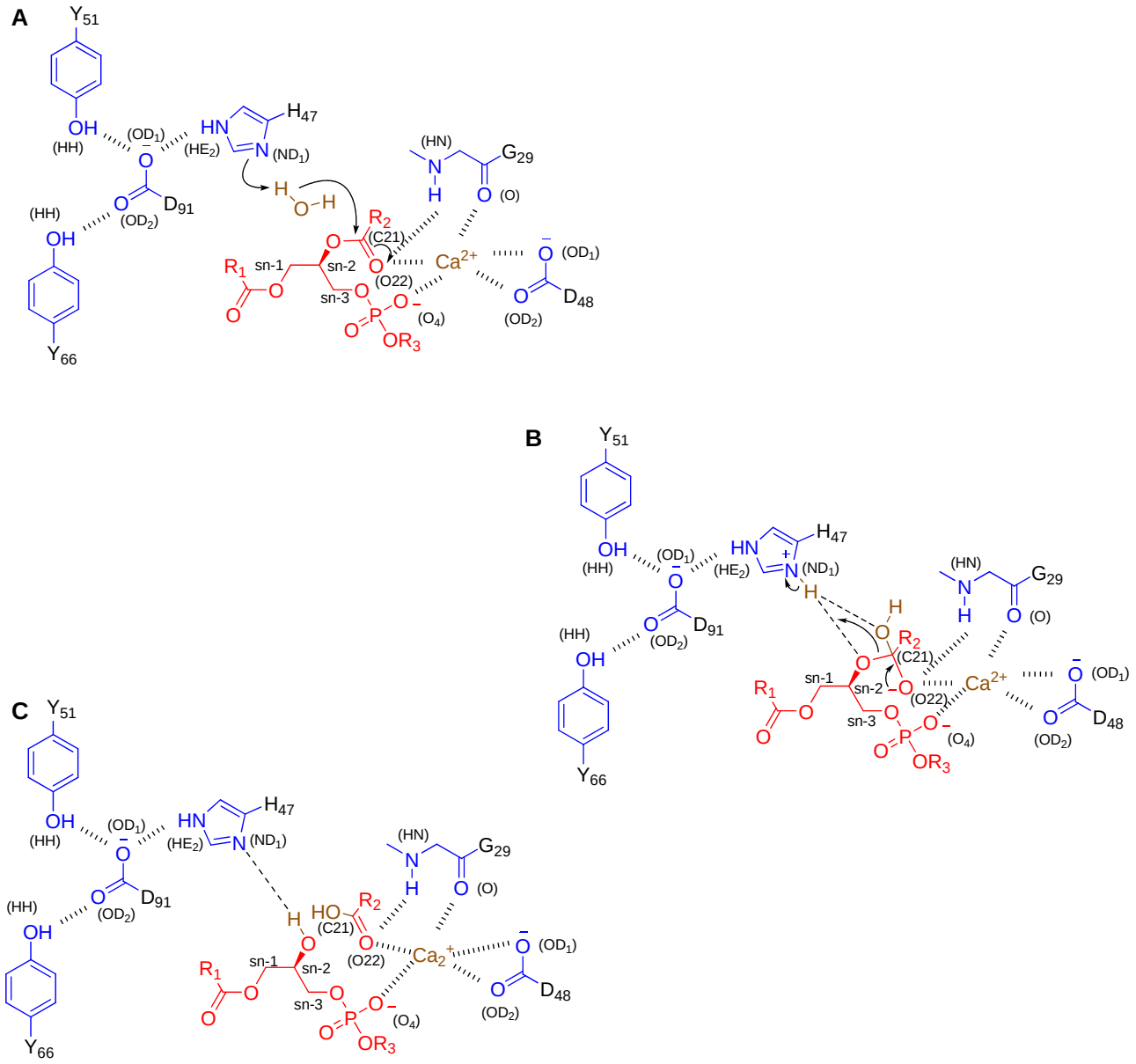


Figure 3: Mechanism of the sPLA$_2$-catalysed hydrolysis of phospholipids. A: Ca^{2+}-mediated formation of the Michaelis-Menten intermediate and initiation of the hydrolysis. B: Collapse of the tetrahedral intermediate, reconstitution of the carbonyl bond and proton exchange between the *sn*-2 oxygen and the protonated His 47 lead to the products (C). The dashed lines highlight the important interactions (hydrogen bonds and ionic interactions) in the reaction. Key protein residues are drawn in blue, the substrate in red, the calcium ion and the water molecule both in brown. Amino acid labels and atom types indicated in parentheses refer to the Protein Data Bank nomenclature. Adapted from refs. 30 and 31.

Investigation of $sPLA_2$ as a drug release trigger initially focused on liposomal formulations of common phospholipids, 1,2-di-hexadecanoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) lipids formulated with 1,2-di-hexadecanoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DPPE-PEG_{2000}). Upon snake venom $sPLA_2$ activity, the phospholipids were hydrolysed in the *sn*-2 position, resulting in lyso-phospholipids and free fatty acids and the liposomes were destabilized, delivering their contents.[32] The study also shows that the products of hydrolysis enhance the permeability of the target cells, thereby constributing to the cytotoxicity of the system.[32]

Later on, studies carried out in our group aimed at engineering new liposomes, which could deliver their active compounds content in cancer tissue upon $sPLA_2$ destabilization. The studies were aiming for an improved DDS, and thus liposomes design had to be compatible with degradation by human $sPLA_2$. It is known that human $sPLA_2$ does not bind (or binds very weakly) to zwitterionic membranes, but has a higher affinity for anionic membranes.[33] Therefore, the lipids were designed with a phosphatidylglycerol (PG) head group. Another interest in the project was to introduce the active compound in the hydrophobic bilayer, via a covalent ester bond at the *sn*-2 position. The liposomal formulation of anticancer drugs obtained with this design has the great advantage over other formulations that release of the cytotoxic compound can only occur upon hydrolysis of the *sn*-2 position of the lipids. This should decrease loss of the contained drug by leakage during the liposomes´ circulation in the blood stream and increase the specificity of the drug release at the tumour by virtue of the elevated activity of $sPLA_2$ in tumour tissues, thus decreasing side effects. Several lipophilic compounds with known anticancer activity have been linked via an ester bond to the *sn*-2 position of phospholipids, formulated as liposomes and the ability of the enzyme to liberate the active compounds was monitored. The study focused on chlorambucil,[42] all-*trans* retinoic acid (ATRA),[63,70] α-tocopheryl succinate, a derivative of vitamin E,[34] and 15-deoxy-$\Delta^{12,14}$-PGJ_2, a prostaglandin derivative .[71] The synthesized prodrug lipids are shown in Figure 4.

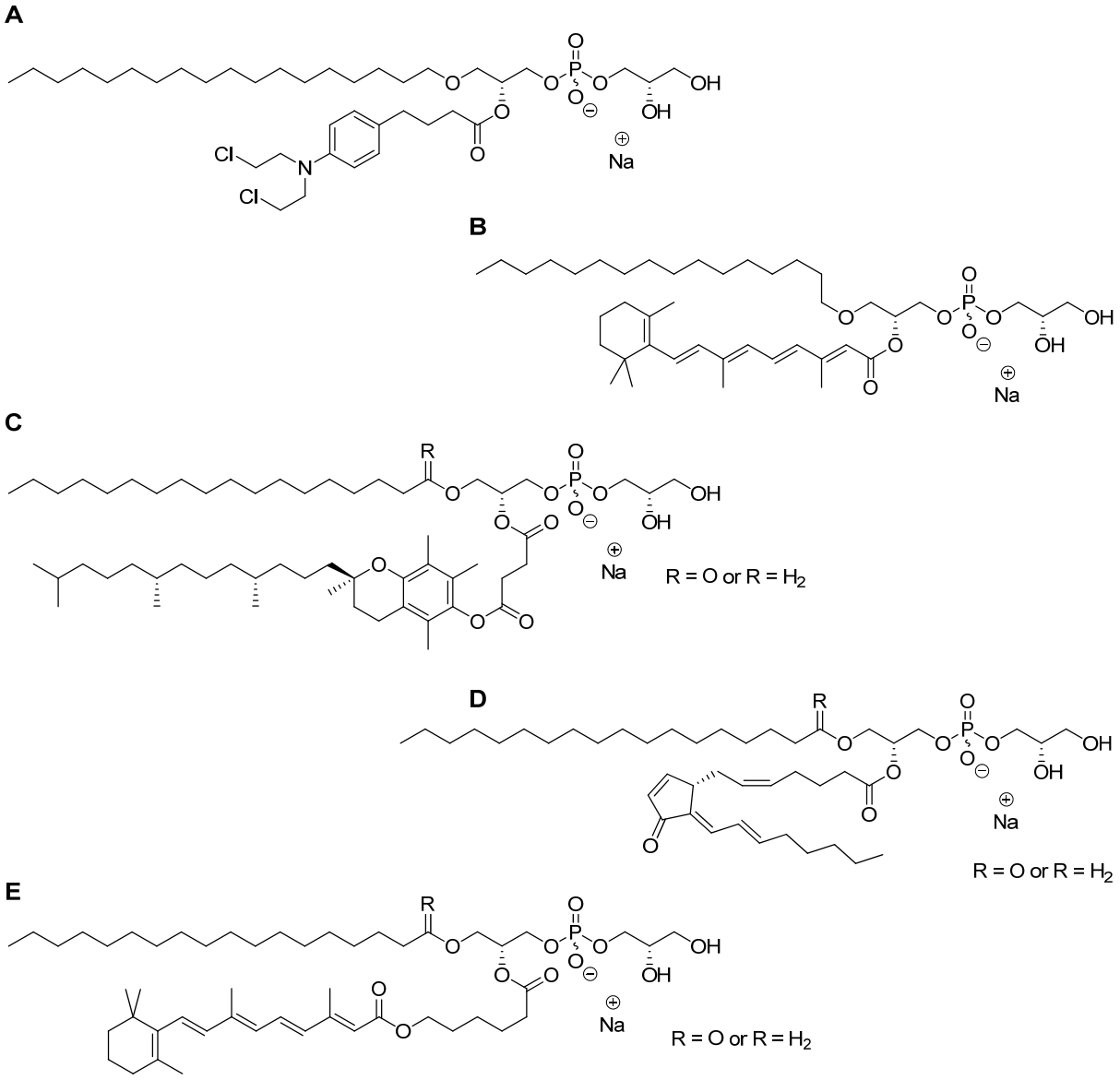


Figure 4: Phospholipid synthesised as potential prodrugs for triggered release by $sPLA_2$. A: Chlorambucil derivative;[42] B: ATRA derivative, not hydrolysed by $sPLA_2$;[63] C: α-tocopheryl succinate derivative, not hydrolysed by $sPLA_2$;[34] D: 15-deoxy-$\Delta^{12,14}$-PGJ_2 derivative;[71] E: ATRA derivative effectively hydrolysed in the presence of $sPLA_2$.[70]

All phospholipid prodrugs were synthesised and shown to form stable liposomes upon formulation, generally in the 100 nm range, except for liposomes formed from conjugates containing prostaglandins (Figure 4D),[71] which were in the 50-80 nm range following extrusion. Hydrolysis by snake venom $sPLA_2$ was investigated by MALDI-TOF-MS for all formulated liposomes and revealed a distinct difference in the ability of the different *sn*-2 ester to undergo hydrolysis: the chlorambucil prodrug (Figure 4A) was an excellent substrate and was almost fully hydrolysed in 24 h and the same was observed for the prostaglandin prodrugs (Figure 4D).[42,71] However, the α-tocopheryl succinate prodrug (Figure 4C) was not hydrolysed by $sPLA_2$,[34] neither was the ATRA conjugate (Figure 4B).[63] For the *sn*-2 ATRA phospholipid, molecular dynamics (MD) simulations

of the lipid-enzyme complex indicated that the α,β-unsaturation and/or the β-methyl substituent led to an unproductive enzyme-substrate complex, which rendered hydrolysis impossible. This was circumvented by preparation of the ester derivative of ATRA having an additional six carbon spacer, in the form of a saturated ester at the *sn*-2 position (Figure 4E), affording a lipid that was efficiently hydrolysed by $sPLA_2$ within hours.[70]

These observations prompted us to investigate the effect of unsaturation and substitution in close proximity to the *sn*-2 position on the ability of modified lipids to serve as substrates for $sPLA_2$. Hence, we set out to prepare the phospholipids shown in Figure 5. As evident, these potential substrates cover the complete range of saturated, *E*- and *Z*-unsaturated esters as well as the presence of a methyl substitution both at the α- and the β-position of the unsaturated esters. We envisioned that a study of the ability of these lipids to undergo hydrolysis catalysed by $sPLA_2$ would provide detailed information about the structural requirements for enzyme activity; data that would both help explain our previous observations and also aid in the future design of prodrug-lipid systems for triggered release by $sPLA_2$. Furthermore, the data could be compared to simulation results from MD investigations of the same substrates, aiding in validating and potentially refining modelling tools for prediction of the enzyme activity.

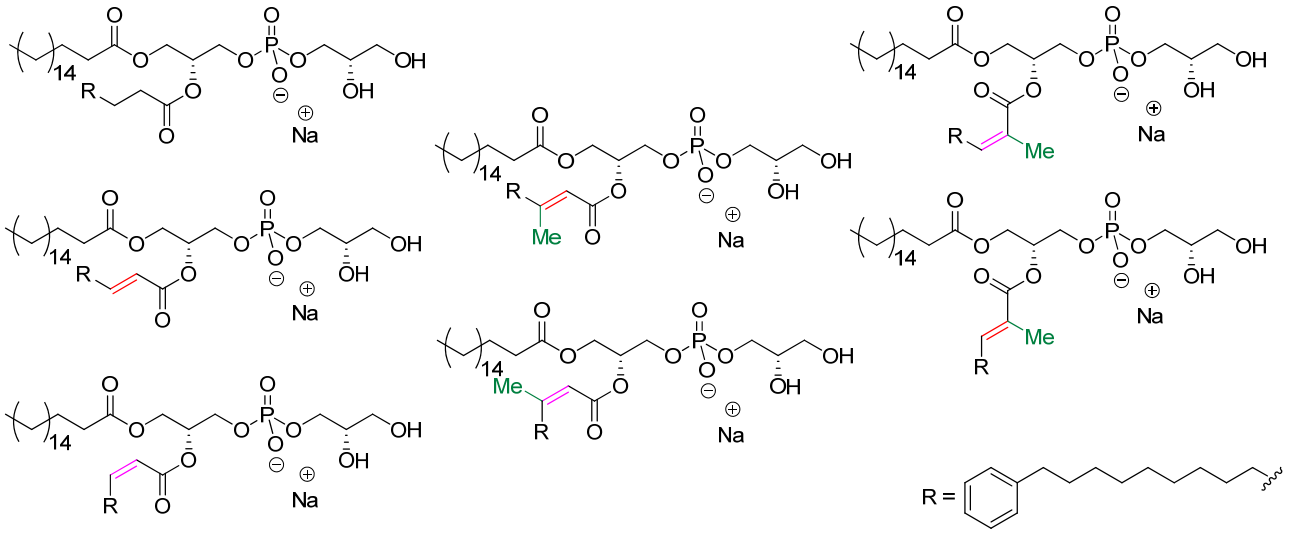


Figure 5: Target phospholipids to be tested as potential substrates for $sPLA_2$ in order to investigate the effect of unsaturation and substitution in the vicinity of the *sn*-2 position on the ability of the enzyme to hydrolyse modified lipids.

1.2. Synthesis of the potential substrates

Well-established routes have been described for the synthesis of symmetrical glycerol derivatives, as well as for mixed chain glycerol derivatives and glycerophospholipids.[35,36,37] Since the phospholipids we want to synthesise and investigate only differ at the *sn*-2 position, a synthetic route allowing the insertion of the *sn*-2 ester in a late stage as described by Murakami *et al* [38] would be preferable, however, that strategy is not commonly applied, and only few articles refer to such syntheses. Furthermore, since the intermediate phosphotriester is chiral, and the PG headgroup has a well-defined stereocenter, such an approach would force us to handle mixtures of diastereoisomers over several steps. Therefore, it was chosen to follow a more extensively described route, installing the stearoyl chain in the *sn*-1 position *via* selective monoacylation on a 3-*O*-protected-glycerol, as previously reported by Greimel *et al.*[39] followed by acylation of the *sn*-2 position, deprotection of the *sn*-3 position and insertion of the phosphatidylglycerol head group. The control of the stereocenter in the *sn*-2 position is provided by the starting material: enantiomerically pure 1,2-*O*-isopropylidene-*sn*-glycerol or 2,3-*O*-isopropylidene-*sn*-glycerol can be used as precursors in the synthesis of phospholipids. The chosen strategy still presents some challenges such as the necessity of multiple protection and deprotection steps, as well as the risk of acyl migration during the deprotection of the isopropylidene group under acidic conditions or during deprotection of the *sn*-3 position.[38,40]

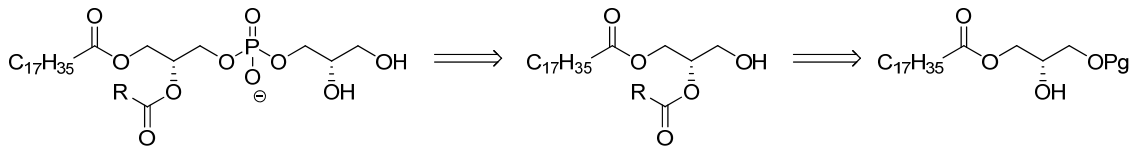


Figure 6: Retrosynthetic analysis, strategy chosen for the synthesis of the 1, 2-diacyl-*sn*-glycerophospholipids. R represents an aliphatic chain and differs for each target substrate. Pg stands for protecting group, the nature of which is not defined here.

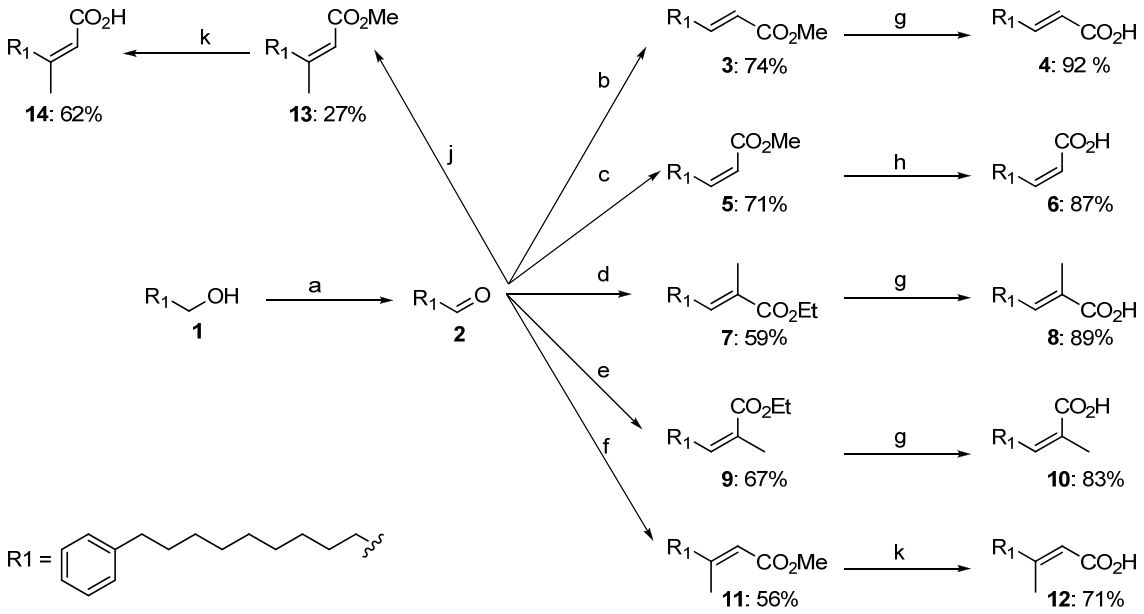
The 1-stearoyl-3-*O*-protected-*sn*-glycerol is a common precursor for every phospholipid to be synthesised, and efficient methods for introduction of the phosphatidylglycerol head group have been described for various mixed chain diacyl glycerol derivatives.[41,42] The aliphatic chain in the *sn*-2 position differs in each substrate and the corresponding fatty acids were prepared.

1.2.1. Synthesis of the acids

All the required fatty acids were synthesised from the same precursor: 10-phenyldecanol (**1**) and for all of them, the first step of the synthesis is the Swern oxidation[43,44] to the corresponding aldehyde (see Scheme 1). 10-Phenyldecanal (**2**) was obtained following the procedure described by Mancuso *et al.* for long-chain alcohols.[44] The aldehyde obtained was not stable and degraded upon storage, even at low temperatures. It was also observed that the crude aldehyde was more stable and could

be kept longer at -18 °C (2 to 3 days), while it would degrade overnight at that temperature when it had been purified by flash chromatography. Thus, only relatively small scales (up to 1.5 g) were synthesised at a time, and the resulting crude aldehyde was used immediately.

Scheme 1: Synthesis of acids from phenyldecanol.[a]



[a] Reagents: (a): $(ClCO)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C; (b): methyl diethylphosphonoacetate, NaH, DME, 45-50 °C; (c): methyl *P,P*-bis(2,2,2-trifluoroethyl)phosphonoacetate, NaH, DME/THF, -78–20 °C; (d): triethyl 2-phosphonopropionate, NaH, DME, 50-70 °C; (e): ethyl *P,P*-bis(2,2,2-trifluoroethyl)phosphonopropionate, NaH, DME/THF, -78–20 °C; (f): (i) MeMgI, Et_2O; (ii) $(ClCO)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C; (iii) methyl diethylphosphonoacetate, NaH, DME, 45-65 °C; (g): aq. NaOH (10%), EtOH; (h): aq. NaOH (10%), ^{t}BuOH; (j) MeMgI, Et_2O; (ii) $(ClCO)_2$, DMSO, Et_3N, CH_2Cl_2; (iii) methyl *P,P*-bis(2,2,2-trifluoroethyl)phosphonoacetate, NaH, DME, 45-65 °C; (k): TMSOK, Et_2O.

The α,β-unsaturated esters displaying the *E* configuration were synthesised by Horner-Wadsworth-Emmons (HWE) olefination,[45,46,47] utilizing stabilized phosphonate carbanions, while the esters displaying the *Z* configuration were obtained by means of the Still-Gennari modification of the HWE reaction, which consists in coupling an aldehyde or a ketone with *bis*(trifluoroalkyl) phosphonoesters under strongly basic conditions (KHMDS/18-crown-6).[48] The *E*-esters could also have been synthesised using the regular Wittig conditions for stabilized ylides.[49]

(E)-12-Phenyldodec-2-enoic acid (**4**) was obtained by following the experimental described by Keenan *et al.*,[50] using methyl diethylphosphonoacetate and NaH in freshly distilled dimethoxyethane (DME) at 50 °C for 2h, followed by hydrolysis in EtOH under basic conditions (10 % NaOH). During the HWE reaction, both the desired *E*- and the *Z*- α,β-unsaturated esters were isolated, however the stereoselectivity was very satisfying, since the desired ester **3** was isolated in 74 % yield, while only about 5 % of the *Z*-ester was isolated.

Still *et al.*[48], described the synthesis of aliphatic *Z*-α,β-unsaturated esters in high yields and good selectivity (*Z:E* ratio of 12:1 and 90 % yield) using methyl *P,P*-bis(2,2,2-trifluoroethyl)phosphonoacetate and KHMDS/18-crown-6 in THF, however only disappointing results were obtained when this method was attempted to synthesise methyl (*Z)*-12-phenyldodec-2-enoate (**5**) (see Table 1, entry 1), and several attempts have been carried out in order to optimize the reaction. Increasing the equivalents of the reagents or the temperature did not improve the yield, however, satisfying yields were obtained using NaH in DME at low temperature (inspired from the synthesis of the *E*-isomer), see Table 1, entry 4, despite that the contrary was originally reported in the literature.[48]

Table 1: Conditions attempted for the synthesis of methyl (*Z*)-12-phenyldodec-2-enoate 5.

Entry	Solvent	Base	*bis*(Trifluoroalkyl) phosphonoesters	Temperature	Yield[a]
1	Anhydrous THF	KHMDS (1 equiv.) 18-crown-6 (5 equiv)	1 equiv.	-78 °C	24 %
2	Anhydrous THF	KHMDS (1.5 equiv.) 18-crown-6 (7.5 equiv)	1.45 equiv.	-78 °C	34 % (10 % *E*-isomer)
3	Anhydrous THF	KHMDS (1.5 equiv.) 18-crown-6 (7.5 equiv)	1.45 equiv.	0 °C	22 % (27 % *E*- isomer)
4	Anhydrous DME	NaH (1.5 equiv)	1.45 equiv.	-78 °C	71 %

[a] Isolated yields after flash chromatography.

Hydrolysis of **5** in EtOH, NaOH (10 %) was competing with transesterification, and following the same procedure as for **4**, **6** was only isolated in 50 % yield along with 25 % of ethyl (*E)*-12-phenyldodec-2-enoate. Attempts were carried out using LiOH in THF/H_2O,[51] but only very little conversion of **5** was observed even with reaction times of several days. Switching the solvent from EtOH to the more sterically hindered ^{t}BuOH in the initial procedure prevented the transesterification, and hydrolysis of **5** in ^{t}BuOH, NaOH (10 %) afforded the desired acid **6** in 87 % yield after recrystallization from EtOH/H_2O. However, the reaction time had to be extended to 5 days before full conversion of the starting material was observed.

Esters **7** and **9** were synthesised from aldehyde **2** using triethyl-2-phosphonopropionate and ethyl-2-[bis(2,2,2-trifluoroethyl)phosphono]propionate, respectively. Again, the reaction conditions required some adjustments: for the synthesis of ester **7**, the reaction demanded more equivalents of NaH and phosphonopropionate as well as more elevated temperature for a longer reaction time (see entry 3, Table 2).

Table 2: Conditions tested for the synthesis of ethyl (*E*)-12-phenyldodec-2-enoate 7.

Entry	Temperature	NaH	Triethyl-2-phosphonopropionate	E[a]	Z[a]
1	70 °C for 4 h, then 20 °C for 18 h	1.75 equiv.	1.75 equiv	41%	12%
2	65 °C for 18 h	3 equiv	2.92 equiv.	38%	16%
3	70 °C for 18 h	1.75 equiv.	1.75 equiv.	59 %	8%

[a] Isolated yields after flash chromatography.

Taking the previous observations into account, ester **9** was synthesised using ethyl-2-[bis(2,2,2-trifluoroethyl)phosphono]propionate and NaH in a mixture of DME and THF at 20 °C. Low temperatures are generally required to obtain good *Z*-selectivity, however, the desired reaction did not occur at -78 °C. Better yields than the ones obtained here were reported for olefination of alphhatic aldehydes, using methyl bis(2,4-difluorophenyl)phosphonoacetate,[52] or different diphenylphosphonoacetic acid esters,[53] and an excess of sodium ions has been reported to increase the *Z*-selectivity using the latter.[54]

The configuration of the esters was determined by the difference in shifts of the vinylic protons: those being more deshielded by the electron withdrawing ester functionality in the *E*-α,β-unsaturated esters (with a downfield chemical shift of 6.92 ppm for **7**) than in the the *Z*-α,β-unsaturated esters (with a chemical shift of 5.92 ppm for **9**), and the structural assignment was supported by NOE measurements (see Figure 7).

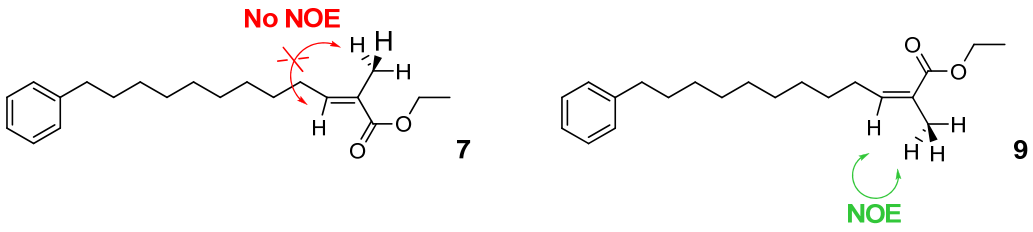


Figure 7: NOESY experiments verify the identification of esters 7 and 9: an NOE was seen between the α-methyl protons and the vinylic proton for the *Z*-ester 9, but not for the *E*-ester 7.

Several attempts to synthesise methyl diethyl-2-phosphonopropionate from methyl diethylphosphonoacetate were carried out using NaH and MeI in DME inspired by Keenan *et al.*,[50] but varying equivalents, reaction times and reaction temperatures repeatedly led to inseparable mixtures of the starting phosphonate, the desired mono-methylated phosphonopropionate and the bis-methylated 2-methyl-phosphono-propionate compound, and the desired compound could not be isolated. The three compounds were easily differenciated by ^{13}C NMR, the carbon α to the phosphorus having characteristic shifts [^{13}C NMR (75 MHz, $CDCl_3$): δ (34.08, $^{1}J_{C\text{-}P}$ = 134 Hz) for

the starting material, (39.02, $^{1}J_{C\text{-}P}$ = 132.8 Hz) for the desired compound and (43.88, $^{1}J_{C\text{-}P}$ = 136 Hz) for the bis-alkylated compound]. As a consequence, the desired phosphonopropionate was obtained from Sigma-Aldrich.

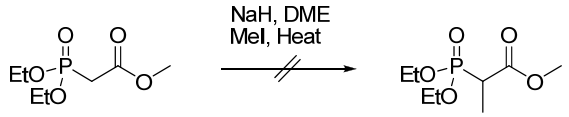
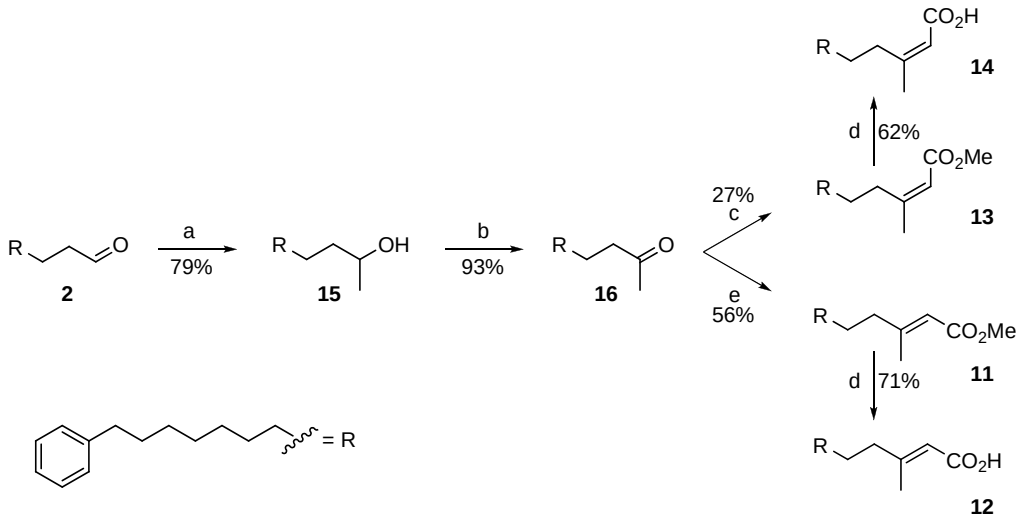


Figure 8: Attempted synthesis of methyl diethylphosphonopropionate.

Based on the results observed previously with hydrolysis of **3** and **5**, the hydrolysis of **7** and **9** was attempted in ^{t}BuOH and aqueous NaOH to avoid transesterification, however after 9 days, only very little hydrolysis had occurred, LiOH in THF/H_2O did not provide better results, and eventually it was found that aqueous NaOH in EtOH was the best system for those two substrates.

The two β-methyl-α,β-unsaturated esters were synthesised from 11-phenylundecan-2-one (**16**) using the same phosphonate as for **3** and **5** (see Scheme 2).

Scheme 2: Synthesis of (*E*)- and (*Z*)-3-methyl-12-phenyldodec-2-enoic acids.[a]



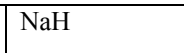
[a] Reagents: (a):MeI, Mg, Et_2O; (b): $(ClCO)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C; (c): NaH, methyl *P*,*P*-bis(2,2,2-trifluoroethyl)phosphonoacetate, DME, 65 °C; (d): TMSOK, Et_2O; (e): methyl diethylphosphonoacetate, NaH, DME, 65 °C.

10-Phenyldecanal was methylated in a Grignard reaction[55] in 79 % yield,[56] methylation using MeLi[50] resulted in a lower yield (65 %). The resulting secondary alcohol **15** was oxidized *via* a

Swern reaction[43,44] in excellent yield to 11-phenylundecan-2-one (**16**). The ketone was significantly less reactive than the aldehyde formerly used, the subsequent HWE olefinations were challenging to perform, and it was especially difficult to get a good *Z*-selectivity. Synthesis of methyl (*E)*-3-methyl-12-phenyldodec-2-enoate (**11**) according to Keenan *et al*,[50] only afforded 22% of the ester, and the reaction required heating to 65 °C for 18 h, with 3 equiv. of NaH and 2.92 equiv. of methyl diethylphosphonoacetate to afford 56 % of the desired ester, and 16 % of the *Z*-ester (**13**).

In order to synthesise *Z*-3-methyl-12-phenyldodec-2-enoate **13**, it was first attempted to carry out the reaction at low temperature to favour *Z*-selectivity, but the reaction did not perform even at 20 °C. Based on the experiments conducted for *Z*-12-phenyldodec-2-enoate **5**, it seemed that NaH in DME was the best system for our substrates, and only reaction temperature and reagents equivalents were screened (see Table 3). The best results were obtained using 3.14 equivalents of NaH, 1.92 equivalents of methyl *P*,*P*-bis(2,2,2-trifluoroethyl)phosphonoacetate at 65 °C for 18 h. Yet, *Z*-selectivity was not obtained and even in the best case, both *E*- and *Z*-α,β-unsaturated esters were obtained in a roughly 1:1 mixture.

Table 3: Conditions attempted for the synthesis of methyl (*Z*)-3-methyl-12-phenyldodec-2-enoate 13.

Entry	NaH		Temperature	Z[a] (13)	E[a] (11)
1	1.5 equiv.	1.45 equiv.	-78 °C for 3h, then 20 °C for 18h	0%	0%
2	1.5 equiv.	1.45 equiv.	40 °C for 18h	7%	9%
3	2 equiv.	1.98 equiv.	65 °C for 18h	12%	10%
4	3.14 equiv.	1.92 equiv.	65 °C for 18h	27%	30%

[a] Isolated yields after flash chromatography.

The stereochemistry of the two esters could be determined from their ^{1}H NMR spectra. It has been observed that β-methyl groups in α,β-unsaturated esters have characteristic shifts: β-methyl protons *cis* to an ester group has a slight downfield chemical shift at δ 2.1-2.2, while β-methyl protons *trans* to an ester resonates around δ 1.8-1.9.[57] ^{1}H NMR spectra and the corresponding stereochemical assignments are in agreement with this observation: the chemical shift is δ 2.10-2.16 for the β-methyl protons for the 3-methyl-*E*-ester (**11**) and δ 1.88 for the 3-methyl-*Z*-ester (**13**).

Hydrolysis of the two esters **11** and **13** turned out to be challenging as well, and several conditions were attempted to get reasonable yields (see Table 4). The conditions described previously for the synthesis of the acids **4**, **6**, **8**, and **10** were inefficient for the latest acids, applying heat to the mixture of ester **11** in EtOH and aqueous NaOH resulted in partial migration of the alkene to give the β-methylene-carboxylic acid (approximately 1:4 ratio between 3-methylene-12-phenyldodecanoic acid and the desired acid), in a mixture that proved challenging to separate (Table 4, Entry 2).

Table 4: Experiments conducted for the hydrolysis of esters 11 and 13.

Entry	Ester	Experimental conditions	Outcome
1	3-Methyl-*E*-ester (**11**)	Aq. NaOH (3 equiv.), EtOH, 20 °C	Very little conversion after 2 days[a]
2	3-Methyl-*E*-ester (**11**)	Aq. NaOH (3 equiv.), EtOH, 40 °C, 24 h	Desired acid **12**: 69 %[b] 3-methylene-12-phenyldodecanoic acid: 20%[b]
3	3-Methyl-*E*-ester (**11**)	LiOH (3 equiv.), THF/H_2O, 20 °C	Less than 50 % conversion after 8 days[a]
4	3-Methyl-*Z*-ester (**13**)	LiOH (3 equiv.), THF/H_2O, 20 °C	Less than 50 % conversion after 8 days[a]
5	3-Methyl-*E*-ester (**11**)	Aq. NaOH (3 equiv.), ^{t}BuOH, 20 °C	Very little conversion after 5 days[a]
6	3-Methyl-*Z*-ester (**13**)	Aq. NaOH (3 equiv.), ^{t}BuOH, 20 °C	Very little conversion after 5 days[a]
7	3-Methyl-*E*-ester (**11**)	TMSOK (3 equiv.), anhydrous Et_2O, 20 °C, 24 h	Desired acid **12**: 71 %[b] *Z*-Isomer **14**: 6 %[b]
8	3-Methyl-*Z*-ester (**13**)	TMSOK (3 equiv.), anhydrous Et_2O, 20 °C, 24 h	Desired acid **14**: 62 %[b] *E*-Isomer **12**: 7 %[b]

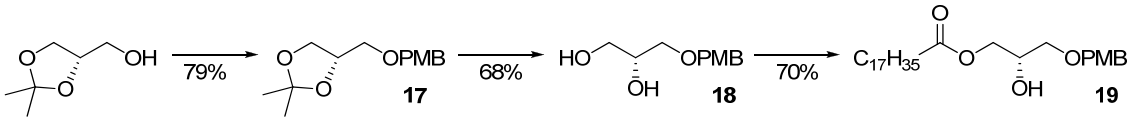
[a] Observed by TLC; [b]Isolated after flash chromatography.

Mild conditions using TMSOK to convert esters into anhydrous acid salts were developed by Laganis *et al.*,[58] and applied efficiently for methyl esters saponification by Fuwa *et al.*,[59] and was attempted on our compounds too. Although the esters were converted faster than with the NaOH/EtOH hydrolysis conditions (entries 5 and 6, Table 4), these new conditions led to partial isomerisation of the olefin, and both *E*- and *Z*- isomers were isolated, yet in a lower extent than in the case of the olefin migration described above (see Table 4, entries 7 and 8). Eventually, the desired acids could be isolated in satisfying yields using TMSOK as reported by *Fuwa et al.*[59], with 71% and 62% yield for *E*-12-phenyldodec-2-enoic acid **12** and *Z*-12-phenyldodec-2-enoic acid **14**, respectively.

1.2.2. Synthesis of 1,2-diacyl-*sn*-glycerol

The acids above obtained were attached to the *sn*-2 position of a monoacyl glycerol. Thus, 1-stearoyl-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (**19**) was synthesised in 3 steps from 1,2-*O*-isopropylidene-*sn*-glycerol (see Scheme 3), by PMB-protection in the *sn*-3 position followed by isopropylidene protecting group hydrolysis under acidic conditions, as reported by Lim *et al*.[60] in 53 % yield over the two steps (65 % reported in the referenced literature).[60] Selective esterification of the *sn*-1 position via a Steglich esterification,[61] afforded the desired monoacyl glycerol **19** in 70 % yield using stearic acid, DCC and catalytic amounts of DMAP at 0 °C, according to the procedure described by Greimel *et al.*.[62]

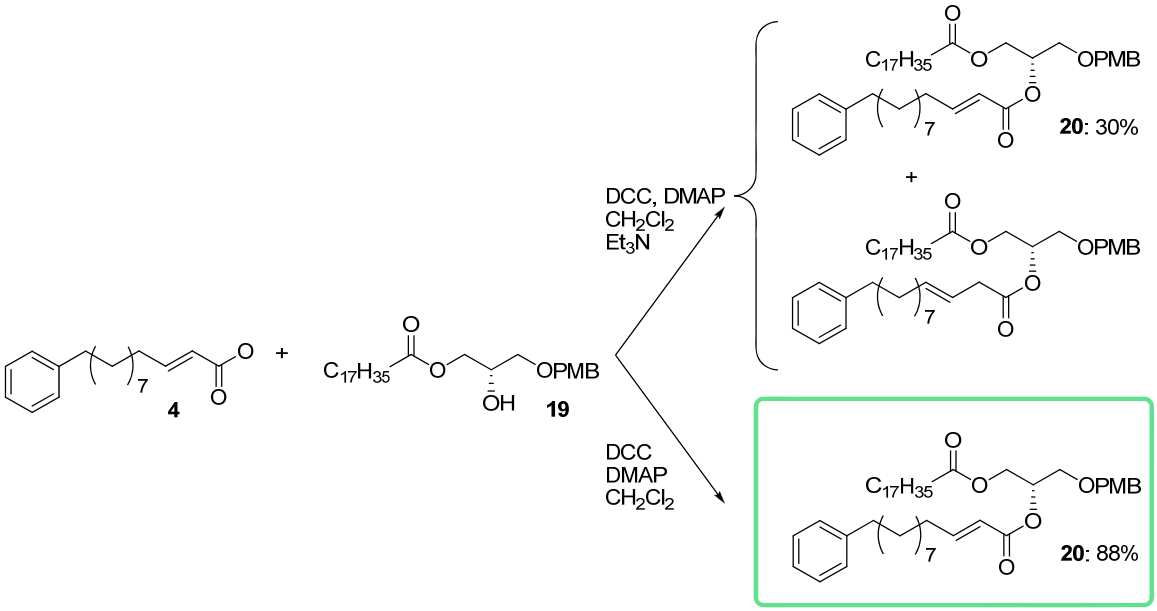
Scheme 3: Preparation of 1-stearoyl-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol 19.[a]



[a] Reagents. (a): NaH, PMBCl, DMF, 0 – 20 °C; (b): 1M HCl, MeOH; (c): Stearic acid, DCC, DMAP, CH_2Cl_2, 0-20 °C.

Christensen *et al.* observed that Steglich esterification of α,β-unsaturated carboxylic acids can result in *E/Z* isomerisation in some instances.[63] It was observed that the isomerisation was mediated by DMAP and required a proton source[64] and thus it was proposed that an excess of base (10 equiv. Et_3N) could solve the issue.[63] Hence, the optimized conditions described by Christensen *et al.*[63] were applied for the esterification in the *sn*-2 position of 1-stearoyl-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol **19** with with *E*-12-phenyldodec-2-enoic acid **4**. No *E/Z* isomerisation was observed, however, the desired 1-stearoyl-2-(*E*-12-phenyldodec-2-enoyl)-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (**20**) was isolated in 30% yield only, as a consequence of double bond migration occurring as side reaction (see Scheme 4). The esterification was also conducted with the more classical Steglich conditions, without Et_3N, adapted from the experimental conditions reported by Kubiak *et al.,*[65] this performed smoothly to afford **20** in 88% yield. Very little migration product was observed in the crude mixture, no *E/Z*-isomerisation took place and the desired product could be separated after flash chromatography.

Scheme 4: Synthesis of 1-stearoyl-2-(*E*-12-phenyldodec-2-enoyl)-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (20)



These observations led us to perform a small screening of acylation conditions and methods for some of our acids with the monoacylglycerol **19** or its enantiomer: **23**: (see Table 5 and Table 6).

It was observed that depending on the conditions applied, either the same kind of by-product as for coupling with *E*-12-phenyldodec-2-enoic acid **4**, resulting from olefin migration, or the by-product described by Christensen *et al.*,[63] and resulting from an *E/Z* isomerisation, was formed. It seems that the classical Steglich reaction[61] was best for the reaction with **4** and the same procedure as for synthesis of **20** was tested for the acylation with *E*-3-methyl-12-phenyldodec-2-enoic acid **12**, but disappointingly this resulted in partial isomerisation and a mixture of the desired *E*-α,β-unsaturated ester and the by-product *Z*-α,β-unsaturated ester in the *sn*-2 position in a 3:1 ratio, observation similar to the one reported previously for ATRA (Table 5 entry 8).[63]

Estimating that the other acids would be as likely to undergo the *E/Z* isomerisation, the modified Steglich reaction conditions, adding 10 equivalents of Et_3N was attempted for the coupling of *Z*-12-phenyldodec-2-enoic acid **6**, where a mixture of the desired diacylglycerol was observed together with two by-products resulting from *E/Z* isomerisation and from olefin migration respectively, in a 3:3:1 ratio (see Figure 9 and Table 5, entry 3). The same conditions did not result in a clean reaction either for the coupling of *E*-2-methyl-12-phenyldodec-2-enoic acid **8**, where two spots of similar intensity could be seen on TLC (Table 5, entry 6). It appears from these experiments as well as from the saponification reactions, where olefin migration was also observed during the course of the

reaction in EtOH with aqueous NaOH at 40 °C for 24h (see Table 4, entry 2), that our aliphatic α,β-unsaturated esters are rather sensitive to basic conditions, which promote olefin migration.

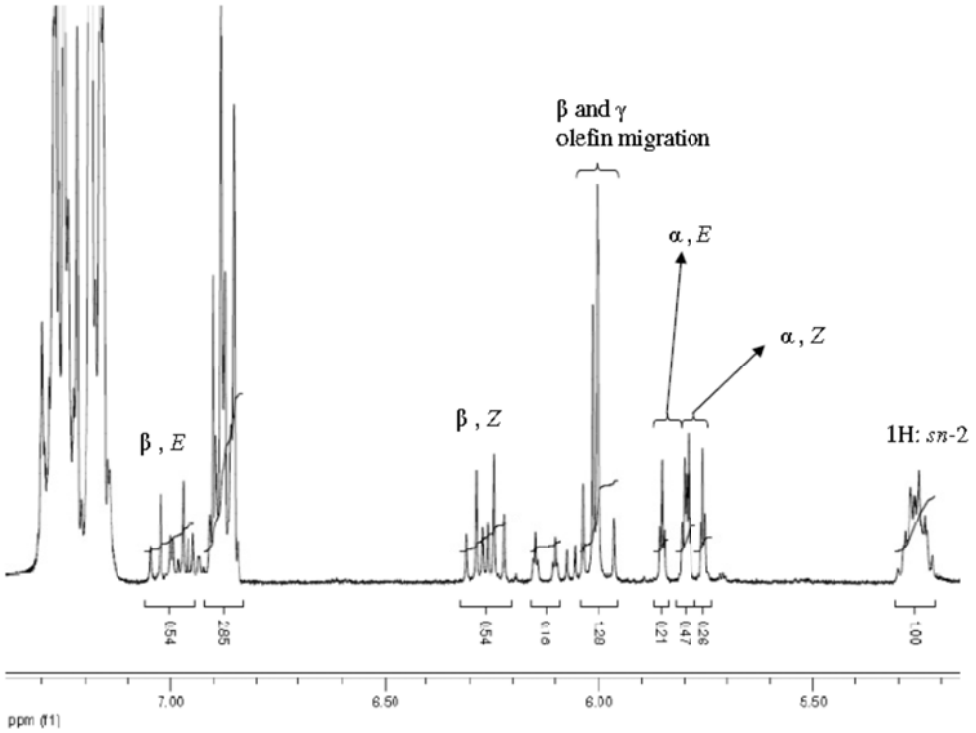


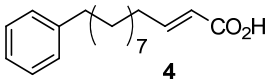
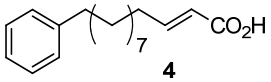
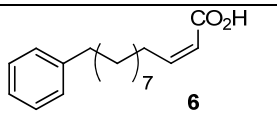
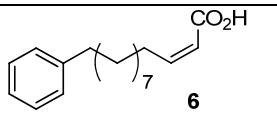
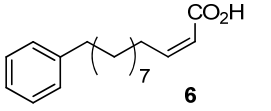
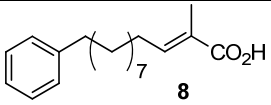
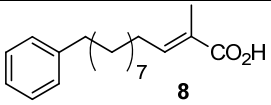
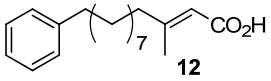
Figure 9: Mixture observed in reaction of *Z*-12-phenyldodec-2-enoic acid 6 with alcohol 19, DCC, DMAP and Et_3N.

Yamaguchi coupling[66] was also considered for the acylation of the *sn*-2 position, but *E/Z* isomerzation was observed with (*Z*)-12-phenyldodec-2-enoic acid **6** (Table 5, entry 4).

Because of the by-products observed in the former conditions applied, where the acids were activated for nucleophilic attack, we decided to try to activate the alcohol instead, and the Mitsunobu[67] reaction was tested with (*E*)-2-methyl-12-phenyldodec-2-enoic acid **8**, Ph_3P, DIAD, and 1-stearoyl-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol **19**, with an excellent outcome, since 1-*O*-(*para*-methoxybenzyl)-2-*(E*-2-methyl-12-phenyldodec-2-enoyl)-3-stearoyl-*sn*-glycerol was obtained in 93 % yield after purification by flash chromatography. However, due to the inversion of

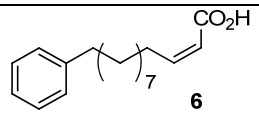
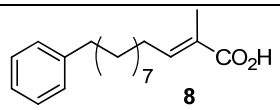
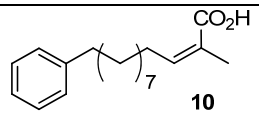
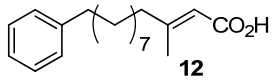
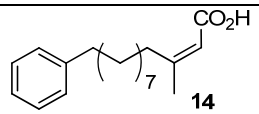
configuration inherent in the Mitsunobu reaction the enantiomer of **19** had to be synthesised if the Mitsunobu reaction were to be used for synthesis of our substrates.

Table 5: Reactions conducted for synthesis of the di-acyl-glycerols.

Entry	Acid and alcohol	Reaction Conditions	Outcome
1	 **4** alcohol: **19**	DCC, DMAP, CH_2Cl_2, 10 equiv. Et_3N	Desired diacylglycerol **19**: 30% [a] Olefin migration by-product [b]
2	 **4** alcohol: **19**	DCC, DMAP, CH_2Cl_2	**20**: 88% [a]
3	 **6** alcohol: **19**	DCC, DMAP, CH_2Cl_2, 10 equiv. Et_3N	Desired diacylglycerol, isomerized diacylglycerol and olefin migration by-product in approx. 3:3:1 mixture [c]
4	 **6** alcohol: **19**	2,4,6-Trichlorobenzoyl chloride, DMAP, THF	Complete isomerisation to the *E*-ester [c]
5	 **6** alcohol: **19**	2,4,6-Trichlorobenzoyl chloride, DMAP, THF, 10 equiv. Et_3N	2 spots on TLC in about 1:1 mixture [d]
6	 **8** alcohol: **19**	DCC, DMAP, CH_2Cl_2, 10 equiv. Et_3N	By-product and desired compound in about 1:1 mixture [d]
7	 **8** alcohol: **19**. test reaction	Ph_3P, DIAD, THF	Desired diacylglycerol, 92 % [a]
8	 **12** alcohol: **19**:	DCC, DMAP, CH_2Cl_2	Desired diacylglycerol and E/Z isomerisation product in a 3:1 mixture[c]

[a] isolated yield after flash chromatography; [b] isolated after flash chromatography; [c] estimation from crude ^1H NMR. [d] estimated from TLC.

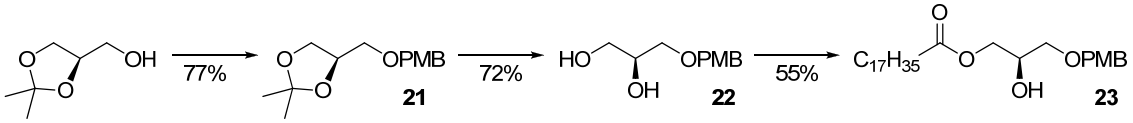
Table 6: Synthesis of the di-acyl-glycerols by the Mitsunobu reaction.

Entry	Acid and alcohol	Reaction Conditions	Outcome
1	 **6** alcohol: **23**:	Ph_3P, DIAD, THF	Desired diacylglycerol **24**: 93 % [a]
2	 **8** alcohol: **23**:	Ph_3P, DIAD, THF	Desired diacylglycerol **25**: 94 % [a] NB: olefin migrationoccurs upon long reaction times.
3	 **10** alcohol: **23**:	Ph_3P, DIAD, THF	Desired diacylglycerol **26**: 76 % [a]
4	 **12** alcohol: **23**:	Ph_3P, DIAD, THF	Desired diacylglycerol **27**: 68 % [a]
5	 **14** alcohol: **23**:	Ph_3P, DIAD, THF	Desired diacylglycerol **28** 74 % [a]

[a] isolated yield after flash chromatography; [b] isolated after flash chromatography; [c] estimation from crude ^1H NMR. [d] estimated from TLC.

Thus, 1-*O*-(*para*-methoxybenzyl)-3-stearoyl-*sn*-glycerol **23** was synthesised in 30% yield over three steps from 2,3-*O*-isopropylidene-*sn*-glycerol following the same procedure as for the synthesis of **19**.

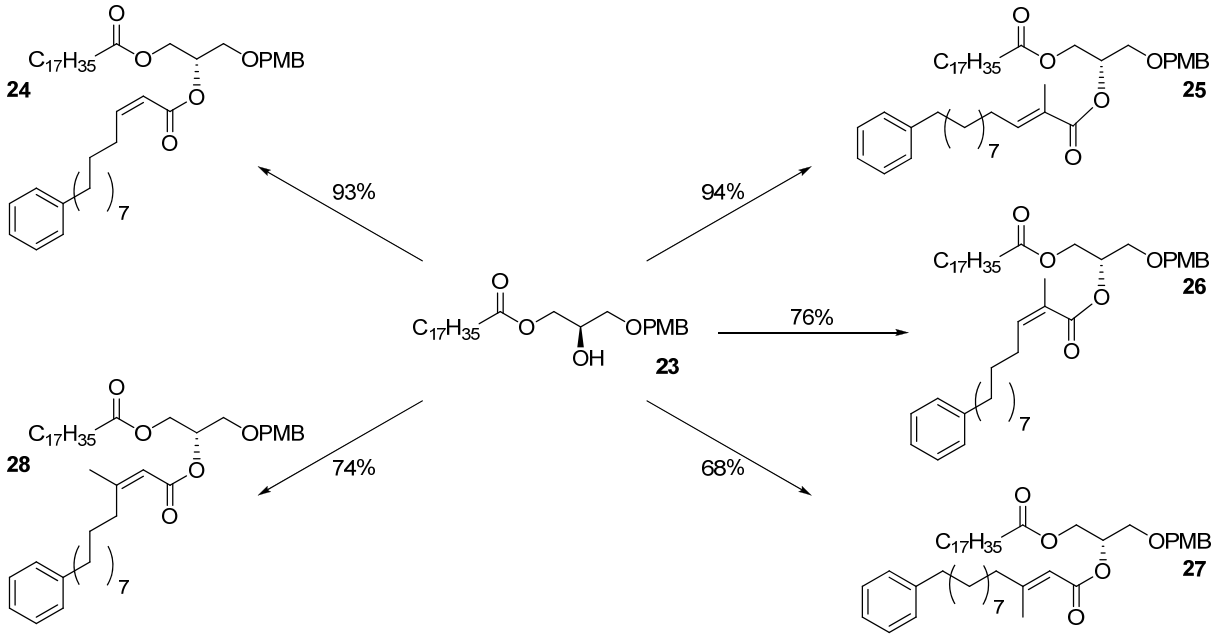
Scheme 5: Synthesis of 1-*O*-(*para*-methoxybenzyl)-3-stearoyl-*sn*-glycerol 23.[a]



[a] Reagents. (a): NaH, PMBCl, DMF, 0 – 20 °C; (b): 1M HCl, MeOH; (c): Stearic acid, DCC, DMAP, CH_2Cl_2, 0-20 °C.

The Mitsunobu reaction proved to be efficient for the synthesis of esters **24** to **28**, affording the desired diacylglycerols in yields ranging from 68% to 94% (see Table 6 and Scheme 6).

Scheme 6: Synthesis of the di-acyl-glycerols by the Mitsunobu reaction.[a]



[a] Reagents: Ph_3P, DIAD, THF, acids **6, 8, 10, 12, 14** for the synthesis **of 24, 25, 26, 27** and **28** respectively.

For the reaction to afford satisfying yields, two equivalents of the acids were required. This was due to by-product formation, which also accounts for the lower yields observed for compounds **26**, **27** and **28**. Indeed, an *N*- acylhydrazine dicarboxylate by-product has been reported in the literature (structure A Figure 10),[68]however, we did not observe this, but rather a related by-product, for which the ^{1}H NMR spectrum is in agreement with either tetraacylhydrazine B or *N*-acylcarbamate C Figure 10.

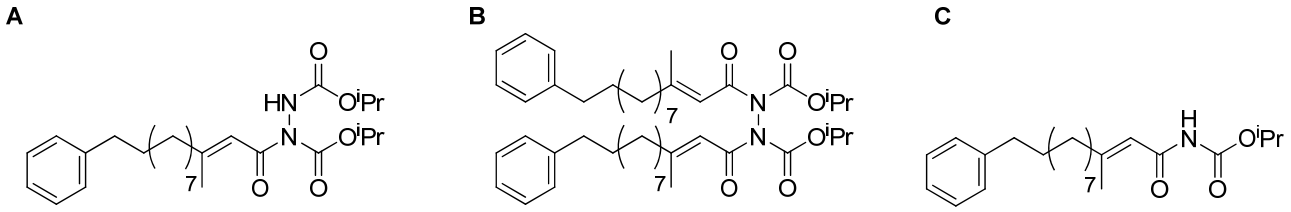


Figure 10: Formation of side products in the Mitsunobu reaction. A: side product reported in the literature;[68] B and C: plausible structures for the by-product isolated in our reactions.

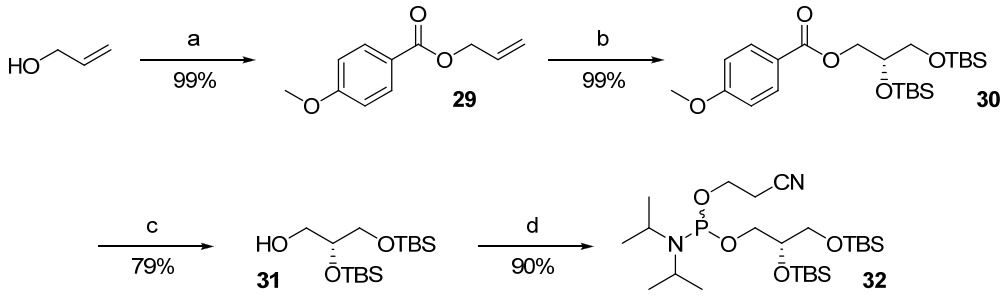
1.2.3. Synthesis of glycerophospholipids

With the 1-stearoyl-2-α,β-unsaturated-acyl-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol derivatives in hand, we could deprotect the *sn*-3 position and install the head groups. Removal of the PMB protecting group was accomplished with DDQ in a biphasic system of CH_2Cl_2 and phosphate buffer

(pH 7)[69] in order to avoid any potential acyl migration to the *sn*-3 position (see Scheme 8). The deprotection proceeded in satisfying yields (77%, 80% and 80% respectively for compounds **24**, **20** and **26** respectively), although slightly lower yield were obtained for compounds **25**, **27** and **28**, with yields of 60%, 60% and 62% respectively (see Scheme 8). The lower yields were obtained for compounds that eluted very close to the anisaldehyde resulting from the oxidative cleavage of PMB on flash chromatography, resulting in more challenging purification of the desired compounds.

A new route to access the PG lipids using 2,3-di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycero-2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite **32** was developed by Pedersen *et al.*,[42] and successfully applied in the synthesis of several asymmetric diacyl phosphatidylglycerol phospholipids.[70,71] The strategy was thus applied to our substrates, and the phosphoramidite **32** precursor for the PG head group was synthesised from allyl alcohol in 70 % yield over 4 steps (see Scheme 7). The alcohol was protected with *para*-methoxybenzoyl chloride to give **29** in excellent yield. The resulting allyl ester was subjected to Sharpless asymmetric dihydroxylation,[72,73] which performed in excellent yield and enantioselectivity (ee 97%, chiral HLPC) to afford 1-(*para*-methoxybenzoyl)-*sn*-glycerol. The crude diol was protected with TBSCl to afford **30** in excellent yield and deprotection of the *sn*-1 position with diisobutylaluminium hydride (DIBAL-H) at -78 °C gave the primary alcohol **31**. Finally, this was reacted with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite under basic conditions, to afford the phosphoramidite **32** as 1:1 diastereoisomeric mixture, as described in the literature,[42] however, the original characterization of the compound was incorrect, as the cyanoethyl protons were not reported.

Scheme 7: Synthesis of the phosphoramidite 32, precursor for the PG head group.[a]

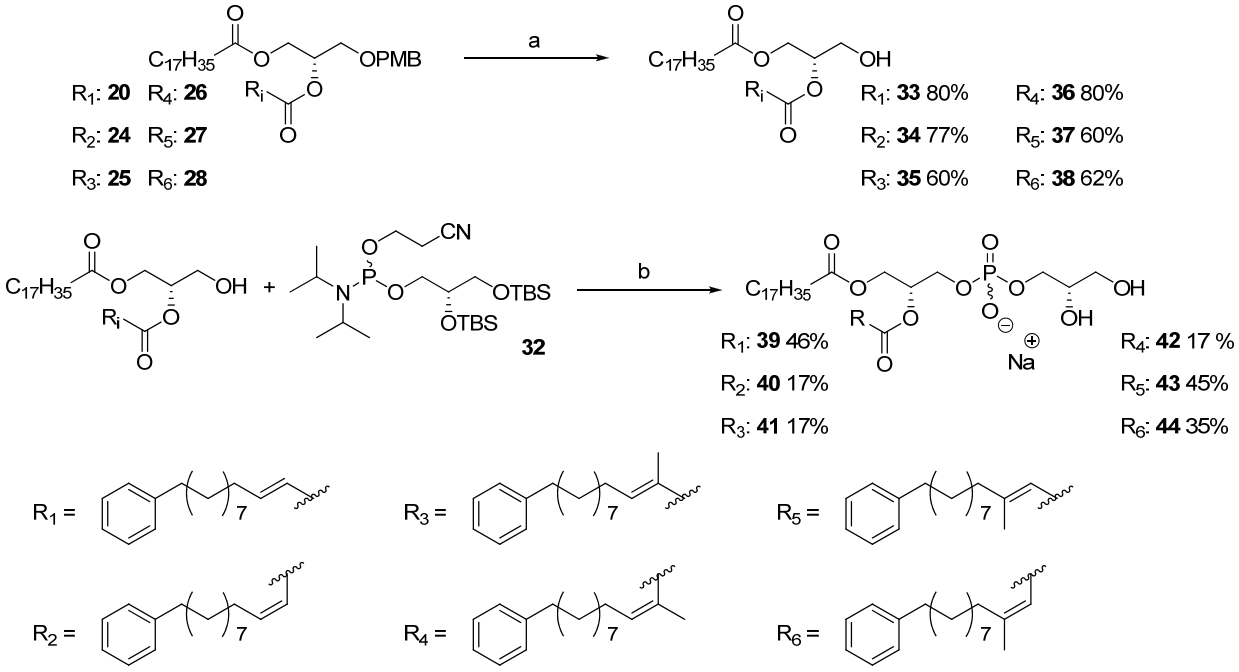


[a]Reagents: (a) *para*-Methoxybenzoyl chloride, Et_3N, DMAP, CH_2Cl_2; (b) (i) $K_2OsO_4 \cdot 2H_2O$, $(DHQD)_2PHAL$, $K_3Fe(CN)_6$, K_2CO_3, ^{t}BuOH, H_2O; (ii) TBSCl, imidazole, DMF; (c) DIBAL-H, CH_2Cl_2; (d) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, DIPEA, CH_2Cl_2.

With the 1,2-diacyl-*sn*-glycerol derivatives and phosphoramidite **32** in hand, we could carry out the synthesis of the desired lipids. The reaction sequence described by Pedersen *et al.*[42] was the inspiration for coupling of the PG head-group and the subsequent deprotections (see Scheme 8). The phosphoramidite coupling to the diacylglycerol was catalysed by tetrazole in CH_2Cl_2,[74] and followed by oxidation of the phosphite to the phosphate by ^{t}BuOOH. The cyanoethyl protecting

group was removed with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2, and the TBS groups were cleaved with 40% aq. HF in a mixture of CH_2Cl_2 and MeCN at 0 °C (see Scheme 8). The yields observed were generally consistent from substrate to substrate, except for the final HF-mediated deprotection step: addition of HF was performed at 0 °C, but when the reaction mixture was allowed to reach 20 °C as proposed in the original literature,[42] we observed hydrolysis of the phosphate head group, which was not the case when the reaction mixture was kept at 0 °C and this dramatically decreased the efficacy of the reaction (yields in the range of 30% were obtained for the last deprotection, while yields neighbouring 80% were generally obtained when the reaction mixture was kept at 0 °C). This explains the differences in yields obtained for the different lipids: lipids **40**, **41** and **42** where reaction temperatures evolved from 0 to 20 °C, while lipids **39**, **43**, **44** were kept at 0 °C during the final TBS-deprotection step.

Scheme 8: Synthesis of phospholipids 39 to 44[a]

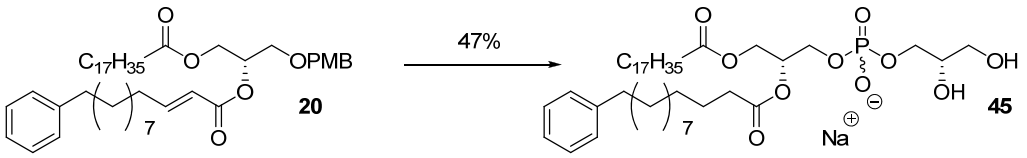


[a] Reagents: (a): DDQ, buffer (pH 7), CH_2Cl_2; (b) (i): tetrazole, CH_2Cl_2, MeCN; (ii) ^{t}BuOOH; (iii) DBU, CH_2Cl_2; (iv) HF, H_2O, CH_2Cl_2, MeCN.

The synthesis of the fully saturated diacyl-glycerophospholipid was attempted by direct hydrogenation of the lipid **39** using activated palladium on charcoal (Pd/C) and H_2 in EtOAc, after 16 h, full conversion of **39** was observed, yet a crude NMR showed the presence of a by-product, which could not be separated by flash chromatography. Therefore it was decided to reduce the olefin in an earlier step. To this end, 1-stearoyl-2-(12-phenyldodecanoyl)-*sn*-glycero-3-phospho-(*S*)-glycerol **45** was synthesised from the diacyl-glycerol **20** in 47% yield over 6 steps. The PMB group was first removed using DDQ in CH_2Cl_2/buffer (pH 7), then the PG-headgroup was installed in the

sn-3 position by reaction with **32** and tetrazole in CH_2Cl_2/MeCN, the phosphite was oxidised to the phosphate by ^{t}BuOOH and the labile cyanoethyl group was displaced by treatment with DBU. The resulting TBS-protected phosphatidylglycerol derivative was at that stage reacted with Pd/C and H_2 in EtOAc, a reaction that performed cleanly and quantitatively and was completed after 14h. Finally, **45** was obtained after treatment with 40% aq. HF in CH_2Cl_2/MeCN at 0 °C.

Scheme 9: Synthesis of 1-Stearoyl-2-(12-phenyldodecanoyl)-*sn*-glycero-3-phospho-(*S*)-glycerol.[a]



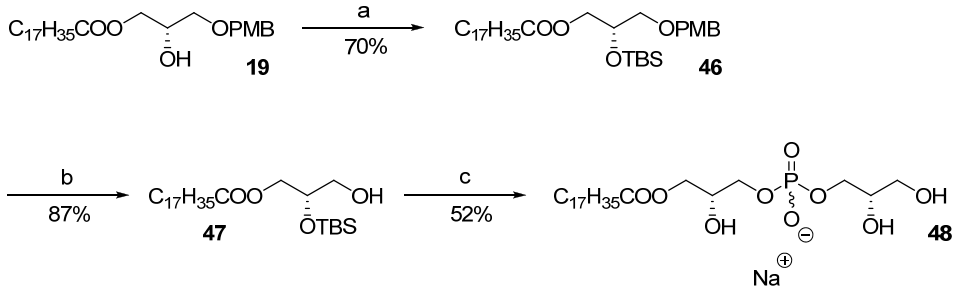
[a] Reagents: (a): DDQ, buffer (pH 7), CH_2Cl_2; (b) (i): (2,3-di-*O*-*tert*-butyldimethylsilyl)-*sn*-glycero-2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite **32**, tetrazole, CH_2Cl_2, MeCN; (ii) ^{t}BuOOH; (iii) DBU, CH_2Cl_2; (iv) Pd/C, H_2, EtOAc; (v): HF, H_2O, CH_2Cl_2, MeCN.

1.2.4. Synthesis of the lysolipid

The lysolipid (**48**) was synthesised from the mono-acylated glycerol derivative **19** (see

Scheme 10). The *sn*-2 position was first protected with TBSCl, *via* imidazole catalysis in DMF to give **46**.[75] TBS was chosen for protection of the *sn*-2 position for its stability to the conditions for PMB deprotection and head group attachment, but also for the convenient possibility to remove it and the TBS protection groups on the head group simultaneously. The following reactions have been performed as for the other lipids discussed above: The PMB group was removed with DDQ in CH_2Cl_2/buffer (pH 7) to give **47**, addition of the protected PG head group was performed with (2,3-di-*O*-*tert*-butyldimethylsilyl)-*sn*-glycero-2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite **32**, in CH_2Cl_2/MeCN and catalysed by tetrazole and the phosphite was oxidized to the phosphate by ^{t}BuOOH. The cyanoethyl group was cleaved by DBU in CH_2Cl_2, and the three TBS groups were removed simultaneously upon treatment with 40% aq. HF in CH_2Cl_2, MeCN at 0 °C, affording the desired lysolipid 1-stearoyl-*sn*-glycero-3-phospho-(*S*)-glycerol **48** in 52% yield over the last four steps. Lysolipid **48** was prepared as a reference compounds, since it is the product of $sPLA_2$ hydrolysis of all the substrates **39-45**.

Scheme 10: Synthesis of 1-stearoyl-*sn*-glycero-3-phospho-(*S*)-glycerol.[a]



[a] Reagents: (a): TBSCl, imidazole, DMF; (b): DDQ, buffer (pH 7), CH_2Cl_2; (c) (i): (2,3-di-*O*-*tert*-butyldimethylsilyl)-*sn*-glycero-2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite **32**, tetrazole, CH_2Cl_2, MeCN; (ii) ^{t}BuOOH; (iii) DBU, CH_2Cl_2; (iv)): HF, H_2O, CH_2Cl_2, MeCN.

1.3. Liposome formulation and enzyme activity

The phospholipids **39** to **44** and **45** were first formulated as multilamellar vesicles in 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid (HEPES) buffer by the dry lipid film technique:[76] the phospholipids were solubilized in $CHCl_3$ and the solvent was evaporated *in vacuo* to form a dry lipid thin film. HEPES buffer was added in order to obtain 2 mM suspensions of the lipids, and the lipids were solubilized upon heating the mixture to 50 °C and periodically vortexed. This resulted in a milky suspension of multilamellar vesicles that was extruded through a 100 nm polycarbonate filter at 20 °C in order to obtain the desired monolamellar liposomes as a transparent solution. The thereby obtained particles sizes were measured by Dynamic Light Scattering (DLS), and we observed that all the phospholipids were forming particles of about 100 nm, which is the typical liposome size region and indicates formation of unilamellar vesicles, with a low polydispersity, which confirms the homogeneity of the vesicles constituents (see Table 7).

Table 7: DLS analysis of the formulated phospholipids 39 to 45

Compound	Particle size	
	Diameter (nm)	PdI[a]
39	94	0.253
40	116	0.070
41	84	0.139
42	96	0.295
43	109	0.113
44	106	0.077
45	108	0.196

[a]PdI = polydispersity index

DSC scans (10–70 °C) of the phospholipid **39** to **45** displayed no phase transitions in the tested temperature range indicating that the phospholipid bilayers are in a fluid state at room temperature as well during the $sPLA_2$ experiments conducted at 37 °C. This is not surprising since the phase transition temperatures decrease when the length of the aliphatic chains decreases (the phase transition temperatures of DSPG, DPPG and DMPG are 55 °C, 41 °C and 23 °C, respectively), and drastically decreases when unstauration is introduced in the aliphatic chains (the phase transition temperatures of POPG, DLPG and DOPG are -2 °C, -3 °C and -18 °C, respectively). The saturated lipid (**45**) was the most likely phospholipid to possess a phase transition temperature above 15 °C, but even that compound did not show any transition phase in the tested temperature range

The ability of $sPLA_2$ to degrade the synthesised lipids **39** to **45** was studied by incubating the formulated liposomes with human tears at 37 °C, and sampling was done after 1, 2, 4, 8, 24, 48 and 96 h. The tear fluid was obtained after breathing of vapour of onions, and it has been shown that $sPLA_2$ content in tears was amongst the highest in human secretions,[77] being highest in the age-group 20-29 years (81.6 ± 32.0 μg/mL),[78,79] fitting with the age of the subject. The samples were

analysed by matrix-assisted laser desorption/ionization - time of flight - mass spectrometry (MALDI-TOF-MS), a method that proved very efficient, fast and sensitive for the detection of lipids.[80,81,82] The matrix applied on the sample target consisted in 2,5-dihydroxybenzoic acid (DHB) and CF_3COONa in methanol and an internal standard 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylglycerol (DMPG), that would not interfere with the masses of the synthetic lipids, was also added to the matrix. Since constant quantities of matrix and sample were applied on the MALDI target, we estimated that the average signal measured for the phospholipids could be normalised with the internal standard, and the normalised signals were compared to study the extent of hydrolysis on each potential substrate. This method has been efficiently used for similar purpose by Linderoth *et al.*[83] and by Pedersen *et al.*.[42,70]

HPLC analysis was also considered as a potential tool to obtain a more quantitative picture of $sPLA_2$ hydrolysis capacity of each potential substrate. This is the reason why a phenyl ring was introduced on the *sn*-2: we looked for a UV-active functionality that would not disturb the hydrophobic chains packing. Thus, a rather small phenyl ring was seen as the best compromise, any larger aromatic system (such as a naphthalene group) or a system of conjugated olefins would most probably influence the packing of the phospholipids and might hinder formulation into liposomes and/or change the electronics in the vicinity of the *sn*-2 position, thereby influencing the properties we wished to investigate.

The MALDI-TOF hydrolysis profiles of the reference substrate (saturated lipid **45**), a good $sPLA_2$ substrate (*E*-unsaturated lipid **39**) and a poorly-hydrolysed lipid (**43**, with a (*E*)-β-methyl substituted *sn*-2 fatty acid) are shown in Figure 11. All results are summarized in Table 8 and it is evident from the data that while unsaturation is tolerated to some degree by $sPLA_2$ (entry 2 and 3), α- or β-methyl substitution of the *sn*-2 fatty acid affords poor (entries 5 and 7) or completely resistant (entries 4 and 6) substrates for the enzyme.

Table 8: Hydrolysis of phospholipids 39-45 by $sPLA_2$ as monitored by MALDI-TOF MS.

Entry	Lipid	Structure	Ratio[a]	Hydrolysis after 96 h[b]
1	**45**	Saturated	0.04	96%
2	**39**	(*E*)-unsaturated	0.04	96%
3	**40**	(*Z*)-unsaturated	0.32	68%
4	**41**	(*E*)-α-methyl	-[c]	-[c], <1% after 48h[d]
5	**42**	(*Z*)-α-methyl	0.85	15%
6	**43**	(*E*)-β-methyl	1.04	<1%
7	**44**	(*Z*)-β-methyl	0.72	28%

[a.] The ratio between the MS signal from the $sPLA_2$ treated liposome solution (average of two independent experiments) and the control of the same liposome without added enzyme after 96 h, after normalisation using the internal standard

(DMPG); [b.] Calculated from the ratio. [c] Data at 96 h was not available due to technical complications. [d] Based on comparison of MS signals at 48 h to 0 h.

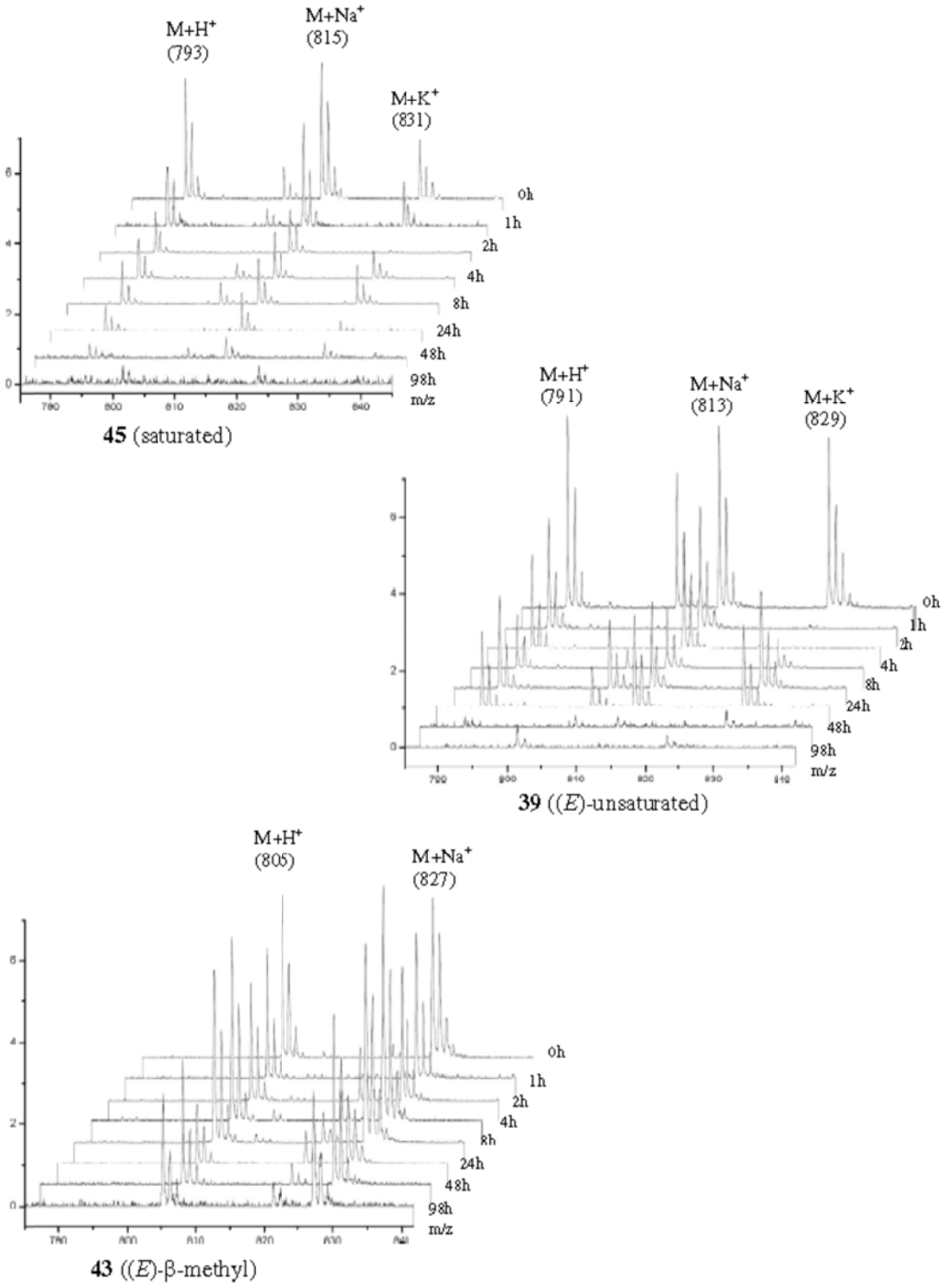


Figure 11: MALDI-TOF monitoring of human sPLA$_2$ hydrolysis profile of the reference substrate (saturated lipid 45), a good sPLA$_2$ substrate (*E*-unsaturated lipid 39) and a non-hydrolysed lipid (43, with a (*E*)-β-methyl

substituted *sn*-2 fatty acid). The signals were normalised to that of the internal standard (DMPG, m/z ($M+H^+$) = 689) to allow for comparison of the lipids signal intensities.

1.4. Molecular dynamics simulations

Previously, MD simulations have been used to study the behaviour of $sPLA_2$ and rationalize experimental observations. This was also the case for the inability of the enzyme to hydrolyze the ATRA prodrug, which prompted our study of the influence of unsaturation and substitution at the *sn*-2 position.[70] In order to better understand how well the computational method can predict and explain experimental observations, we have carried out similar MD simulations for the seven phospholipids **39-45**. The MD simulations described in the following were carried out by Dr. Vijayakumar Vinodhkumar and Assoc. Prof. Günther H. Peters, DTU Chemistry.

1.4.1. Molecular dynamics results

The overall stability of the protein structure in the simulations was verified by calculating the time evolution of the root mean square deviation (rmsd) of the amino acid Cα atoms with respect to the protein structure after minimisation. In all simulations, the rmsd reached a constant average value of ~2 Å within a few nanoseconds (<5 ns; data not shown). The rmsd data slightly varied between the different simulations performed for the same complex as well as for the different complexes. However, the variations amounted to only ±0.5 Å indicating that the protein structure is stable over the time course of the simulations.

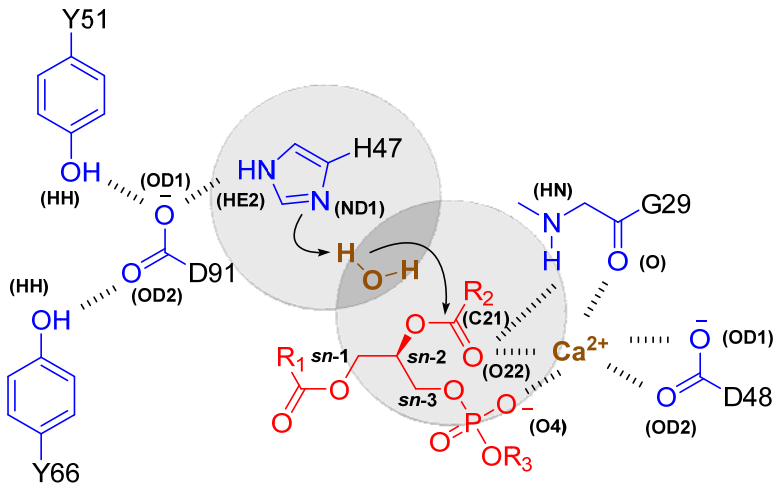


Figure 12: Representation of the active site in $sPLA_2$ with hydrogen bonds and ionic interactions indicated with dashed bonds. Key protein residues are drawn in blue, the substrate in red, the calcium ion and the water molecule both in brown. The two grey circles indicate the H–S region, where the overlap in dark grey represents the water count region. Amino acid labels and atom types indicated in parentheses refer to the Protein Data Bank nomenclature.

At least two conditions have to be fulfilled for the enzyme-catalysed reaction to occur. Firstly, a stable Michaelis-Menten complex has to form, and secondly, water molecules have to reach the active site to perform the nucleophilic attack on the substrate. Previous work has shown that both criteria can be used successfully to predict from simulation if a phospholipid analogue is a potential substrate for $sPLA_2$.[84]. In the previous study of the ATRA prodrug, two parameters were studied: the access of water to the catalytic site and the geometry of the enzyme-substrate complex. It was found from the simulations that the prodrug examined both had a distorted and thus non-productive Michaelis-Menten complex and that the relative water count was lower than for the simulations with the natural substrate (DPPG). Therefore, we apply the same strategy in the following study. We first recorded the entry of water molecules into the binding site. In particular, we were interested in those water molecules that reach the region between His 47(ND1) and the substrate carbonyl carbon S(C21) (Figure 12), since this is where a water molecule needs to be positioned for hydrolysis. The region between these two residues will hereafter be referred to as the H-S region. The relative water count at 3.5, 4.0 and 4.5 Å is plotted in Figure 13. The saturated lipid **45** is a good substrate for $sPLA_2$ (*vide supra*) and its results will be used for comparison to the other lipids. The probability of observing a water molecule at a certain distance from H-S varies slightly between the ester lipids. As can be seen from Figure 13, water molecules were able to enter H-S, and the results for ester lipids **39**–**44** were comparable to **45**. Ester lipids **39** and **43** showed a relative water count that is higher than for **45**. From this, we conclude that both ester lipids should show a higher activity towards $sPLA_2$ than **45**. Since these results are not in agreement with the experimental data (**39** is as good a substrate as **45**, while **43** is not a substrate, see Table 8), we conclude that the criterion of water accessibility to the active site is not sensitive enough to distinguish between the ester lipids. This may be expected since the structural changes between the ester lipids are local and placed close to the carbonyl carbon in the *sn*-2 position and thus unlikely to affect the flux of water molecules.

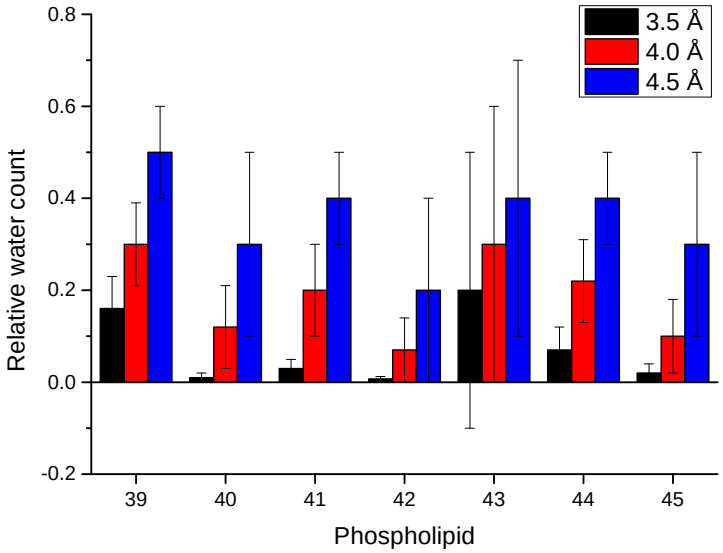


Figure 13: Relative water count extracted from the MD simulations. Before calculating the averages, the data were normalised by the water count at 6 Å, since there were significant differences in the water count within a single series of simulations. Mean and standard deviations (SD) are based on a series of independent simulations of each complex.

Since the water count data were not conclusive, we returned to the stability of the Michaelis-Menten complex and monitored the distances in the complex. We have chosen distances involving the cofactor calcium, the carbonyl group in the phospholipid analogue and selected atoms in amino acid residues that are in the vicinity of the substrate. Mean distances and standard deviations (SD) for the different phospholipids are shown in Figure 14. For simplicity, we report here only distances between calcium and S(O22), as well as the distances Asp 91(OD1)-Tyr 51(OH), His 47(HE2)-Asp 91(OD1) and His 47(ND1)-S(C21). Atom types and S refer to the Protein Data Bank nomenclature and substrate, respectively, and those are displayed in Figure 12. As shown in Figure 14, there are relatively large deviations. Standard deviations > ~0.4 are observed in all four distances for the ester lipids **41**, **42** and **43**. More importantly, most of the ester lipids cause a distortion of the His 47(ND1)-S(C21) distance (Figure 14D), where the distances ±SD for **41** and **42** lie clearly above the distance measured for **45** indicating that these lipids cannot be hydrolysed by $sPLA_2$. Similarly, for **40**, **43** and **44** the mean His 47(ND1)-S(C21) distance lay outside the variation of the distance for the saturated lipid **45**. This is more pronounced for **43** than for **40** and **44** suggesting that **43** is not a substrate, whereas **40** and **44** could be substrates but should not be hydrolysed very efficiently by $sPLA_2$. That **43** is not a substrate is further supported by the relatively large standard deviation observed for the Ca^{2+}-S(O22) distance (Figure 14A). The Michaelis-Menten complexes resulting from the MD simulations are illustrated in Figure 15 for the saturated substrate **45** (A) and the resistant lipid **43** (B).

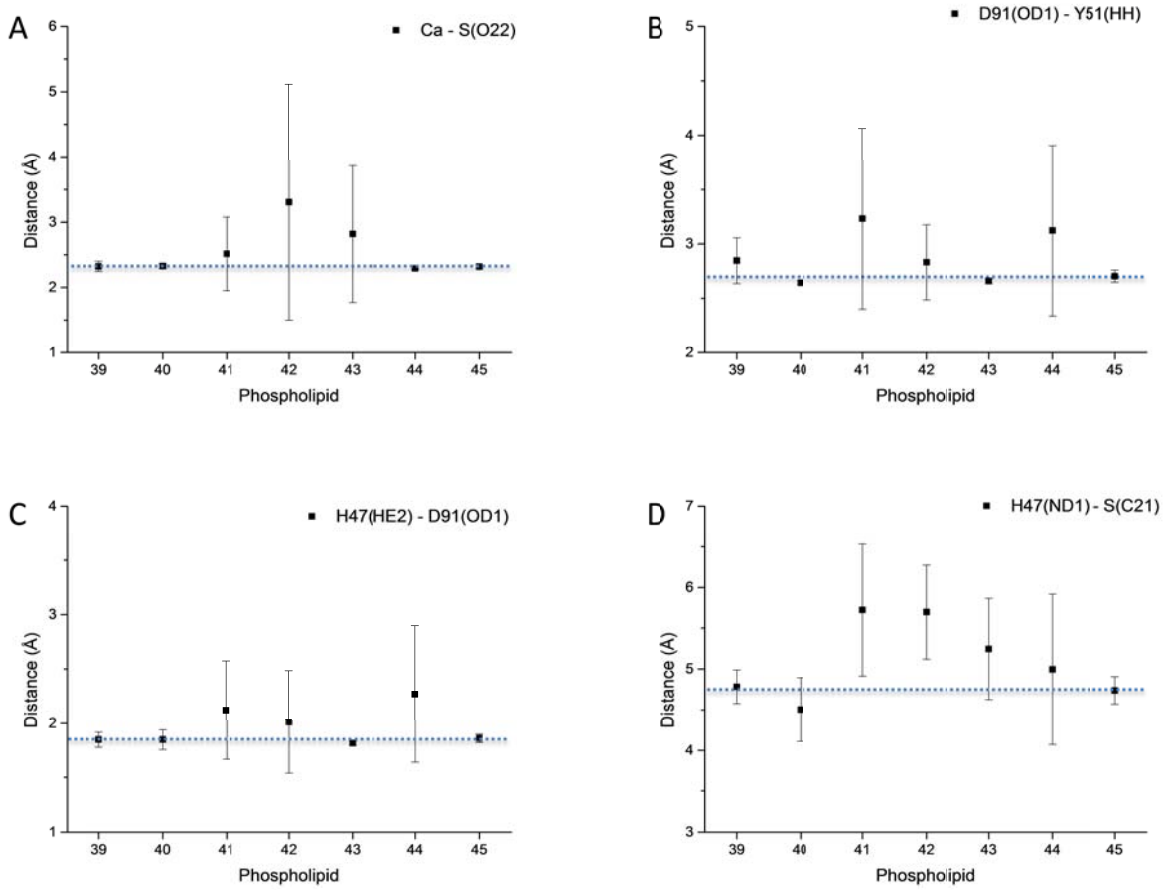


Figure 14: Average distances between atoms for: (A) Ca^{2+}–S(O22; carbonyl oxygen), (B) Aps 91(OD1)–Tyr 51(HH), (C) His 47(HE2)–Asp 91(OD1) and (D) His 47(ND1)–S(C21; carbonyl carbon). Distances were extracted from the simulations of the different $sPLA_2$-phospholipid complexes over the last 5 ns. Mean and standard deviations are based on a series of independent simulations of each complex. The dashed blue line indicates the average distance calculated from the simulations of the substrate 45.

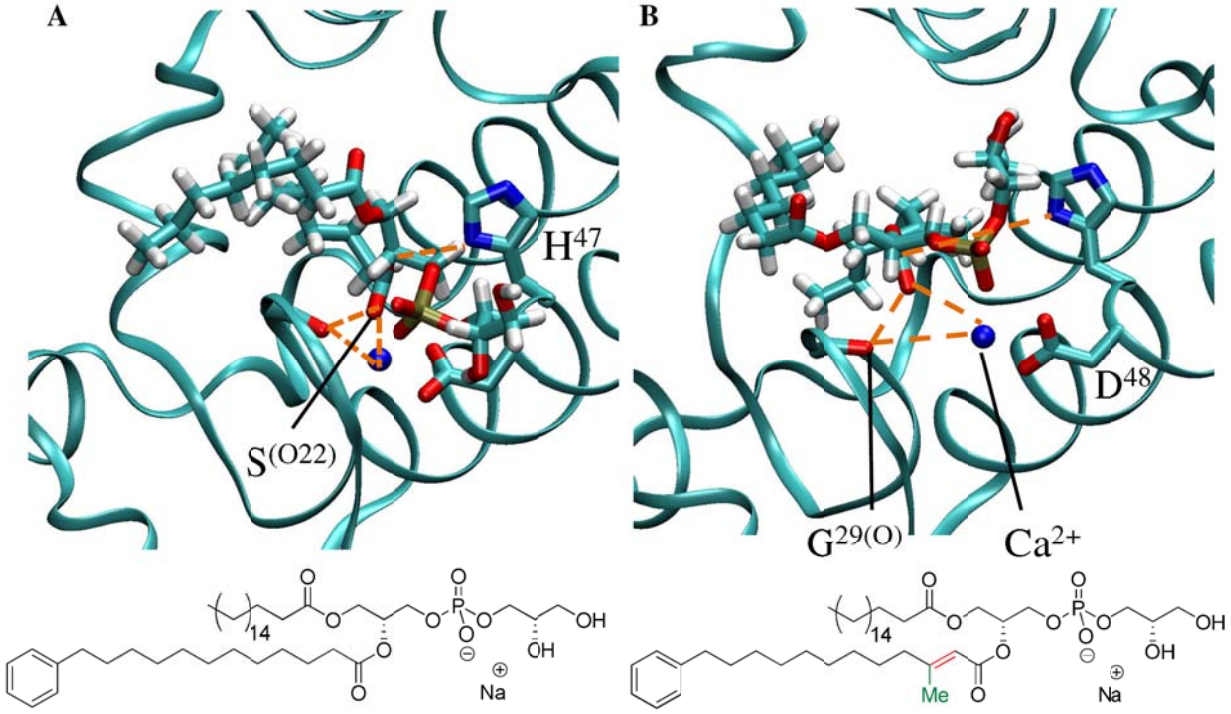


Figure 15: Geometry of phospholipid–sPLA$_2$ complexes after simulation for (A) saturated substrate 45 and (B) the non-hydrolysed lipid 43.

Our simulation results suggest that most of the ester lipids should not be hydrolysed by sPLA$_2$ due to steric hindrance in the active site that distorts the Michaelis-Menten complexes. Phospholipids **45** and **39** are predicted to be the best substrates, based on the MD analysis, which is supported by experiments and the two lipids **40** and **44**, which are hydrolysed slower than the former two, are predicted to be substrate candidates.

1.5. Conclusion

We have synthesised the complete set of *sn*-2 modified phospholipids to probe the structure-activity relationship for human secretory phospholipase A$_2$ activity and hydrolysis. The key steps in the syntheses include the use of HWE olefinations to obtain the unsaturated esters and the Mitsunobu reaction to form the glyceryl esters. Both of these transformations, as well as the hydrolysis of the intermediate methyl esters, have been investigated and optimised for the individual target phospholipids. The substrates include the lipid with a saturated acyl chain, which is similar to the natural substrates, the cis- and trans-α,β-unsaturated fatty acid esters as well as the α- and β-methyl substituted, unsaturated variants. The ability of sPLA$_2$ to hydrolyse all seven phospholipids has been investigated and the experimental results have been compared to molecular dynamics simulations of the geometry of the lipids bound in the active site of the enzyme. The experimental results are in excellent agreement with the MD simulations, which supports this approach to

evaluate putative substrates. Furthermore, we have established that while α,β-unsaturation is tolerated either very well (for *trans* geometry) or somewhat (for *cis* geometry), substitution, even by a relatively small methyl group, is not. Taken together, the results described here will help guide the development of future prodrug substrates that rely on triggered release by $sPLA_2$ in tumour tissue.

2. Synthesis of functionalized lipids for reactivity studies on liposome surfaces

2.1. Surface modified liposomes as pharmaceutical carriers

As mentioned in section 1.1, while liposomes are used as DDS, they also have potential application as imaging agent delivery systems.[86] The main early issue related to the use of liposomes which hindered their clinical applications was their short blood lifetimes, due to elimination by the cells of the reticulo-endothelial system (RES) primarily in the liver, which also resulted in a poor selectivity for the tissues of interest. However, a number of improvements have aimed at and contributed to reducing these problems. Modification of the liposomes surface by grafting biocompatible polymers such at PEG, improved the liposomes circulation longevity and reduced uptake by the RES organs. Indeed, due to their flexibility and high mobility, the PEG chains grafted to the liposomes (generally 3-7% for optimal circulation longevity) mask the surface of the liposomes, and thereby reduce nonspecific interaction with various plasma components. Therefore, the PEG-grafted liposomes, often referred to as STEALTH liposomes in the literature, have extended half-lives in the bloodstream (≥48 h in humans), and their uptake by the liver is reduced.[85] As a result of their extended blood circulation, and because of the disrupted vasculature found in certain pathological conditions, liposomes can accumulate in diseased tissues. This is the case for instance in inflamed tissues and in solid tumours, where larger gaps are found between endothelial cells to enable leukocytes and other signalling molecules to extravasate. Thus, contrary to healthy tissues with tight blood vessels, gaps between adjacent endothelial cells can be as large as 600 to 800 nm in angiogenic tissues, enabling small unilamellar vesicles as liposomes (100 nm to 200 nm diameter) to extravasate and localize in the tissue interstitial space. Moreover, the impaired lymphatic drainage, characteristic for tumours, allows accumulation of the liposomes. This passive concentration of long circulating liposomes in tumours taking advantage of diseased tissues biology is known as the enhanced permeability and retention (EPR) effect.4

If long circulating liposomes can accumulate in a passive way via the EPR effect in inflamed tissues and solid tumours, the selectivity for the diseased tissues, even in those cases, is not complete. In order to obtain better selectivity, targeted liposomes have been suggested, that display on their surfaces ligands capable of recognizing cell surface receptors and binding to the cells of interest. To that end, the most widely used targeting moieties are immunoglobulins and their fragments, but other proteins, peptides, and small molecules such as folic acid or carbohydrates and vitamins have also been used. Because of the short blood stream half-lives and low systemic exposure of the early liposomes, ligand-mediated targeting has been for a long time an elusive goal. However, after the development of the long circulating PEG-modified liposomes, ligand mediated targeting gained interest and applicability.[86,85] Ligand-targeted liposomes can be internalized into endosomes and deliver their content directly into the cell, which can result in a better drug efficiency, when they display ligands against an internalizing receptor. This is the case for the folate-modified liposomes.[86]

Because of that need for PEG-liposomes decorated with various ligands, several strategies have been devised for the development of surface-modified liposomes, these are depicted in Figure 16. Strategies consisting of introducing the ligand directly on the polar head-group of lipids later on formulated with PEG-liposomes have been described in the literature (see Figure 16A),[87] but only display poor target binding because the highly flexible PEG-chains hide the ligand and result in a steric barrier between the ligand and the targeted receptor.[85] Therefore it became more attractive to attach the ligand at the distal end of the PEG chains. Hence, a number of end group functionalized pegylated lipids have been developed. Incorporation of the ligand-bearing PEG-lipid in liposomes have been done in three different manners. In some instances, PEG-lipids with a reactive functional group at the distal end of the PEG chain were incorporated in the liposomal formulation, and then conjugated with the desired ligand to that reactive group on the formulated liposome, as in Figure 16B. This strategy is more suitable for macromolecular ligands such as immunoglobulins. However, a drawback of this strategy lies in the obtention of ligand-bearing liposomes that still display unreacted functional end groups on the inner monolayer in any case, and also in the outer monolayer if the conjugation is not complete. The unreacted functional groups can then take part in side reactions with water or drug molecules. Moreover, there is a possibility of cross-linking through several potential reactive sites of a single ligand. Another approach consists in formulating the liposomes with lipids displaying the ligand of interest at the distal end of the PEG chain and other common lipids such as PC and cholesterol, as was performed by Gabizon *et al.* in their synthesis of folate receptor-targeted liposomes (see Figure 16C).[88] This method is suited for low molecular weight ligands, e.g. peptides, oligosaccharides or vitamins. However, with this method, only a little over half (55-60%) of the ligands linked to the lipids will be on the outer layer and effective in binding to the targeted receptor, since the other half is situated on the inner layer of the liposome, and thus is entrapped in the core of the liposome. Finally, a methodology of insertion of ligand-PEG-DSPE into preformed liposomes, depicted in Figure 16D, has been developed. This is done by incubating the ligand-PEG-DSPE (1.2-1.5%) with the preformed liposomes containing 2-3% PEG-DSPE at 37 °C, and results in positioning of the PEG-tethered ligands exclusively at the outer surface of the lipid bilayer, with similar external surface densities of ligand-derived lipids incorporation as in the lipid mixing followed by extrusion process. This method has been performed with oligosaccharides and peptides conjugates successfully, and overcomes the drawbacks of the two previously mentioned methods.[85]

Various methods have also been developed for the coupling of ligands on functionalized liposomes (case B, Figure 16), employing different end-group functionalized PEG-lipids. Some of these methods are summarized in Figure 17. Click chemistry has also been exploited for that purpose, examples of such applications are presented in Figure 18 (section 2.3).

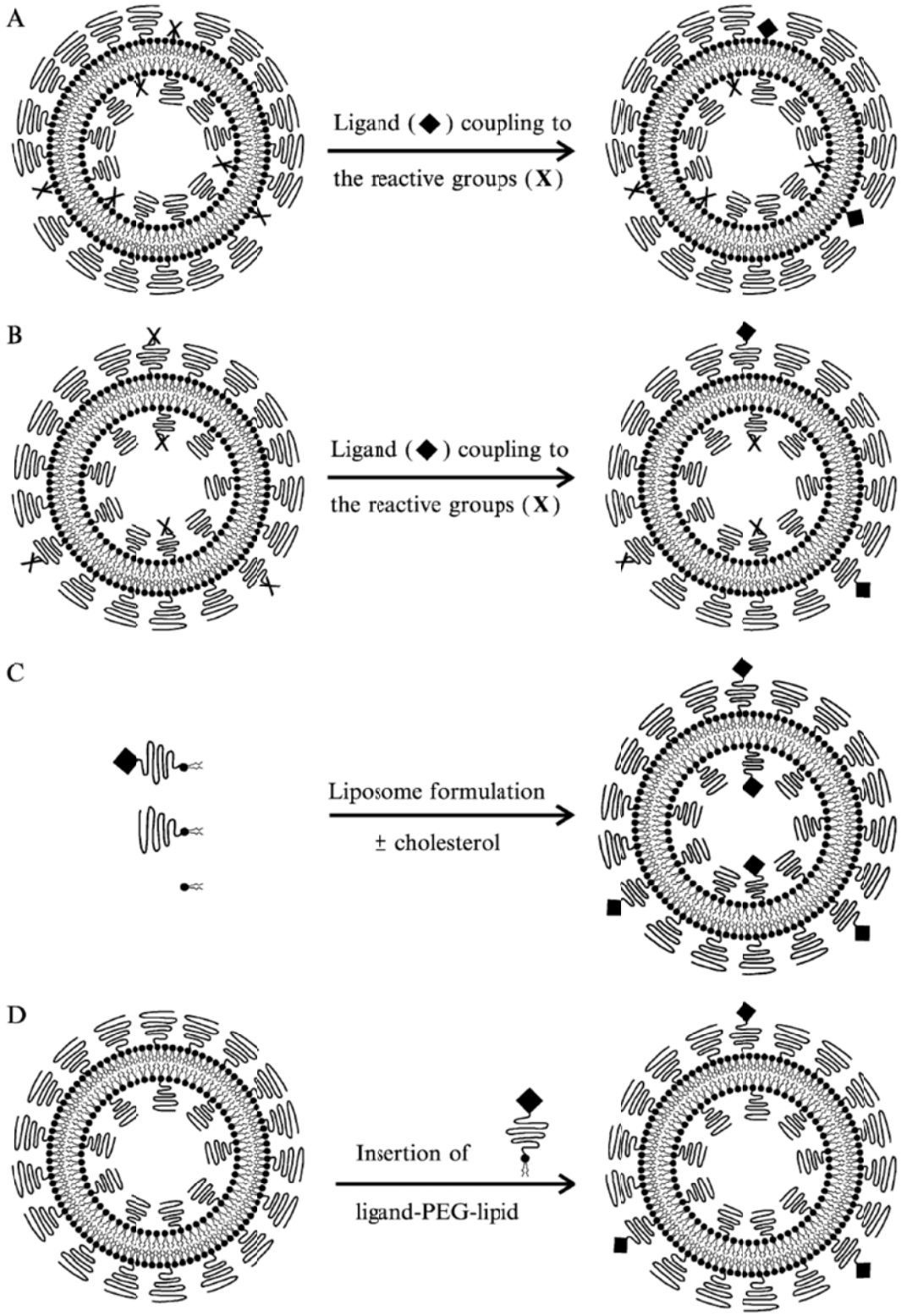


Figure 16: Strategies for the formation of ligand-bearing PEG-lipiosomes. A: ligands are conjugated with a reactive group on the lipids polar head group; B: ligands are conjugated with a reactive group on the distal end of the PEG chain grafted on the liposome; C: Liposomes are formulated with a mixture of ligand-PEG-lipid, common lipids, with or without cholesterol; D: the ligand-PEG lipid is incorporated to a preformed PEG-grafted liposome.[85]

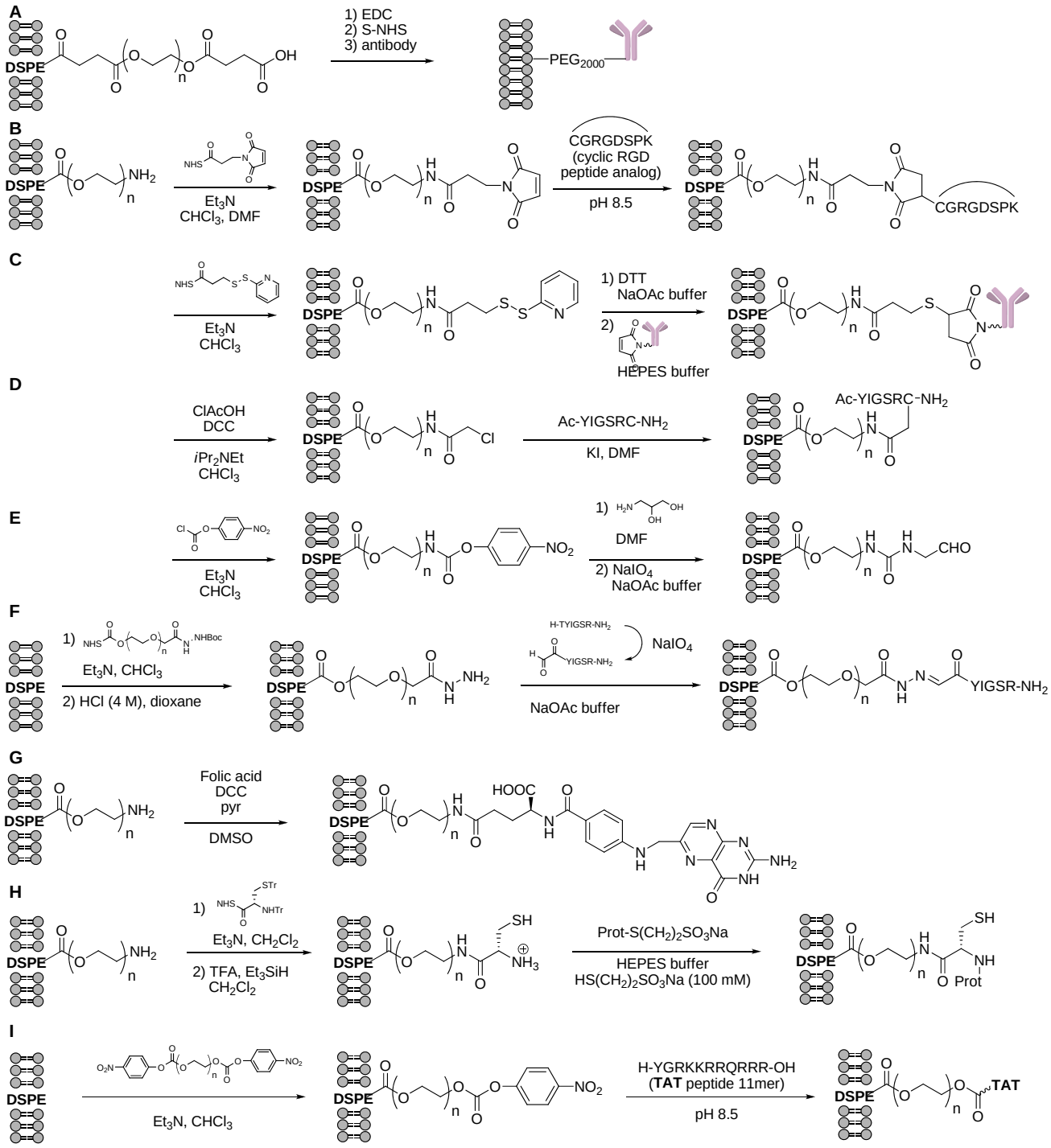


Figure 17: Overview of reported methods for the coupling of ligands on functionalized liposomes. A: direct coupling of an antibody to an activated ester at the distal end of a pegylated DPPE.[89] B: formation of a maleimide functionalized lipid from an aminoterminated PEG-DPPE followed by 1,4-addition of the cysteine thiol of a cyclic peptide.[90] C: synthesis of a pyridyldisulfide functionality that was reduced to the mercaptane and conjugated to a maleimide functionalised antibody.[91,92] D: chloroacetylation of the distal amino group followed by nucleophilic attack of a peptidic cysteine.[91] E: example of the formation of an activated carbamate, which was reacted with 3-aminopropane-1,2-diol and oxidized to afford an aldehyde-terminated lipid.[91] F: direct conjugation of an *N*-Boc protected hydrazide to DSPE, followed by deprotection and hydrazone formation to attach a peptide through an oxidized N-terminal threonine residue.[93,94] G: amidation with folic acid on an amino terminated DSPE-PEG lipid.[95] H: formation of a cysteine lipid applied for native chemical ligation of a protein thioester.[96] I: the application of a double activated PEG-carbonate to first functionalise DSPE followed by the non-specific attachment of a polycationic TAT peptide.[97,98]

Despite the number of methods reported thus far, very few (click chemistry, Staudinger ligation and native chemical ligation) are truly chemoselective, and coupling generally result in poor control of the site of reaction for instance when peptides are involved, hence the interest in investigating and developing other chemoselective (or more chemospecific) ways to couple ligands on functionalized liposomes.

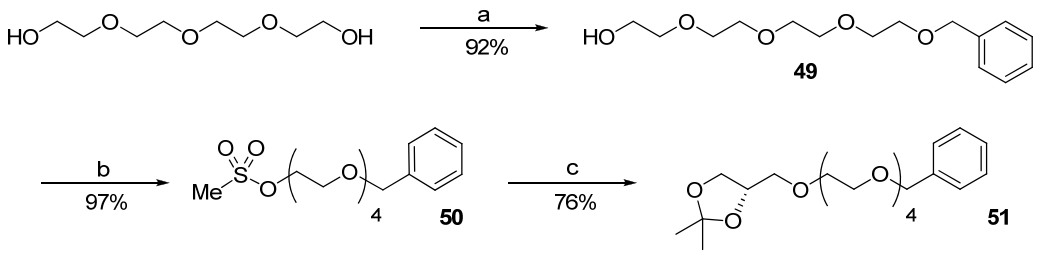
We intended to investigate the click reaction for the coupling of ligands with a functionalized PEG-grafted liposome, and developed a new, selective method for ligand coupling using progargyl amines and isothiocyanate derivatives. The syntheses of the lipids for those two separate studies were based on the same strategy. Therefore the syntheses will be discussed together in the following section. The study on the click chemistry will be developed in section 2.3, while the new conjugation method by formation of iminothiazolidines will be discussed in section 2.4.

2.2. Synthesis of the functionalised lipids of interest

As described in paragraph 1.2, enantiomerically pure glycerolipids can be obtained from 1,2-*O*-isopropylidene-*sn*-glycerol. Contrary to the previous project, we chose to work here with ether lipids, which requires new reaction conditions, but will also provide more stable target compounds. General synthetic routes to 1,2-di-*O*-alkyl-sn-glycerolipids have been well established[99] also starting from 1,2-*O*-isopropylidene-*sn*-glycerol.[100,101,102,103] We envisioned that ether bond formation would be more difficult on a 1,2-di-*O*-stearyl-sn-glycerol, where the sn-3 position can be "buried" in the aliphatic chains, and thereby hindered. Thus, we decided to install the tetraethylene glycol (TEG) at an early stage. Therefore, the commercially available TEG was protected at one end *via* monobenzylation, and activated at the other end by introduction of a methanesulfonate leaving group in excellent yield according to a procedure described by Kunze *et al.*,[103] by treatment with methanesulfonyl chloride (MsCl) and Et_3N in CH_2Cl_2 (see Scheme 11). Insertion of the benzyl-protected TEG to the *sn*-3 position of the 1,2-protected glycerol moiety also performed smoothly (see Scheme 11), after some improvement of the protocol reported by Kunze *et al*.[103] Indeed with the conditions described in the literature, **51** was isolated in 64% yield. It was attempted to add a slight excess of the mesylate, but this resulted in lower yield. On the contrary, using 1.5 equivalents of the protected glycerol resulted in an improvement, with **51** isolated in 76% yield.

The enantiomer of **51**, 1-*O*-(13-phenyl-3,6,9,12-tetraoxatridecanyl)-2,3-*O*-isopropylidene-*sn*-glycerol was reported by Kunze *et al.*,[103] with synthetic schemes and compound names being in accordance with the enantiomeric series. However, the isopropylidene glycerol reported as the starting material in the synthesis is drawn as the (*R*)-(–)-enantiomer, but reported in the experimental section to have an optical rotation of +15.0, consistent with the (*S*)-(+)-enantiomer. Consequently, the authors have in fact prepared the same series of compounds as seen in Scheme 11, but reported them as the other antipodes. The characterization data confirms this, specifically, the optical rotations of the starting protected glycerol as well of compound **51** are both in agreement with the data reported by Kunze *et al*.[103]

Scheme 11: Synthesis of 1,2-*O*-isopropylidene-3-*O*-(13-phenyl-3,6,9,12-tetraoxatridecanyl)-*sn*-glycerol (51).[a]



[a] Reagents: (a): NaH, BnBr, DMF; (b): MsCl, Et_3N, CH_2Cl_2; (c): KOH, DMSO, 1,2-*O*-isopropylidene-*sn*-glycerol.

Different methods were tested to perform the monobenzylation in good yield (see Table 9).The first method applied was to have a slight excess of the TEG (1.7 equivalents) compared to BnBr in THF, following a protocol described by Coppola *et al.*,[104] but the yields obtained varied from one reaction to another, and could get as low as 51%. It was also attempted to obtain the monobenzylation upon phase transfer catalysis, using tetrabutylammonium hydrogen sulphate (TBAHS) in a biphasic system of aqueous NaOH (1.3M) and CH_2Cl_2, as reported by Csuk *et al.*,[105] however whereas high yields were reported for the substrates in the original paper, only 20 to 25% were obtained on our system (see Table 9, entry 2). We finally decided to use a large excess of the TEG (5 equivalents) compared to the BnBr in DMF, which gave a much better result with the monobenzylated TEG **49** was obtained in 92% yield. The excess reagent was not lost, since the unreacted TEG was recovered after purification by flash chromatography (see Table 9, entry 3).

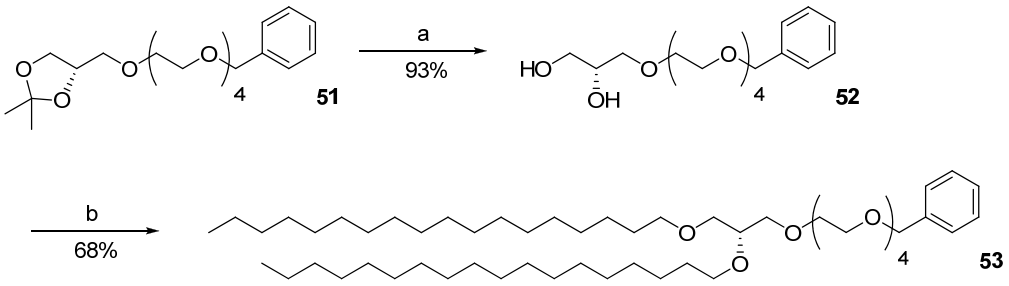
Table 9: Conditions tested for monobenzylation of TEG.

Entry	Experimental conditions	Outcome
1	1.7 equiv. TEG, 1.7 equiv. NaH, 1 equiv. BnBr, THF, 20 °C	**49**: 51% to 76% yields[a] (not consistently reproducible)
2	Phase transfer catalysis:1 equiv. TEG, aq. NaOH (1.3 M), 0.2 equiv. TBAHS, CH_2Cl_2, 40 °C, 24h	**49**: 20% to 25%[a] Only partial conversion of TEG
3	5 equiv. TEG, 5 equiv. NaH, 1 equiv. BnBr, DMF, 20 °C	**49**: 92%[a] Unreacted TEG recovered (68%)

[a] Isolated yields after flash chromatography

Once the protected TEG had been added to the *sn*-3 position, the isopropylidene was cleaved by hydrolysis, in the same way as for the synthesis of compounds **18** and **22** in excellent yield, and the octadecanyl chains were attached to the *sn*-1 and *sn*-2 positions in one step (see Scheme 12).

Scheme 12: Synthesis of 1,2-di-*O*-octadecyl-3-*O*-(13-phenyl -3,6,9,12-tetraoxatridecyl)-*sn*-glycerol 53.[a]



[a] Reagents: (a): HCl (1M in MeOH), MeOH; (b): bromooctadecane, KOH, DMSO.

Several reactions were conducted for the alkylation of the *sn*-1 and *sn*-2 positions before effective and efficient experimental conditions could be found. Williamson ether syntheses were first attempted, using bromooctadecane, NaH (2.5 equivalents) as base in different solvents and temperatures (THF at 20 °C, THF at 66 °C, DME at 85 °C and DMF at 153 °C), but only small amounts of the desired product was isolated, and instead the main product resulted from elimination of bromide (see Table 10, entries 1-4). Neither the recourse to tetrabutylammonium iodide (TBAI) catalysis, nor to other base with crown ether (KH with 18-crown-6) improved the previous results (see Table 10, entries 5 and 6). The use of a stronger base system, KOH in DMSO revealed much more effective, both with bromooctadecane and with its tosyl counterpart (see Table 10, entries 7 and 8), and reactions with both electrophiles gave comparable results. However, octadecyl *para*-toluenesulfonate **54** had to be synthesised in one step from octadecanol according to the procedure described by Yang *et al*,[106] and it was preferred to work further with the bromine derivative.

Table 10: Reaction conditions tested for the synthesis of 1,2-di-*O*-octadecyl-3-*O*-(13-phenyl-3,6,9,12-tetraoxatridecanyl)-*sn*-glycerol 53.

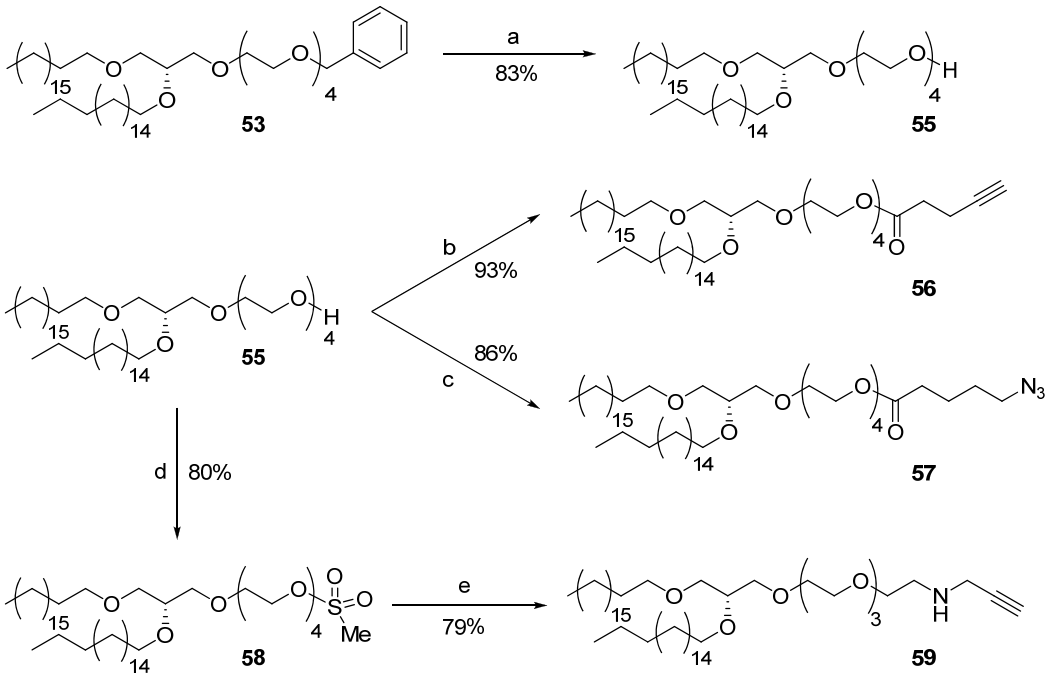
Entry	Reaction condition	Outcome
1	4 equiv. $C_{18}H_{37}Br$, 2.4 equiv. NaH, THF, 20 °C, 19h	**53**: <10% isolated Mainly $C_{16}H_{33}$-CH=CH_2 isolated [a]
2	4 equiv. $C_{18}H_{37}Br$, 2.4 equiv. NaH, THF, 66 °C, 19h	idem [a]
3	4 equiv. $C_{18}H_{37}Br$, 2.4 equiv. NaH, DME, 85 °C, 19h	idem [a]
4	4 equiv. $C_{18}H_{37}Br$, 2.4 equiv. NaH, DMF, 153 °C, 19h	idem [a]
5	3 equiv. NaH, 3 equiv. $C_{18}H_{37}Br$, 5% equiv. TBAI, THF, 0 to 20 °C, 19h	idem [a]
6	3 equiv. KH, 10 equiv. 18-crown-6, 3 equiv. $C_{18}H_{37}Br$, 5% equiv. TBAI, THF, 0 to 20 °C, 19h	idem [a]
7	3 equiv. $C_{18}H_{37}OTs$[b], 5 equiv. KOH, DMSO, 20 °C, 14h	**53**: 65%[a] $C_{16}H_{33}$-CH=CH_2 also isolated
8	4 equiv. $C_{18}H_{37}Br$, 5 equiv KOH, DMSO, 20 °C, 22h	**53**: 60 to 68%[a] $C_{16}H_{33}$-CH=CH_2 also isolated

[a]Isolated after flash chromatography; [b]synthesised from $C_{18}H_{37}OH$ according to the procedure described by Yang *et al.*[106]

With 1,2-di-*O*-octadecyl-3-*O*-(13-phenyl-3,6,9,12-tetraoxatridecanyl)-*sn*-glycerol **53** in hand, the desired functionalized lipids could be synthesised over 2 to 3 steps. The benzyl group was first cleaved by palladium catalysed hydrogenolysis to give **55**, and the resulting alcohol was either reacted with 4-pentynoic acid, EDC·HCl and DMAP to give 1,2-di-*O*-octadecyl-3-*O*-(3,6,9,12-tetraoxa-13-oxo-heptadec-16-yn-1-yl)-*sn*-glycerol **56** or with 5-azidopentanoic acid, EDC.HCl and DMAP to give 1,2-di-*O*-octadecyl-3-*O*-(17-azido-3,6,9,12-tetraoxa-13-oxo-heptadecan-1-yl)-*sn*-glycerol **57**.[107] The Keck esterification performed in very satisfying yields to afford both lipids **56** and **57** in 93% and 86%, respectively (see Scheme 13).

1,2-Di-*O*-octadecyl-3-*O*-(12-aza-3,6,9,12-tetraoxa-pentadec-14-yn-1-yl)-*sn*-glycerol **59** was obtained after mesylation of **55** followed by mono-N-alkylation of propargylamine inspired by a procedure reported by Xiong *et al.*,[108] in 63% yield over the two steps (see Scheme 13). It is to be noted that bis-alkylated progargyl amine was also isolated after flash chromatography upon longer reaction times, despite the fact that an excess propargylamine was reacted compared to the mesyl derivative, reducing the yield of the alkylation reaction down to 38% yield after 24 h reaction, instead of 79% yield when the reaction was stopped after 3.5 h.

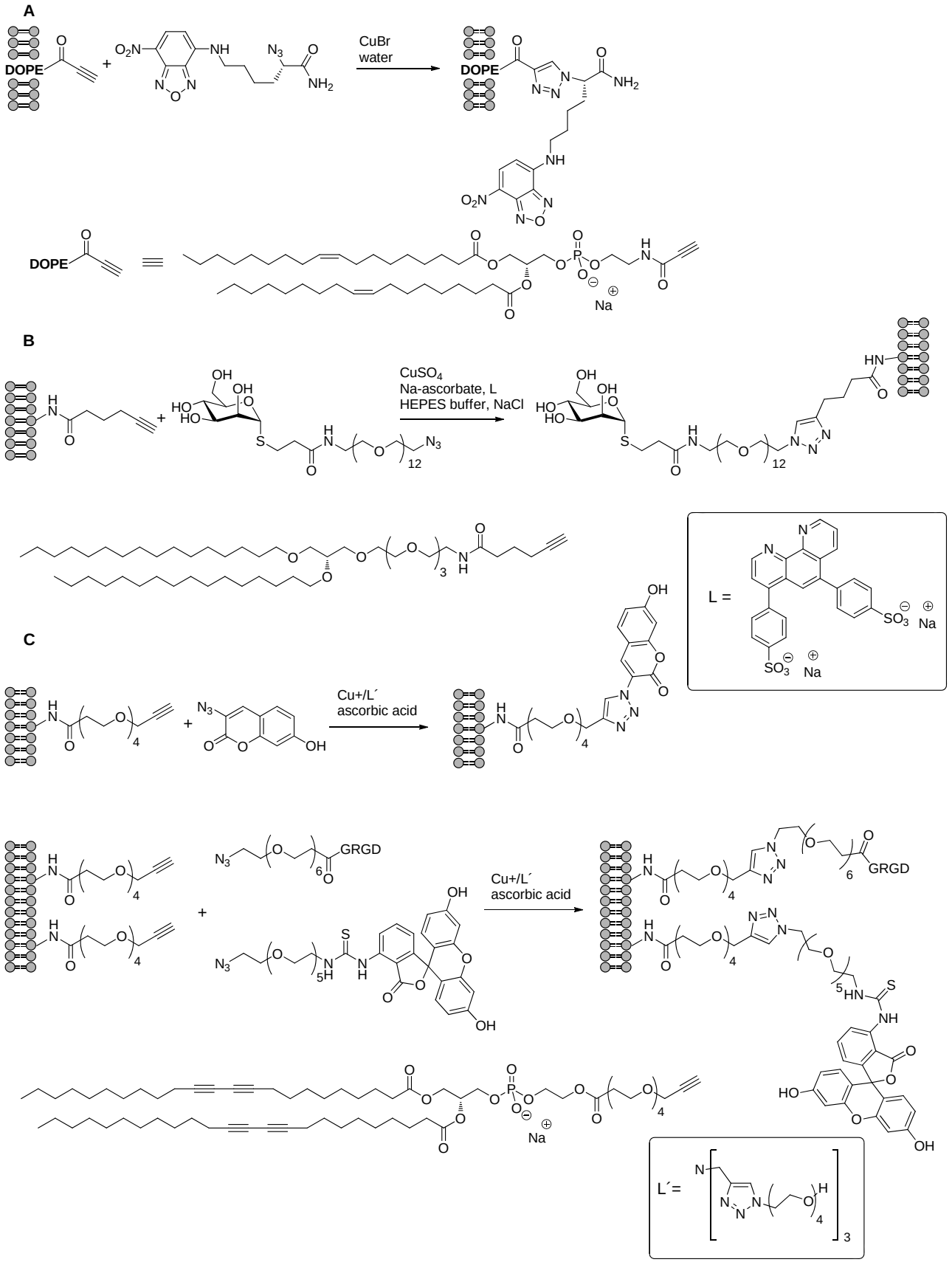
Scheme 13: Synthesis of functionalized lipids 56, 57 and 59.[a]



[a] Reagents: (a): Pd/C, H_2, EtOAc; (b): 4-pentynoic acid, EDC.HCl, DMAP, CH_2Cl_2; (c): 5-azidopentanoic acid, EDC·HCl, DMAP, CH_2Cl_2; (d): Et_3N, MsCl, CH_2Cl_2, (e): propargyl amine, Et_3N, Bu_4NI, THF.

2.3. Study of the copper catalysed click reaction on functionalized lipids

As described in section 2.1, a number of methods has been developed and used to modify liposome surfaces and attach targeting agents. However, most covalent anchoring methods used in the literature are not specific, and result in a lack of control in the formation of covalent bonds between the liposomes and the ligand of interest (generally peptides or polypeptides). On the contrary, the [3+2] cycloaddition between azides and alkynes offers several advantages in comparison with other coupling methods, especially since both the copper(I)-catalyzed reaction[109,110] and the strain-promoted cycloaddition[111] have been reported to occur in aqueous media at room temperature. Furthermore, the chemoselectivity of the reaction and the unreactive nature of both alkynes and azides towards functionalities present in biomolecules prevent unwanted side reactions, or coupling at undesired residues. Therefore, the reaction has been increasingly used as a biocompatible tool to modify biomolecules, and liposomes have been functionalised by means of the [3+2] azide-alkyne cycloaddition. Examples are presented in Figure 18, and include functionalization of conventional (e.g. DOPE) (Figure 18, A), pegylated (Figure 18, B), or engineered (Figure 18, C) liposomes with a terminal alkyne with small molecules, PEG-carbohydrate conjugates or peptides displaying an azide functionality,[112,114,115] but also reaction between two membrane-bound alkyne and azide moieties (Figure 18, D).[116] The CuAAC reaction has also been performed on free lipids and phospholipids at various positions (head-group or lipophilic chains),[113] but since our interest lies in the functionalization of formulated liposomes, this area is not further described.


A
DOPE
CuBr
water
B
CuSO4
Na-ascorbate, L
HEPES buffer, NaCl
C
Cu+/L´
ascorbic acid
GRGD
L =
L´=

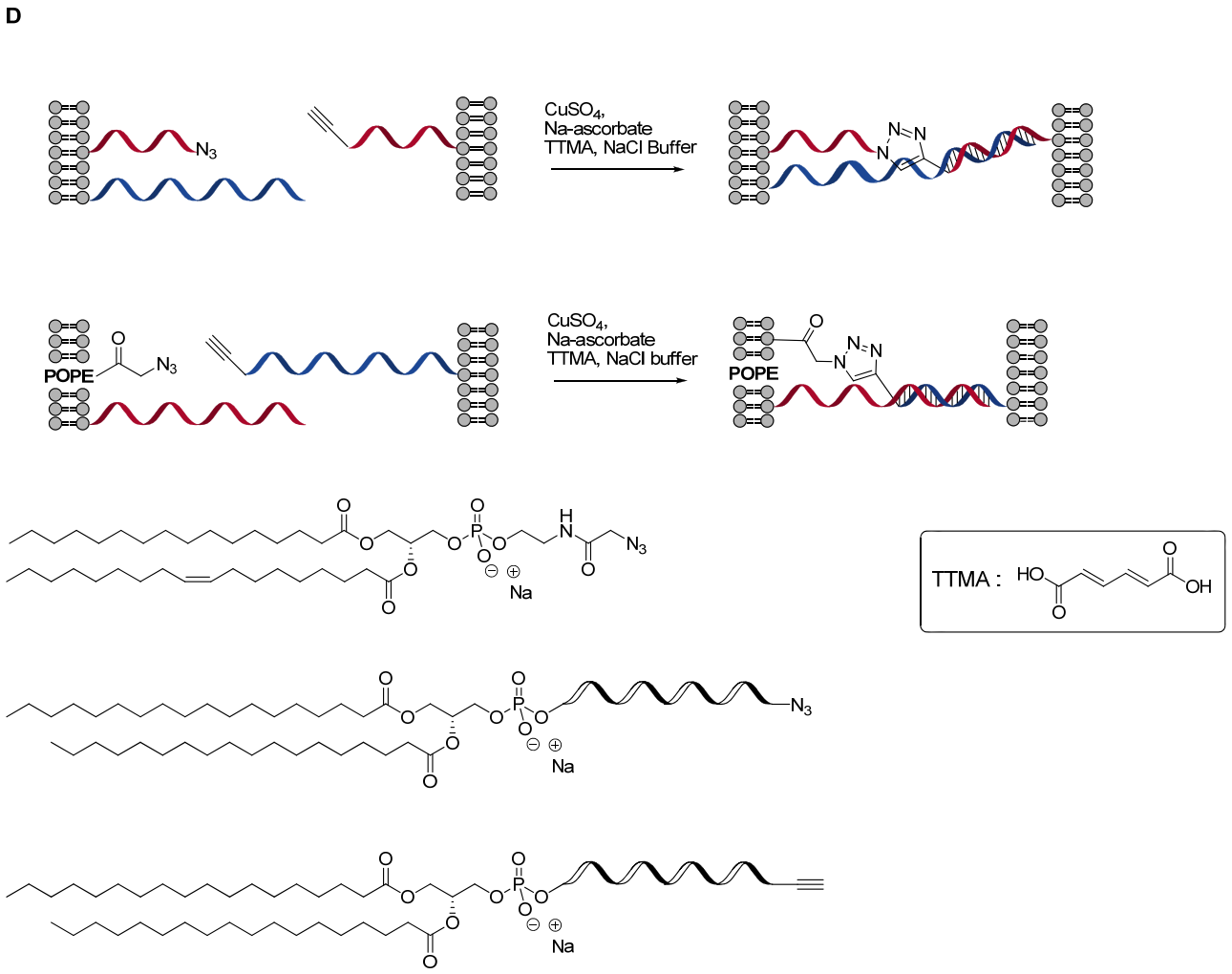
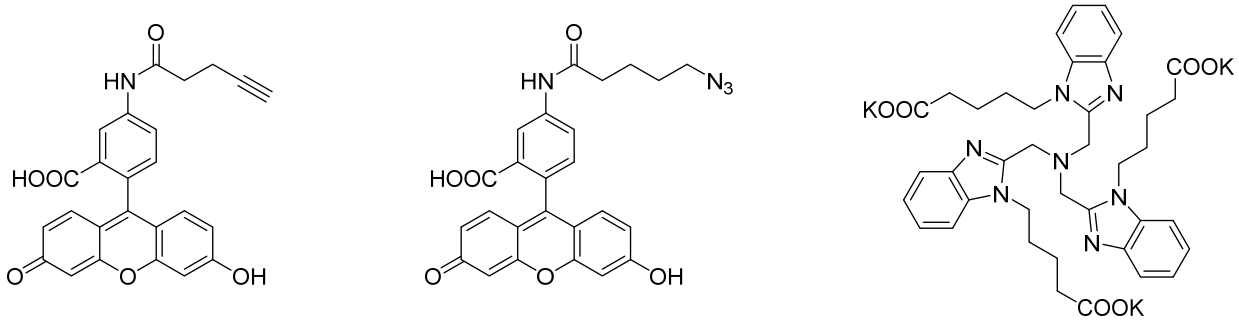


Figure 18: Reported click reactions performed on liposomes bearing surface functionalities. A: Cavalli *et al.* monitor the CuAAC reaction on liposomes using FRET.[112] B: Hassane *et al.* introduced azide-containing sugars onto alkyne-displaying liposomes *via* CuAAC.[114] C: Cai *et al.* derivatized the surface of highly stable polymerized liposomes.[115] D: Van Lengerich *et al.* observed DNA-mediated vesicle interactions between vesicles tethered to a supported lipid bilayer via CuAAC.[116]

Despite those examples of liposomes functionalization by CuAAC, general optimization of the reaction on liposomes has not been investigated for these substrates, and in particular, the importance of the positioning of the two reactive functionalities has not been studied. Given that most conjugation reactions take place between a surface functionality (i.e. a modified lipid incorporated in a liposome) and a coupling partner (small molecule, peptide, etc.) in solution in the presence of a soluble catalyst, we found it prudent to investigate, whether the reaction is equally efficient with surface alkynes and soluble azides and with an inverted orientation of the functional groups. Therefore, we formulated liposomes containing the two functional lipids **56** and **57**, presenting an alkyne and an azide, respectively, and investigated their reaction with the fluorescein conjugated azide and alkyne (Figure 19) as the coupling partners.



Fluorescein-alkyne *Fluorescein-azide* *Ligand*

Figure 19: The coupling partners for the functionalised liposomes and the water soluble ligand added in some of the experiments. The fluorescein-alkyne conjugate is known[117] and the azido containing derivative was synthesised from fluoresceinamine and 5-azidopentanoic acid.

The functionalized lipid **56** or **57** (5%) was mixed with POPC (95%) and formulated as liposomes by extrusion in PBS buffer (c = 25 mM) using the dry lipid film technique, yielding milky solutions. The functionalized liposomes (c = 12.5 mM) were incubated with the corresponding coupling partner (the fluorescein-azide with **56** and the fluorescein-alkyne with **57**) (0.20 mM; 0.65 equiv.), sodium ascorbate (0.56 mM) and copper sulfate (0.10 mM; 0.33 equiv.) in the absence or presence of a ligand for Cu (0.20 mM, 0.66 equiv.). Samples were taken for HPLC analysis after 0 (before addition of copper sulfate), 120, 480 and 1440 minutes. The time-course profile of the reaction can be seen in Figure 20. As can be seen, there was very little conversion in the coupling between the fluorescein-alkyne and the azide functionalised liposomes. When the reaction partners were reversed, approximately 50% of the fluorescein-azide was converted within the first two hours, but then the reaction stalled.

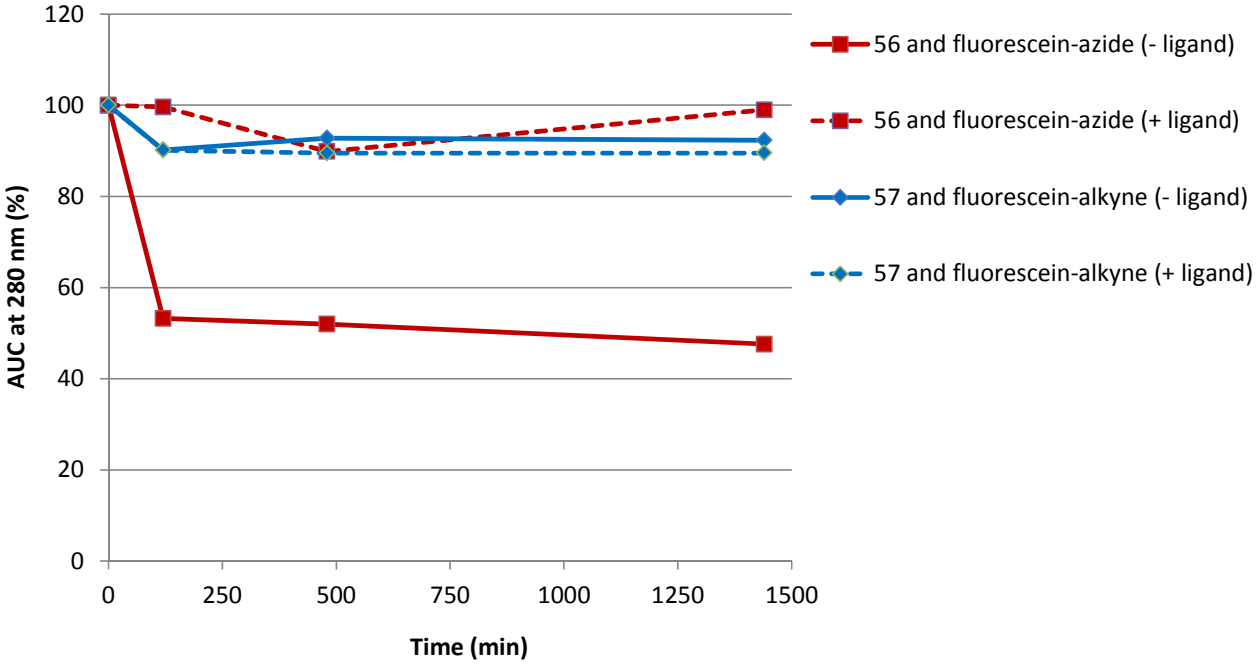


Figure 20: Time-course of the conjugation reactions. AUC for the fluorescein-alkyne (red) and the fluorescein-azide (blue) when incubated with liposomes consisting of POPC and 56 or 57, respectively, from HPLC data at t=0, 120, 480 and 1440 min.

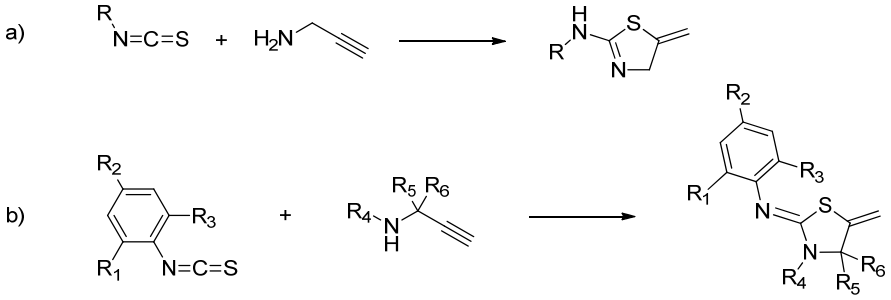
When liposomes consisting of only POPC were subjected to the reaction conditions, no conversion of the coupling partners were observed and the same was the case in the absence of Cu (data not shown). As mentioned previously, we have observed earlier that the CuAAC reaction with liposome coupling partners is significantly more sluggish when the alkyne is in solution and the liposomes contain the azide functionality, compared to the reverse situation, which is confirmed by the observations made here. It has previously been reported that the CuAAC reaction of liposomal substrates perform better in the presence of ligands for copper[114,115] but when the reactions were performed in the presence of the water-soluble Cu-ligand shown in Figure 19[118] (two equiv. relative to copper), both combinations of coupling partners resulted in very poor conversion in 24 h (see Figure 20). It is also noteworthy that the very popular CuAAC reaction does not afford full conversion of the fluorescein-azide when reacted with the alkyne functionalised liposomes, despite the fact that the azide is the limiting reagent. Rather remarkably, the reaction appears to stall after about two hours and this phenomenon warrants further investigation.

2.4. Study on the reaction of propargyl amines with isothiocyanates.

Synthesis of 2-amino-4-methylene-thiazolines upon extended reaction of propargylamines with isothiocyanates at reflux in benzene was first reported by Eloy and Deryckere in 1973.[119] A decade before, Easton *et al.* had also reported the isolation of similar compounds, in their attempt to

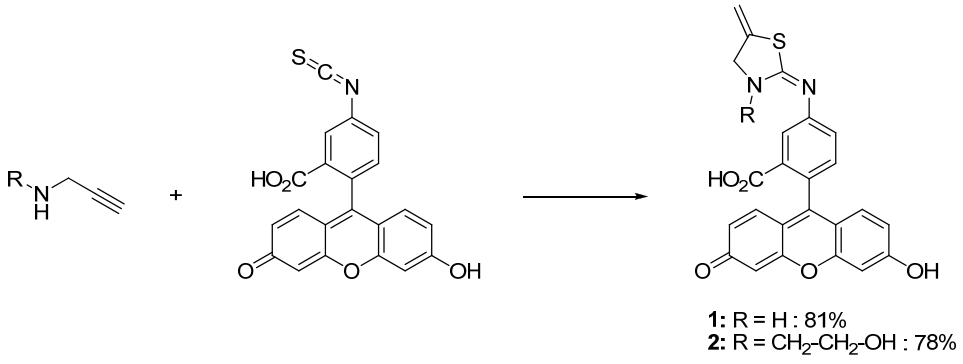
synthesise thioureas by reaction of secondary propargylamines with isothiocyanate.[120] A later study on the synthesis of iminothiazolidines also reported the synthesis of the heterocycles upon reaction of propargyl amines with aryl isothiocyanates at reflux in dioxane.[121]

Scheme 14: Reaction of isothiocyanate with propargyl amines: a) aminothiazolines and b) iminothiazolidines have been reported.[119,121]



Earlier work carried out in the group showed that the above reactions, though only reported to occur under rather harsh conditions (several hours under reflux in dioxane[121] or under reduced pressure above 80 °C[120]), could in fact be performed under very mild and biocompatible conditions, and a series of experiments had been carried out to investigate the applicability of the reaction under biocompatible conditions.[122] The reaction with fluorescein isothiocyanate (FITC) and either primary or secondary propargyl amine (2-(prop-2-yn-1-ylamino)-ethanol) have been performed in a mixture of water and *tert*-butanol at 20 °C, to afford the desired thiazolidines in 81% and 78% yields respectively (see Scheme 15).[122]

Scheme 15: Reaction of primary and secondary propargyl amines with fluorescein isothioscyanate.[a] [122]

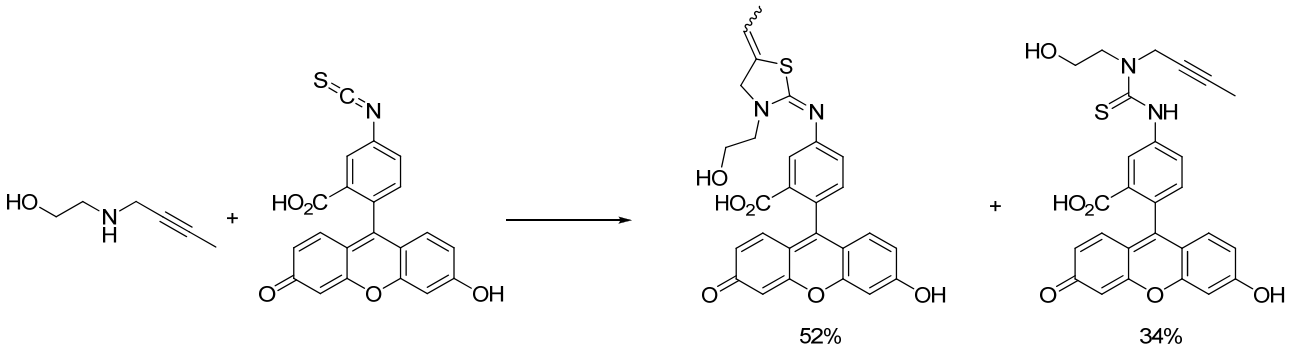


[a] Conditions: **1**: H_2O/^{t}BuOH, 20 °C, 6 h; **2**: H_2O/^{t}BuOH, 20 °C, 2 h.

The reaction was described to compete with formation of thiourea when internal propargyl amines are used, and the desired thiazolidine was isolated in 52% yield along with 34% of the thiourea,[122] (see Scheme 16). However, we identified a stepwise mechanism, in which the thiourea is an intermediate, and prolonged reaction times showed full conversion to the desired thiazolidine,

which was isolated in 82 % yield. The reaction scope is therefore extended to any propargyl amine, knowing that longer reaction times are necessary for internal propargyl amines.

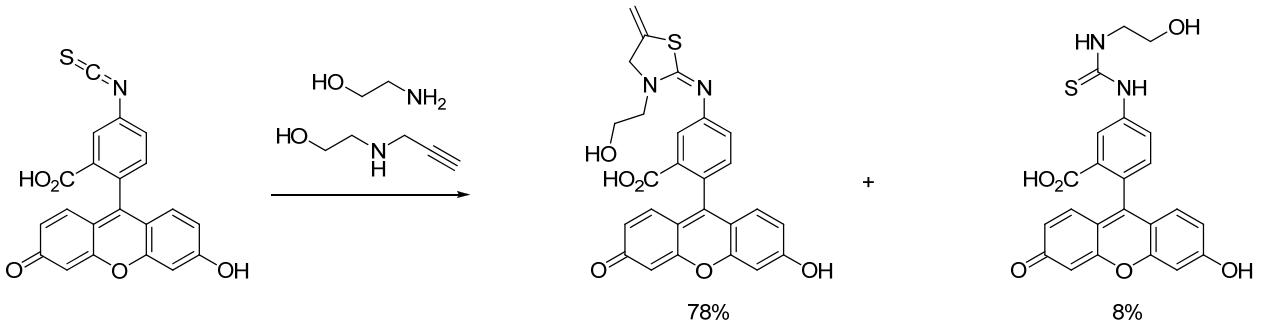
Scheme 16: Reaction of internal propargyl amine with fluorescein isothiocyanate after 4h.[a] [122]



[a] Conditions: $H_2O/^tBuOH$, 20 °C, 4 h.

Finally, an experiment was performed to investigate the competition of a primary amine and a terminal propargyl amine in their reaction with FITC. It was observed that the propargyl amine reacted faster than the primary amine, to afford the desired thiazolidine along with the thiourea resulting from attack of the primary amine on FITC in a 10:1 mixture (respectively isolated in 78% and 8% yield after flash chromatography, see Scheme 17).[122] Thus, the reaction is selective towards propargyl amines, which attests the relevance of the cyclization reaction in biological systems, since it is feasible to imagine using the reaction in the presence of other amino functionalities.

Scheme 17: Competition reaction of a primary amine and a terminal propargyl amine with fluorescein isothiocyanate. [a] [122]



[a] Conditions: $H_2O/^tBuOH$, 20 °C, 2 h.

The perspective of a new selective and biocompatible reaction provided by these interesting data prompted us to investigate the reaction on biological systems and possibly develop a new tool for functionalization of macromolecules in aqueous media.

2.4.1. Investigation of conjugation of fluorescein isothiocyanate with functionalised liposomes

Functionalized liposomes bearing a terminal propargyl amine were chosen as test substrates, since these would validate if the reaction was useful for macromolecular constructs in water. We first wanted to investigate the conjugation reaction of liposomes containing lipid **59** (see section 2.2, Scheme 13) displaying a terminal propargyl amine with FITC, following and quantifying the reaction by fluorescence spectroscopy, the principle is outlined in Figure 21.

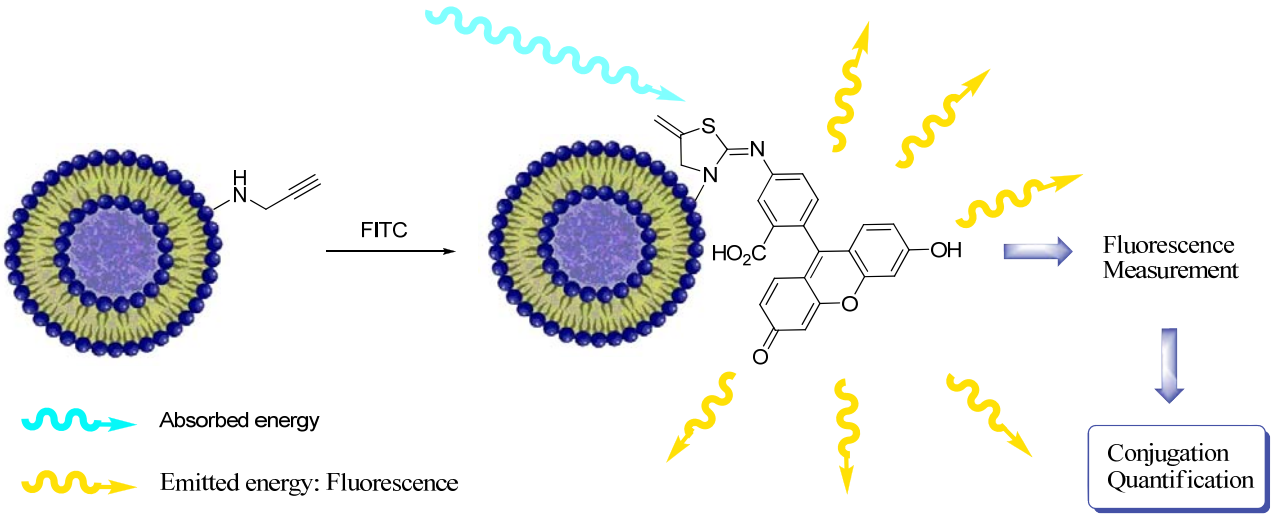


Figure 21: Principle of conjugation quantification by fluorescence measurement; Liposomes containing lipid 59 could be reacted with FITC, resulting in fluorescent liposomes. Measurement of the resulting fluorescent quantifies the conjugation.

However, preliminary experiments on fluorescence measurement and purification of free FITC indicated issues with regard to quantification of the conjugation reaction. Indeed, in order to quantify the conjugation in the way described above, unreacted FITC has to be removed from the liposomal solution, without altering the liposomes, which is not straightforward.

Two devices were investigated for the elimination of free FITC based on size exclusion: dialysis using Slide-A-Lyzer® dialysis cassettes (10.000 MW cut off), which is a well established method for dialysis of small volumes and Amicon® Ultra-15 centrifugal filter devices (100.000 MW cut off), which has the advantage of the time efficiency (liposomal solutions have been cleaned in minutes to hours, while days are needed with the dialysis cassettes). Therefore, liposomomes were formulated with 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine (DSPC) and 1% fluorescent lipid 1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-(carboxyfluorescein) in phosphate buffered saline (PBS) solution at 65 °C, and fluorescein was attempted removed from a solution containing those liposomes (see structures in Figure 22).

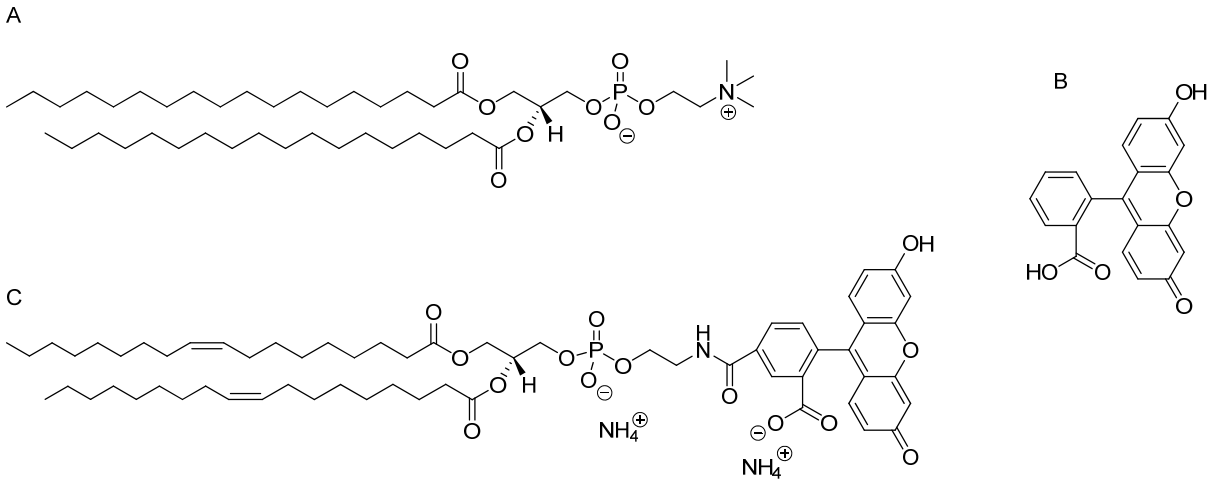


Figure 22: Compounds used for the preliminary experiments. A: 1,2-Distearoyl-*sn*-glycero-3-phosphatidylcholine (DSPC); B: Fluorescein; C: 1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-(carboxyfluorescein).

During optimization of the centrifugation conditions using the centrifugal filter devices, we observed a considerable overall loss of fluorescence (*i.e.* the fluorescence measured in the filtrate and in the solution remaining in the device did not correspond to the initial fluorescence in the mother solution). The same outcome was observed in an additional centrifugation experiment with a solution containing only the fluorescent liposomes, indicating that the liposomes were most probably partly trapped in the filter of the device during centrifugation. For this reason, we discarded the initially attractive idea of a fast removal of FITC by centrifugation.

The later observation prompted us to adjust the strategy for quantification of the conjugation reaction: Realising the difficulty to recover all the liposomes also from dialysis cassettes, either because some of the liposomes could also be trapped in the membrane of the dialysis cassettes, or because some liposomes might be lost when the dialysed solution is removed from the cassette, we decided to integrate a fluorescent phospholipid as an internal standard to the liposomes (see Figure 23).

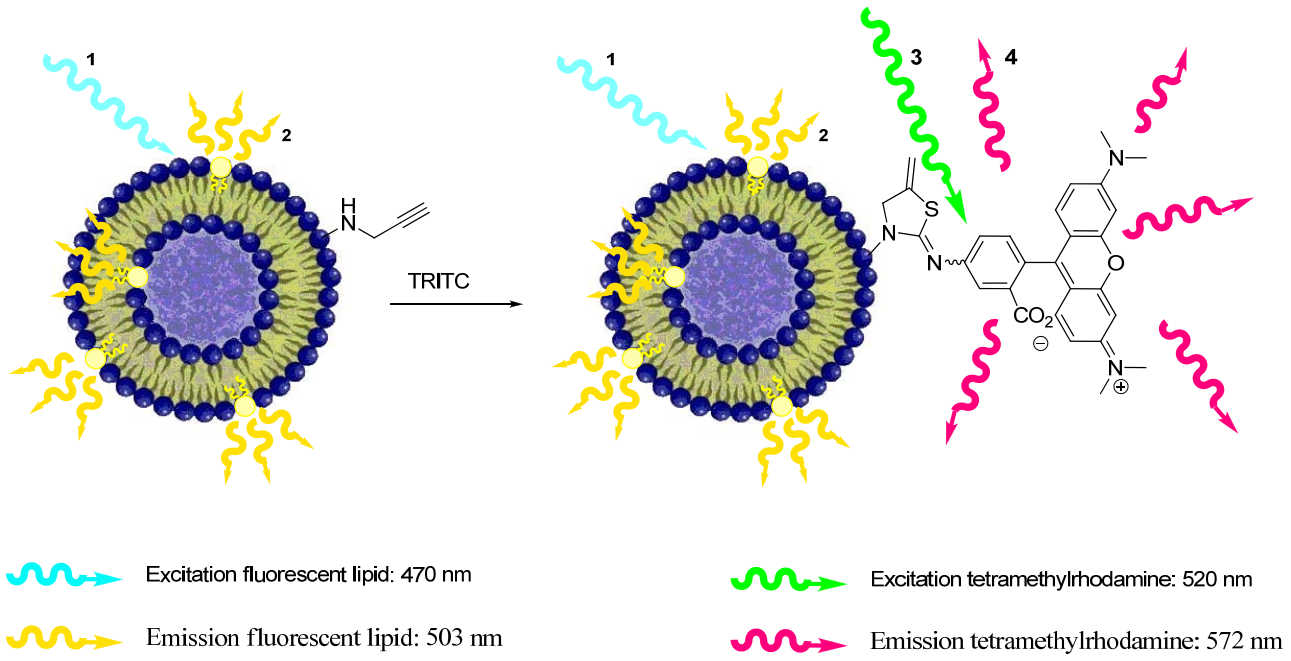


Figure 23: Conjugation quantification using an internal fluorescent standard. 1: The internal fluorophore contained in the lipid is excited with a wave length of 470 nm. 2: The internal fluorophore contained in the lipid emits fluorescence with a maximum at a wave length of 503 nm. 3: After reaction with the propargyl amine functionalities, the tetramethylrhodamine fluorophore is covalently linked to the liposomes, and can be excited at a wavelength of 520 nm. 4:.After excitation, the tetramethylrhodamine fluorophore emits fluorescence with a maximum at a wavelength of 572 nm.

This allows us to measure the fluorescence due to the conjugation product and compare it to the intrinsic fluorescence of the lipid, using the ratio to quantify the progress of the conjugation reaction. However, in order to do so, the fluorescent lipid and the isothiocyanate-fluorophore reacting with the propargyl amine should have distinct excitation and emission spectra, and especially, the emission of one of the fluorophore should not overlap with the excitation spectrum of the second.

Therefore, we chose to work with a lipid displaying 4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene, for which the excitation maximum is at 495 nm and the emission maximum is at 503 nm, and tetramethylrhodamine ITC, for which the excitation maximum is at 544 nm and the excitation maximum is at 572 nm. The structures and emission spectra can be seen in Figure 24 B to E.

Thus, liposomes containing 0.5% Bodipy® 495/503 lipid (Figure 24B) and 1% lipid **59** (Figure 24A) with DSPC were formulated in PBS buffer at 65 °C, and liposomes containing only 0.5% of the Bodipy® lipid, but no lipid **59** were formulated in PBS buffer at 65 °C for control. As for the precedent project, the lipids were first formulated as multilamellar vesicles but this time in PBS buffer, by the dry lipid film technique:[123] the phospholipids were solubilised in $CHCl_3$ and the solvent was evaporated *in vacuo* to form a dry lipid thin film. PBS buffer was added in order to obtain 2 mM suspensions of the lipids, and the lipids were solubilised upon heating the mixture to

60 °C with periodic vortexing. This resulted in a milky suspension of multilamellar vesicles that was extruded through a 100 nm polycarbonate filter at 65 °C in order to obtain the desired monolamellar liposomes as a transparent solution.

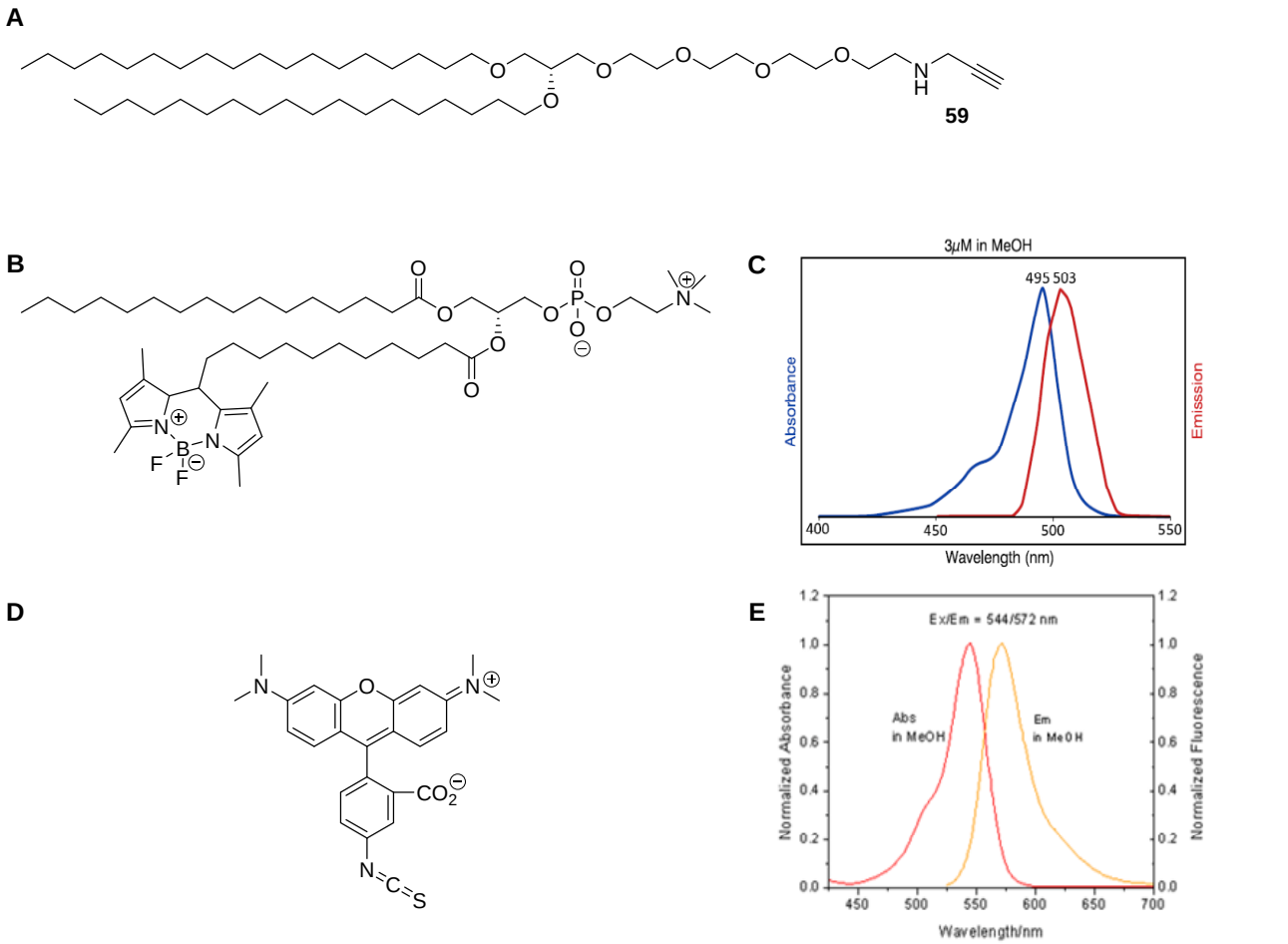


Figure 24: Synthesised functionalised lipid 59 (A) and fluorescent dyes used for conjugation quantification. B: Bodipy® lipid 495/503. C: Excitation and emission spectra of Bodipy® lipid 495/503. D: Tetramethylrhodamine isothiocyanate. E: Excitation and emission spectra of tetramethylrhodamine isothiocyanate.

Three sets of experiments were conducted to study the conjugation reaction on functionalised liposomes: In the first one, one equivalent (compared to the propargyl amine) of TRITC in MeOH (0.003M) was reacted with the liposomal solution containing 1% of **59** and 0.5 % of Bodipy® lipid, in the second one, ten equivalents (compared to the propargyl amine) of TRITC in MeOH (0.003M) was reacted with the liposomal solution containing 1% of **59** and 0.5 % of Bodipy® lipid, and in the third one, the same amounts as for ten equivalents of TRITC in MeOH (0.003M) was reacted with the liposomal solution containing only 0.5 % of Bodipy® lipid, but no propargyl amine lipid **59** as a control for passive diffusion of the tetramethylrhodamine dye into the liposomal membrane. Sampling was done after 2 h, 8 h, 24 h, 48 h and 96 h, and the resulting solutions were purified by

dialysis over 8 days, changing the buffer every 24 h. Fluorescence measurements are shown in Figure 25.

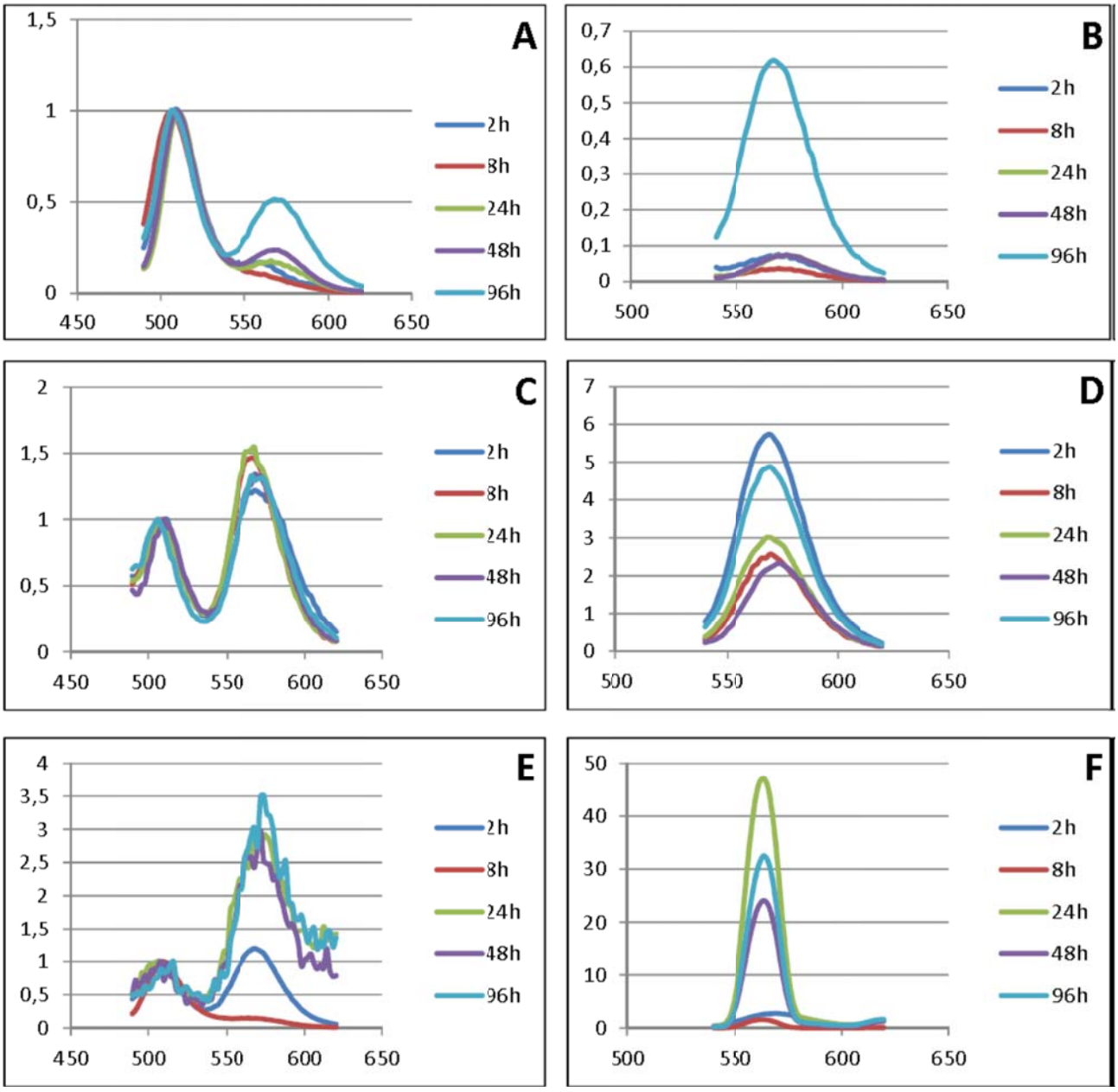


Figure 25: Fluorescence measurements performed on the dialysed samples. A: Control reaction, performed with DSPC liposomes containing 0.5% Bodipy® lipid and TRITC, excitation at 470 nm. B: Control reaction, performed with DSPC liposomes containing 0.5% Bodipy® lipid and TRITC, excitation at 520 nm. C: Test reaction, performed with DSPC liposomes containing 0.5% Bodipy® lipid, 1% 59 and 1 equiv. TRITC, excitation at 470 nm. D: Test reaction, performed with DSPC liposomes containing 0.5% Bodipy® lipid, 1% 59 and 1 equiv. TRITC, excitation at 520 nm. E: Test reaction, performed with DSPC liposomes containing 0.5% Bodipy® lipid, 1% 59 and 10 equiv. TRITC , excitation at 470 nm. F: Test reaction, performed with DSPC liposomes containing 0.5% Bodipy® lipid 1% 59 and 10 equiv. TRITC, excitation at 520 nm. Measurements have been normalised to the reference fluorescence read at 503 nm.

Figure 25 A and B shows the fluorescence spectrum of the control reaction with 20 equivalents TRITC compared to Bodipy® lipid (conducted on liposomes containing 0.5 % Bodipy® lipid only) with excitations at 470 nm and 520 nm respectively. From Figure 25 A, we can see fluorescence of the Bodipy® lipid, after 2 h, 8 h and 24 h, the maximum fluorescence at 503 nm is characteristic for the Bodipy lipid only, attesting that the TRITC was effectively cleared up by dialysis. However, a second emission at 572 nm increasing with time can be observed, which indicates that TRITC has been incorporated in some extent to the liposomes free of propargyl amine, probably by diffusion through the liposome membranes. This is further assessed by Figure 25 B, where excitation at 520 nm also results in fluorescence at 572 nm increasing with time and especially powerful after 96 h. However, for reaction times below 24 h, diffusion of TRITC through the liposome membranes does not seem to be an issue. The fact that fluorescence characteristic for TRICT is observed in Figure 25 A, where the sample was excited with a wavelength of 470 nm, also indicates that fluorescence resonance energy transfer (FRET) occurs. FRET is the mechanism through which energy is transfered between two chromophores in close proximity. A donor chromophore is excited at an initial wavelength, it is thus in its electronic excited state, and emits some of the absorbed energy by fluorescence, but can also transfer part of its energy to an acceptor fluorophore through non-radiative dipole coupling. The acceptor fluorophore then enters its electronic excited state, loses some of the energy through internal conversion, and emits fluorescence at a higher wavelength (see Figure 26). The energy transfered from the donor to the acceptor fluorophore is inversely proportional to the sixth power of the distance, and thus only fluorophores in very close proximity can interact through FRET.

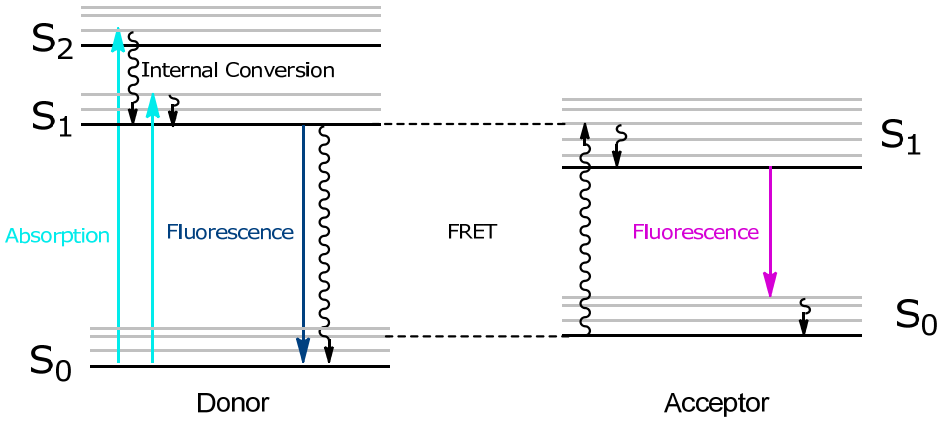


Figure 26: Jablonski diagram showing fluorescence resonance energy transfer (FRET).

Figure 25 C and D show the fluorescence resulting from excitation at 470 nm and 520 nm respectively of the samples from the test reaction (performed with one equivalent of TRITC compared to the propargyl amine in the liposomal solution containing 1% of **59** and 0.5 % of Bodipy® lipid). Figure 25 E and F show the fluorescence spectrum of the test reaction with 10 equivalents TRITC (conducted on liposomes containing 0.5 % Bodipy® lipid and 1% lipid **59**) with

excitations at 470 nm and 520 nm respectively. From graphs D and F and by comparison with graphs B and E, it is clear that the rhodamine derivative has been attached to the functionalised liposomes (containing the propargyl amine functionalised lipid **59**) already with a single equivalent of TRITC (graph D), but to a larger extent when ten equivalents of TRITC are added (stronger fluorescence in graph F). Indeed, the fluorescence observed at 572 nm after excitation at 520 nm is characteristic for tetramethylrhodamine derivatives, and the fluorescence observed in Figure 25 D and F is significantly stronger than in Figure 25 B, which indicates that the passive diffusion of TRITC into the liposomes (observed in Figure 25B) does not account for all the fluorescence observed in Figure 25 D. However, Figure 25 C also shows that FRET occurs, which unfortunately limits the possibility for quantification of the reaction.

Moreover, DLS analysis of the samples showed an increase in the size of the liposomes especially after 48 h and 96 h (see Table 11). This was thought to be due to liposome aggregation, possibly aggravated by the presence of TRITC.

Table 11: DLS analysis of the lipids formulated with DSPC and 1% Bodipy (control) or DSPC, 1% Bodipy and 0.5% 59 after reaction with TRITC.

Liposome size control by DLS		
Sample	Diameter (nm)	PdI[a]
Control		
2 h	254,2	0,316
8 h	214,3	0,366
24 h	383,2	0,390
48 h	743,6	0,344
96 h	1103,5	0,355
Reaction with 1 equiv. TRITC		
2 h	238,1	0,317
8 h	248,9	0,370
24 h	349	0,399
48 h	7,2,2	0,392
96 h	1659,8	0,236
Reaction with 10 equiv. TRITC		
2 h	256,8	0,366
8 h	273,1	0,345
24 h	503,2	0,409
48 h	962,9	0,401
96 h	1521,7	0,345

[a]PdI = polydispersity index

In order to assess whether TRITC enhances liposome aggregation, test reactions have been performed, mixing different concentrations of TRITC (0%, 1%, 5% and 10%) with liposomes formulated with 100% DSPC in PBS, and measuring the liposome diameter after 2 h, 4 h, 24 h and

96 h, but no increase in liposome diameter was observed under those conditions. Thus, the aggregation of the liposomes seems to be mainly due to inhomogeneity in the liposome formulation.

These results, even though the method shows limitations, were very encouraging, since the reaction described above seems to occur readily at 20 °C in PBS buffer.

In order to circumvent the issues faced in the last set of experiments, we chose to perform the next set of experiement with POPC instead of DSPC. Since the conjuagation experiments are carried out at 20 °C, which is well above the T_m for POPC, it was expected that aggregation could be circumvented by this change in the major liposome component. Moreover, the liposomal solutions would be analysed by HPLC instead of fluorimetry, which would also bypass the quantification difficulties introduced by the undesired FRET phenomenon. Another advantage of following the reaction by HPLC is that introduction of the Bodipy ® lipid is no longer necessary, thus liposomes can be formulated with only POPC and lipid **59**, increasing the homogeneity of the liposomes and hopefully their stability.

The propargyl amine functionalized lipid **59** (1%) was mixed with POPC (99%) and formulated as liposomes by extrusion in PBS buffer using the dry lipid film technique, yielding clear solutions. The particle size of the formulated lipids was measured by DLS. The DLS analysis revealed that all of the lipids were able to form particles with a diameter between 120 and 180 nm and with a low polydispersity (mean: 145.2 nm, polydispersity: 0.245), indicating the formation of unilamellar vesicles. The functionalized liposomes (c = 12.5 mM) were incubated with FITC (31.25 μM; 0.5 equiv.) and samples were taken for HPLC analysis after 5, 30, 60, 120, 240 and 1440 minutes. The analysis showed that after 5 min, more than 50% of the FITC (retention time (Rt): 6 min) had been converted to a major (Rt=18.6 min) and a minor (Rt=17 min) product. After 4 h, all the FITC had been converted to the two products and after 24 h, only the fast eluting (Rt 18 min) product was detected, see Figure 27. We performed the reaction between the propargyl amine functionalised lipid **59** and FITC in solution, which enabled us to identify the faster eluting compound (Rt=17 min) as the desired product. HPLC-ESI-MS showed that both peaks had the same mass, 1198.8, which leads us to assign the peak at Rt=18.6 min as the intermediate thiourea.

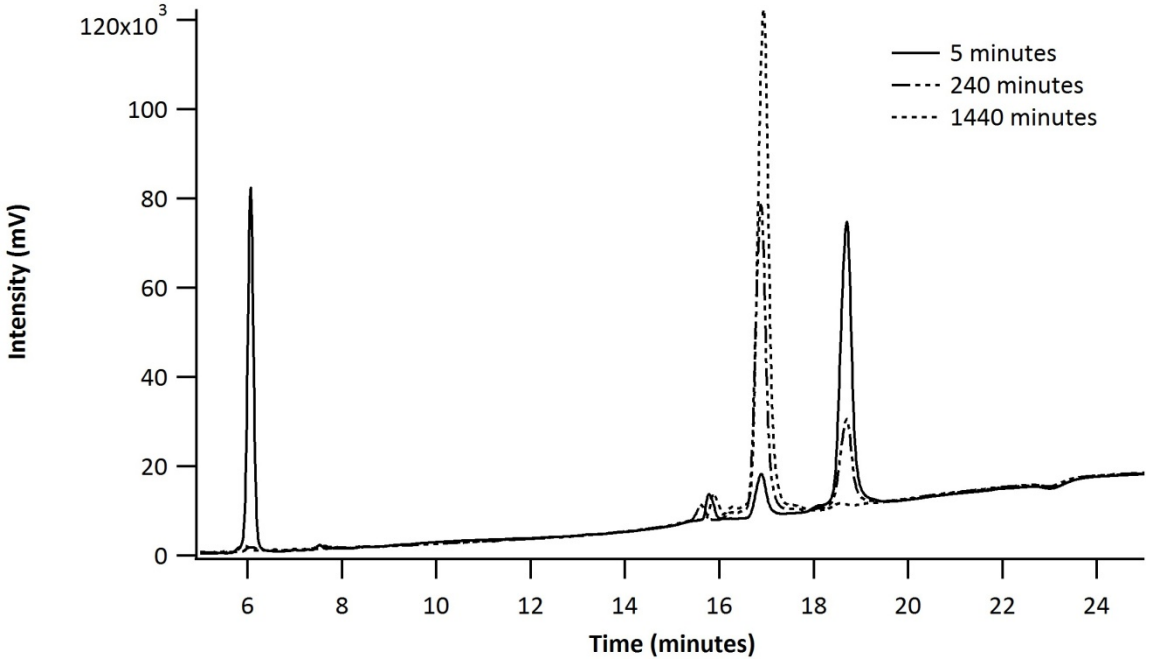


Figure 27: Example of the monitoring of a conjugation reaction by HPLC over time at 254 nm. The decreasing peak with RT=6 min belongs to the free fluorescein isothiocyanate (FITC), while the peaks with RT=17 min and RT=18.6 min are the 2-imino-4-methylenethiazolidine and the intermediate thiourea, respectively.

The time-course profile of the reaction can be seen in Figure 28. Already after 5 min, more than 50% of the FITC has been converted to a mixture of the thiourea and the thiazolidine. After 2 h, the FITC is fully converted and after 4 h, the thiazolidine is the major product. After 24 h, only the product can be detected, demonstrating that the reaction has gone to completion. The integrity of the liposomes after 24 h incubation with FITC were verified by DLS and showed no significant change in the average size or polydispersity of the particles (diameter mean: 147.6 nm, polydispersity: 0.231).

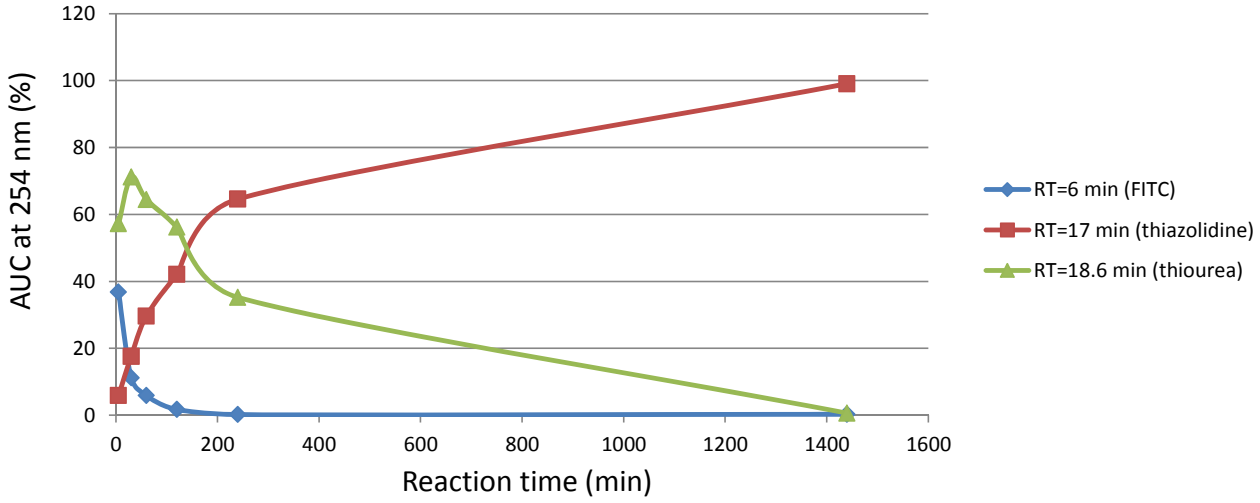


Figure 28: Time-course of the conjugation reaction. AUC for FITC (blue), the intermediate thiourea (green) and the desired 2-imino-4-methylenethiazolidine (red), from HPLC data at t=5, 30, 60, 120, 240 and 1440 min.

2.5. Conclusion

We have devised a short and efficient synthesis of three functionalised lipids, **56**, **57** and **59**, starting from the commercially available 1,2-*O*-isopropylidene-sn-glycerol. The design is based on diether lipids to confer stability and a short PEG chain in the *sn*-3 position to serve as the headgroup and can be viewed as a model for lipids useful for formulation of stealth liposomes with distal functionality (Figure 16 B).

Lipids **56** and **57** enabled us to investigate the importance of positioning of the two functional groups involved in the copper catalysed azide-alkyne click reaction on either the surface of nanoparticles or in solution. We have previously found that the reaction performs best when liposomes display the alkyne coupling partner rather than the azide and the synthesis of **56** and **57** for the first time allowed us to confirm this while comparing structurally similar lipids. The studies supported the hypothesis and interestingly also indicated that the presence of a ligand for copper was detrimental to the click reaction on liposome.

Lipid **59** was used to benchmark a new conjugation reaction in aqueous media, the spontaneous reaction between an isothiocyanate and a secondary propargyl amine. Extensive experimentation went into identifying the best method to follow the reaction on the surface of liposomes and it was gratifying to observe that the reaction occurred cleanly to afford the desired product in high purity within 24 h, when monitored by HPLC.

The results described here will be useful for future research in methodology for functionalising particle surfaces. We have chosen to study liposomes, but the conclusions reached about the click reaction and the novel conjugation method developed should be applicable to other nanoparticle systems. It is also noteworthy that our new conjugation reaction between propargyl amines and

isothiocyanates is superior to the widely used CuAAC “click” reaction, when compared directly on similar systems. The novel conjugation reaction does not require a catalyst and goes to completion in less than 24 hours, where the azide-alkyne coupling is significantly slower and even with 5% functionalised lipid on the liposome surface and applying 50% copper, the reaction stalled at around 55% conversion.

3. Synthesis of 2-mono(10,16-dihydroxyhexadecanoyl)glycerol for investigation of cutin biosynthesis in plants

3.1. Introduction

Land plants are protected from desiccation and pathogens by the presence of a hydrophobic cuticle on their aerial epidermis. This cuticle consists of a polyester of hydroxy fatty acids, called cutin, surrounded by a variety of waxes.[124] While the generic composition of the cutin polymer is known, the mechanism and site of cutin polymerization have remained longstanding question. Our collaborators had identified a candidate protein CD1 (and its coding gene) responsible for the formation of the cutin polymer, along with an expected substrate for the enzyme and precursor for the cutin polymer and they desired to test the activity of the enzyme on the putative substrate 2-mono(10,16-dihydroxyhexadecanoyl)glycerol. However, the scarce availability and low purity of the compound in plants extracts prompted us to pursue a synthetic route to the compound.

3.2. Synthesis of the proposed substrate for CD1

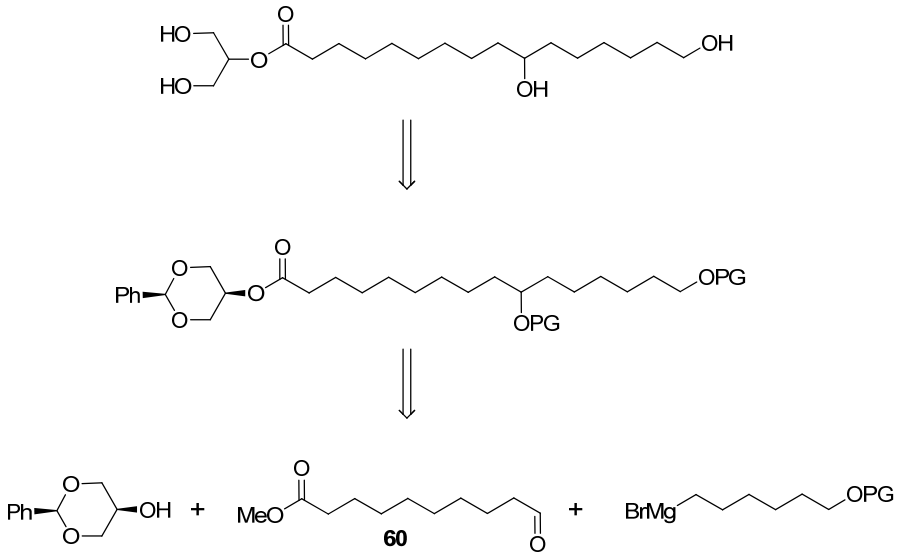
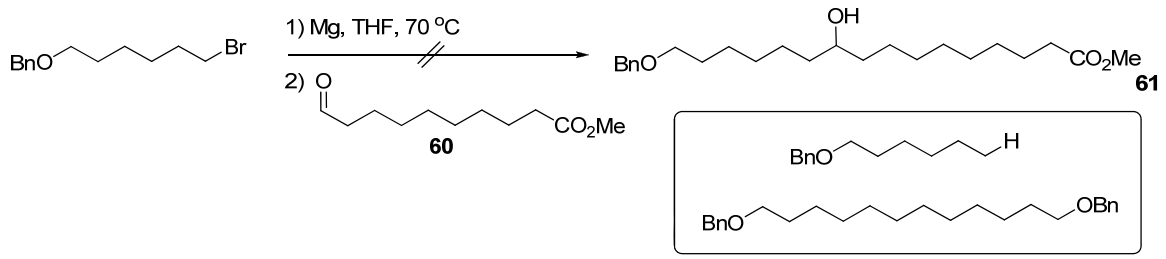


Figure 29: Retrosynthetic analysis for 2-mono(10,16-dihydroxyhexadecanoyl)glycerol.

2-Mono(10,16-dihydroxyhexadecanoyl)glycerol was first attempted synthesised by the route shown in Figure 29. Methyl 10-oxodecanoate **60** was synthesised by Swern oxidation[43,44] from commercially available methyl 10-hydroxydecanoate.

Scheme 18: Attempted synthesis of methyl 16-(benzyloxy)-10-hydroxyhexadecanoate 61 by a Grignard reaction.

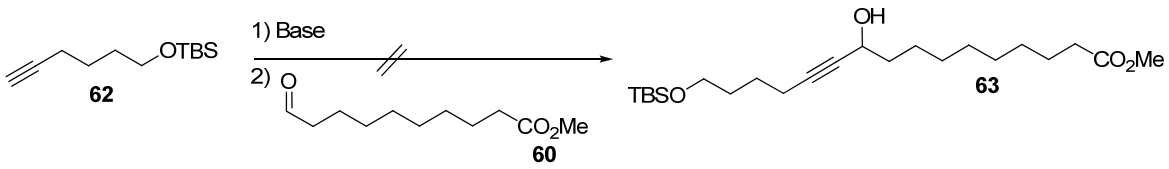


Several attempts have been carried out in order to synthesise 16-(benzyloxy)-10-hydroxyhexadecanoate **61**. The classical Grignard reaction[55] was attempted by first preparing (6-(benzyloxy)hexyl)magnesium bromide. Formation of the organomagnesium compound was titrated by 1M HNO_3 and phenolphthalein, and the Grignard reagent was calculated to be formed in about 90% yield. However, following addition of a solution of the aldehyde **60** in anhydrous THF, after 4 h the Grignard reagent had mainly been converted into its dimer and only 1,12-bis(benzyloxy)dodecane and benzyl-hexyl-ether were isolated (see Scheme 18), even though the reaction was performed under argon, which has been reported to prevent the coupling products arising from a Wurtz-type reaction (Equation 3.1).[125] Barbier conditions[126] were also tested, but the same outcome was observed as with the Grignard reaction, and the desired product was not isolated.

$$2\ RX + Mg \longrightarrow R\text{-}R + MgX_2 \quad (3.1)$$

The strategy was then slightly changed, and we thought an alkyne could be easily deprotonated to attack the electrophilic aldehyde **60**, the resulting ynol could then be hydrogenated in a later step. Therefore (6-*tert*-butyldimethylsilyloxy)-hex-1-yne **62** was synthesised in one step from hexyn-6-ol.

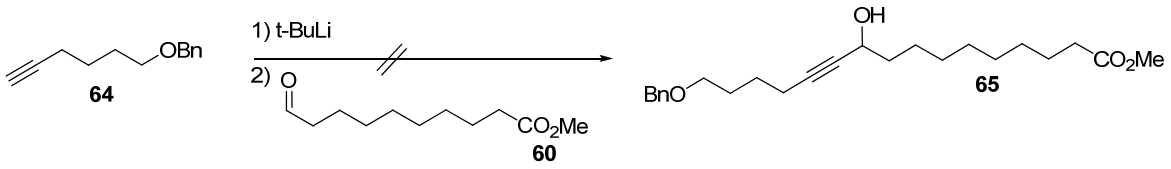
Scheme 19: Attempted synthesis of methyl 16-(*tert*-butyldimethylsilyloxy)-10-hydroxyhexadec-11-ynoate 63.



Several attempts have again been carried out for the synthesis of ynol **63**, but none of them proved successful. The first base used in this reaction was LHMDS, but with reaction temperatures ranging from – 78 °C to 20 °C, no conversion of **60** could be observed. Test reactions were then performed with ^{n}BuLi, ^{t}BuLi and LDA. In those cases, inseparable mixtures of by-products were obtained. Although TBS groups are typically compatible with strong bases under anhydrous conditions, , we speculated that some of the observed by-products might arise from partial deprotection of the TBS

group. Therefore, similar reactions were attempted using the *O*-benzyl-protected alkyne **64**, synthesised in 1 step from hexyn-6-ol (see Scheme 20), however, the desired product **65** could not be isolated.

Scheme 20: Attempted synthesis of methyl 16-(benzyloxy)-10-hydroxyhexadec-11-ynoate 65

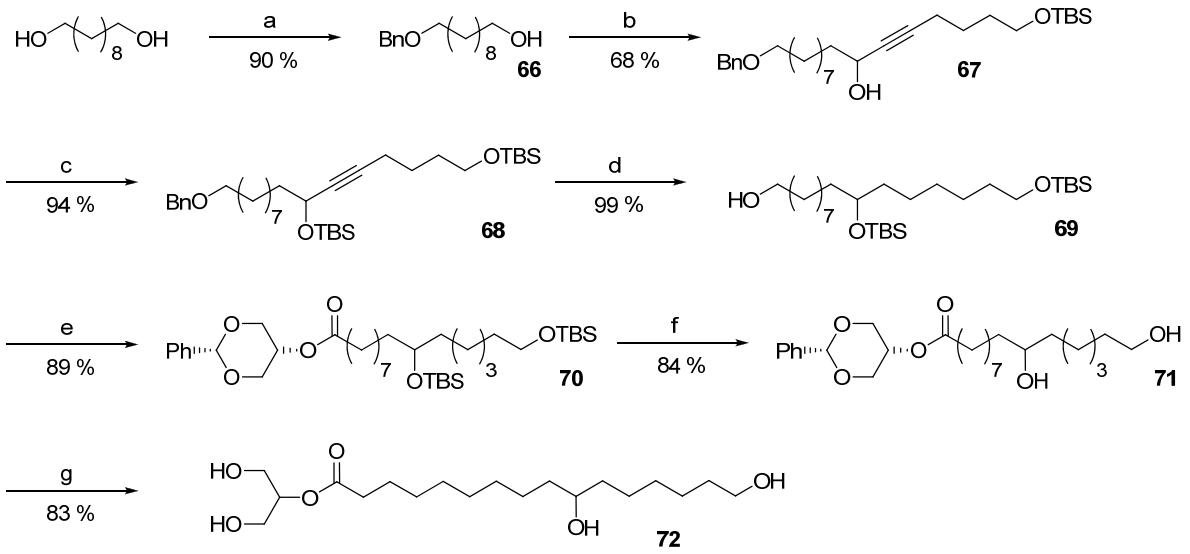


It was observed that for the main by-products that could be purified to some extent, no carbonyl signal could be seen in ^{13}C NMR. Thus, we concluded that the problem observed in those reactions may arise from the ester functionality present in **60**, and we decided to change the strategy and use a simpler aldehyde.

The new synthetic route to 2-mono-(10,16-dihydroxyhexadecanoyl)glycerol is shown in Scheme 21. Starting with decan-1,10-diol, one hydroxyl was protected by monobenzylation in excellent yield, inspired from the previous project, and the other hydroxyl was oxidized *via* Swern oxidation.[43,44] The resulting aldehyde was reacted with (6-*tert*-butyldimethylsilyloxy)-hex-1-yne **62** and ^{t}BuLi in THF. It was extremely gratifying to see that the reaction we could not perform successfully with methyl 10-oxodecanoate **60**, now not only afforded the desired ynol **67** but also proceeded in a good yield (68%).

The resulting secondary alcohol was TBS-protected with TBSCl and imidazole in DMF in excellent yield to give **68**, and the benzyl protecting group was removed by palladium-catalysed hydrogenolysis with concomitant reduction of the alkyne in quantitative yield. The resulting alcohol was first oxidized to the carboxylic acid by use of $PhI(OAc)_2$ and TEMPO in CH_2Cl_2 and H_2O, and the glycerol moiety was only then introduced on the fatty acid in its 1,3-benzylidene-protected form, by Keck coupling of the fatty acid with *cis*-5-hydroxy-2-phenyl-1,3-dioxane, EDC·HCl and DMAP in CH_2Cl_2 to afford the fully protected dihydroxy acid ester **70** in 89% yield.

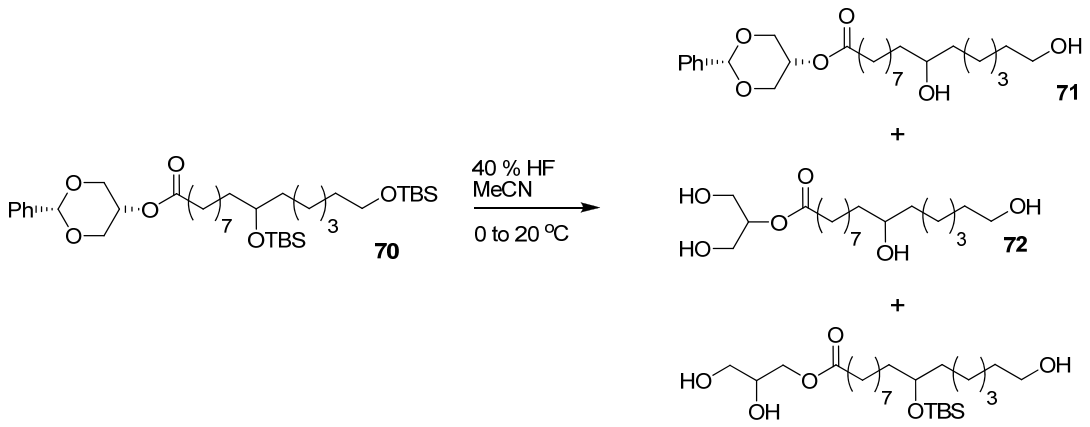
Scheme 21: Final synthetic route to 2-mono-(10,16-dihydroxyhexadecanoyl)glycerol.[a]



[a]Reagents: (a): NaH, DMF, BnBr; (b): i) DMSO, $(ClCO)_2$,CH_2Cl_2, ii) Et_3N, iii) (6-tertbutyldimethylsilyloxy)-hex-1-yne **62**, ^{t}BuLi, THF; (c): TBSCl, imidazole, DMF; (d): H_2, Pd/C, EtOAc; (e): i) $PhI(OAc)_2$, TEMPO,CH_2Cl_2, H_2O, ii) *cis*-5-hydroxy-2-phenyl-1,3-dioxane, EDC·HCl, DMAP, CH_2Cl_2; (f): 20 % aq. HF, MeCN; (g): H_2, $Pd(OH)_2$/C, THF.

The TBS protecting groups were removed with HF in MeCN to afford the diol **71**. For this deprotection step, milder conditions were at first attempted, using TBAF and HOAc in THF at 20 °C, however, after 24 h only the terminal TBS group was cleaved using these conditions. Even applying a large excess of TBAF in THF for an extended time only resulted in trace amounts of the desired product. Therefore, we turned to acidic conditions for the removal of the TBS groups: using more concentrated (40%) aqueous HF in MeCN at temperatures ranging from 0 °C to 20 °C resulted in a mixture of the desired diol **71**, the fully deprotected 2-MHG (**72**), and the isomer resulting from migration of the acyl moiety to one of the primary alcohols (see Scheme 22).

Scheme 22: Attempted cleavage of the TBS protecting groups with 40% HF in MeCN at 0 °C to 20 °C.



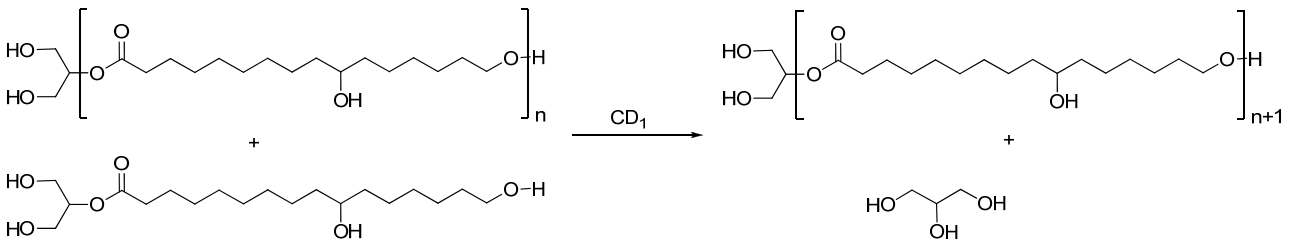
Gratifyingly, we found that using 20% aq. HF and keeping the reaction temperature at 0 °C for three hours cleanly afforded the desired diol in 84% yield. With the dioxane **71** in hands, the last step for the synthesis of 2-MHG was cleavage of the benzylidene group. This step had to be carried out with special care, because of the high propensity of the acyl group to migrate to one of the primary alcohols (as was observed in the attempted TBS-deprotection using 40% HF).

We chose to use mild hydrogenation conditions with Pearlman´s catalyst[127] in THF and obtained the desired lipid **72** in 83% yield after crystallization from ethyl acetate and heptane. The challenge of potential migration from the 2-position to one of the primary alcohols was avoided by the use of THF. Indeed other solvents do not give such good results as the one reported here: dissolving the pure lipid **72** in deuterated methanol resulted in acyl migration after 15 min, which could be monitored by ^{1}H NMR.

3.3. 2-MHG (72) is a substrate for CD1

The CD1 protein is thought to acts as an acyl transferase, operating a transesterification from the glycerol of the 2-MHG to the primary hydroxyl group of another molecule of 2-MHG or of a growing oligomer, as shown in Scheme 23. The acyl donor is the 2-MHG monomer, which is transferred, either to another molecule of 2-MHG (n=1) to start the polymerisation or to the growing polyester chain.

Scheme 23: Proposed mechanism for the synthesis of cutin catalysed by the enzyme CD1.



In order to validate the theory, the synthesised 2-MHG **72** was tested as substrate for the recombinant CD1 protein in an *in vitro* polymerization assay. The products of the assay were analysed by MALDI-TOF-MS, which showed the presence of a series of oligomers with up to seven units of the 2-MHG linked to a glycerol end group (see Figure 30), which assesses both the role of the enzyme and the identity of its substrate.

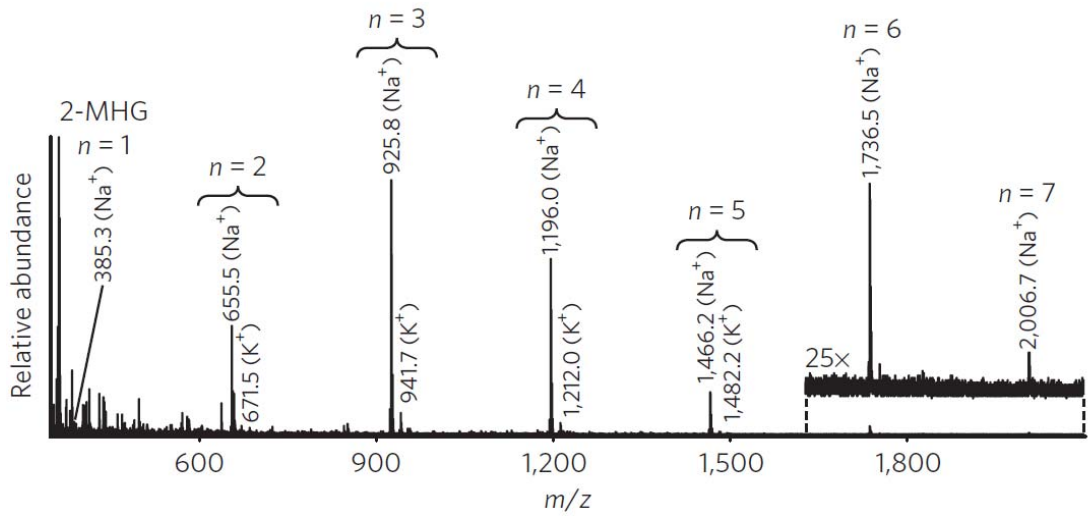


Figure 30: MALDI-TOF positive-mode ion spectra of lipid products from in vitro assays with 2-MHG substrate 72 and purified recombinant CD 1 enzyme. Oligomerisation up to DP 7 can be detected.

3.4. Conclusion

A concice synthesis of the cutin monomer 2-mono(10,16-dihydroxyhexadecanoyl)glycerol (2-MHG, **72**) has been devised. The route takes advantage of the efficient addition of an alkyne-lithium reagent to an aldehyde, followed by protecting group manipulations, oxidation-esterification and finally careful deprotection to afford the sensitive product in its stable, crystalline form.

With the proposed monomer for CD1 catalysed formation of cutin oligomers in hand, our collaborators were able to show that the enzyme does indeed accept **72** as a substrate and catalyse the formation of cutin oligomers. These findings constitute the first biochemical validation of the function of an enzyme involed in cutin biosynthesis in plants.[128]

4. Synthesis of a novel C_3 symmetric phosphacyclophane as a putative ligand for transition metal catalysed reactions

4.1. Introduction

4.1.1. Phosphines and other mono-phosphorus ligands for transition metals

Phosphines, PR_3, are valuable ligands in organometallic chemistry. Their abilility to form very diverse complexes, to stabilize a variety of metals as their phosphine complexes $(R_3P)_nM$-L as well as different oxidation states of the metals, enables phosphine-metal complexes to promote a wide range of catalytic reactions.

Not only electronic effects, but also to a considerable extend steric effects of ligands have been shown to influence the reactivity of complexes, by stabilizing or destabilizing different oxidation states and/or coordination numbers of the metal. This feature is of special interest in the case of phosphine ligands, for which changing the ligand sizes and electron-donor power can be achieved relatively easily and in a predictable manner by varying R. This fine tuning of steric and electronic properties allows eliciting desired properties from their complexes.[129]

Phosphine ligands can donate their phosphorus lone pair, and are good sigma donors, they are also π-acids, which means that the empty σ* orbital of the P-R bond overlaps with the filled metal d_π orbital and accepts electrons from the metal. This effect is known as back bonding. The extent to which the empty σ* orbital accepts electron back donation depends on the nature of the R substituents: the more electronegative the atom attached to phosphorus, the better σ* acceptor from the metal. This effect affects the P-R bond, as well as the M-P bond length and stability.

Electronic effects are defined by the changes in molecular properties as a result of transmission along chemical bond (for instance changing from an electron donating substituent on the phosphorus, such as $P(p\text{-}C_6H_4OCH_3)_3$, to an electron withdrawing substituent as in $P(p\text{-}C_6H_4Cl)_3$). Steric affects are defined by the changes in molecular properties as a result of forces between parts of the molecules (for instance repulsions between bulky substituents). A schematic definition of these effects is depicted in Figure 31.[130] The cone angle is a steric parameter introduced by Tolman, which allows an intuitive depiction of the steric effects, and which has been used to explain the influence of the steric bulk of phosphorus ligands in a variety of complexes. It is defined as the apex angle of the cylindrical cone centred 2.28 Å from the centre of the P atom, and tangent to the Van der Waals radii of the outmost atoms in the space filling model of the $M(PR_3)$ group, as shown in Figure 31.

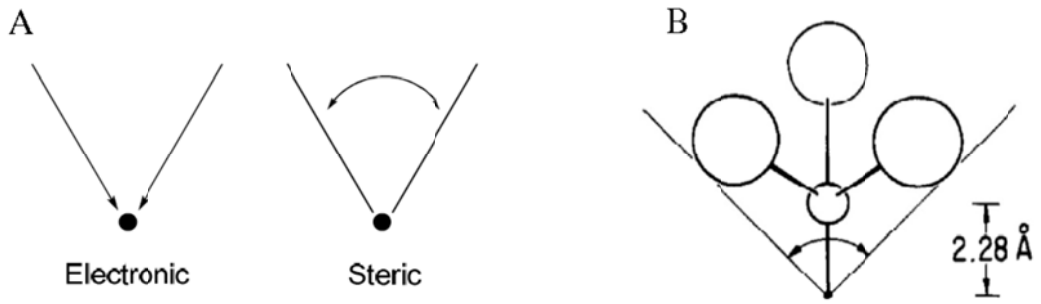


Figure 31: Steric and electronic effects. A: Schematic definition of electronic and steric effects. B: Definition of the cone angle Θ for symmetric ligands.[130]

Electronic and steric effects are defined independently, but are intimately related. For instance, the electronegativity of the substituents´ atoms will influence bond distances and angles.

Some of the consequences of steric and electronic effects of phosphine ligands on the stability and reactivity of complexes are exemplified below:

4.1.1.a) Dissociation of phosphorus ligands and ligand exchange

Steric effects have been shown to be of major importance in the dissociation of phosphorus ligands, and to dominate equilibria of type 4.1.1.1

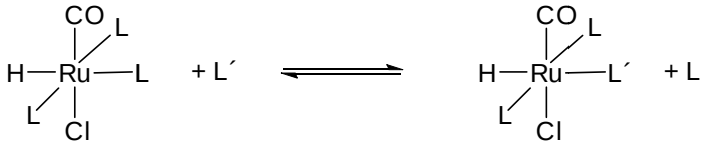
$$MX_2L_3 \overset{K}{\rightleftharpoons} MX_2L_2 + L \qquad (4.1.1.1)$$

where M=Ni or Co, L=PR_3 and X=CN or halogen. The dissociation constant intuitively increases with the bulk of the ligands (in the order $PMe_3 < PEt_3 < PCy_3$).[130,131]

Equilibria 4.1.1.2 has also been studied and is also dominated by the size of the substituents, in the order $PMe_3 \sim PMe_2Ph \sim PMePh_2 < PEt_3 \sim PBu_3 \sim PPh_3 < PBn_3 < P(i\text{-}Pr)_3 < PCy_3 < PPh(t\text{-}Bu)_2$.[132]

$$PdL_4 \rightleftharpoons PdL_3 \rightleftharpoons PdL_2 \qquad (4.1.1.2)$$

Ligand exchange has also been shown to be strongly influenced by sterics in several complexes, where smaller phosphine and phosphate ligands can displace bulkier phosphine ligands of Ru(II) complexes. In equation 4.1.1.3, ligand exchange goes in the order L´ = $P(OMe)_2Ph$ > $PMe_2Ph \sim P(OEt)_3$ > $PEt_2Ph \sim PPr_2Ph \sim PBu_2Ph$.[133] In this series, with the exception of $P(OEt)_3$, the smaller the cone angle, the better the displacement of a bulkier ligand.



L= PEt_2Ph, PPr_2Ph or PBu_2Ph. (4.1.1.3).

In the same way, displacement of other types of ligands by phosphorus ligands is similarly influenced by sterics. Figure 32 shows the results for Ni(0): the degree of substitution of CO ligands from $Ni(CO)_4$ is highest for phosphorous ligands with the smaller cone angle.[130] Similar studies have shown that for $ReBr(CO)_5$ and $MnBr(CO)_5$ complexes, the size of the phosphorus ligands controls the rates and equilibria of CO displacement, and PEt_3 can lead to trisubstitution, while PPh_3 can only achieve disubstitution.[130] Generally speaking, a maximum of two PCy_3 or $P(i\text{-}Pr)_3$, three or four PPh_3, four PMe_2Ph and five or six PMe_3 ligands can bind to a metal for steric reasons.[129] This feature can be extremely useful for the synthesis of complexes containing small but weakly held ligands as in the case of the synthesis of $(N_2)Ni(PEt_3)_3$, while it has been observed that $Ni(PMe_3)_4$ does not coordinate N_2.[130] The reason for this observation is that bulkier phosphorus ligands are more easily displaced, but bulky phosphorus ligands can also stabilize coordinatively unsaturated complexes such as $Pt(PCy_3)_2$ and $Rh(PPh_3)_3^+$, that can then engage in bonding with weakly coordinating ligands.[129] In the same way, complexes of metals with unusual coordination numbers have been synthesised and isolated. In order to achieve the inert gas configuration, zerovalent Ni, Pd and Pt generally prefer a coordination number of four. However, this rule is overcome if bulky ligands are used, and ML_3 complexes have been isolated with L = PBn_3, P^iPr_3,[132] $P(O\text{-}o\text{-}Tol)_3$,[134] and ML_2 complexes have been isolated with L = P^iPr_3, PCy_3 and P^tBu_2Ph.[132,135]

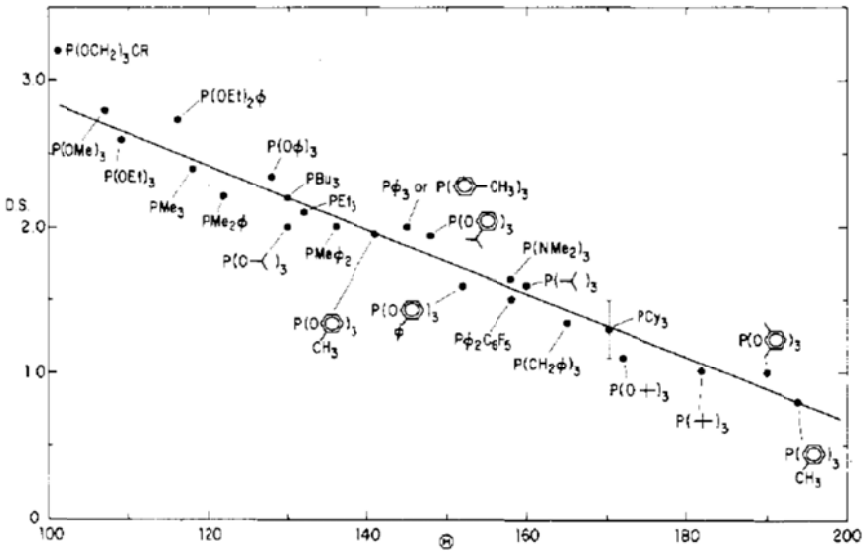


Figure 32: Degree of substitution (DS) of carbonyl groups from $Ni(CO)_4$ by phosphorus ligands as a function of their cone angles Θ.[130]

4.1.1.c) Oxidative addition reactions

As in the cases of ligand dissociation and ligand exchange, oxidative additions are strongly influenced by sterics. Thus from the previous observations, the following trends become intuitive: bulkier phosphorus ligands hamper direct oxidative addition (steric inhibition), but can also accelerate the overall reaction, when a first step consisting in ligand dissociation from an 18-

electrons complex is a prerequisite for the oxidative addition (steric acceleration).[130] For instance, Pd[PPh(*t*-Bu)$_2$]$_2$ readily forms an O_2PdL_2 complex upon reaction with air, but Pd[P(*t*-Bu)$_3$]$_2$ is stable in air. Despite the complex unsaturation (both complexes are 14-electron complexes) the latter is apparently too crowded and even a small O_2 cannot insert due to a lack of room around the metal.[136]

An interesting example studied by Halpern and Phelan shows that while steric factors dominate the phosphorus ligand properties, electronic factors also affect associative reactions: According to their study, associative reaction of benzyl bromide with Co(DH)$_2$L (see reaction 4.1.1.4), is inhibited by bulky phosphines (e.g. L = tricyclohexylphosphine), but performs faster with the electron-donating L = P(*p*-$C_6H_4OCH_3$)$_3$ than with the electron-withdrawing L = P(*p*-C_6H_4Cl)$_3$, between which steric factors are presumably unimportant.[137] Data are shown in Figure 33.

2 Co(DH)$_2$L + BnBr → BrCo(DH)$_2$L + BnCo(DH)$_2$L (4.1.1.4)

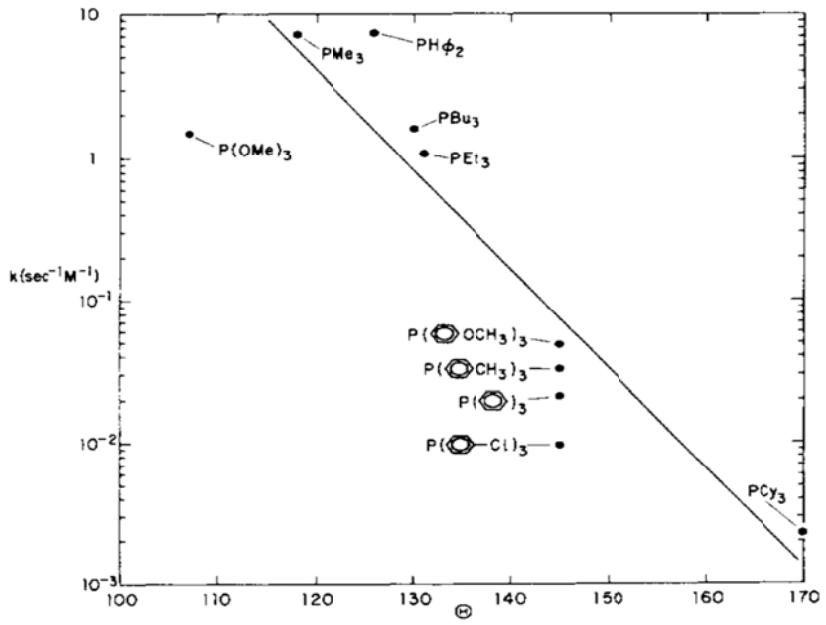


Figure 33: Rate constants of reaction 4.1.1.4 in benzene at 25 °C.[130]

In order to optimize the choice of phosphorus ligand for a complex designed for a given reaction, Tolman has introduced his steric and electronic map of phosphorus ligands, shown in Figure 34.[130] The map consists in plotting the different phosphorus ligands in a two-dimensional graph, defined by the steric parameter Θ (cone angle) on the abscissa axis, and the electronic parameter ν_{CO} on the ordinate axis. The cone angle Θ was defined earlier in section 4.1.1. The electronic parameter, ν_{CO}, is the vibration frequency of CO ligands in the series of complexes LNi(CO)$_3$, where L is the phosphorus ligand studied. The measure of ν_{CO} quantifies the electron donor power of the ligand: the stronger donor the phosphorus ligand, the higher the electron density on Ni, and in turn, the

greater the back donation to the CO ligands, which lowers ν_{CO}. Values of ν_{CO} of $LNi(CO)_3$ have also been predicted computationally for a variety of ligands.[138]

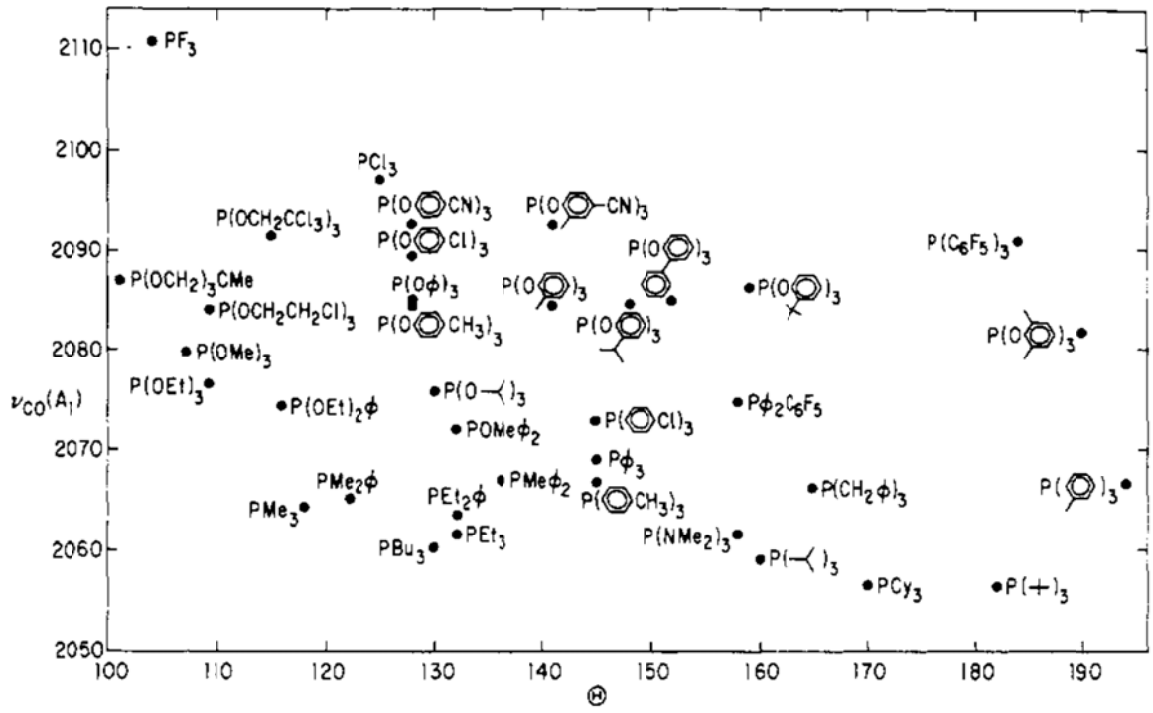


Figure 34: Tolman´s steric and electronic map of phosphorus ligands.[130] ν_{CO} characterizes the electronic effects or electron donor power of the ligand, Θ is the cone angle and depicts the steric effects (ν in cm $^{-1}$, Θ in degrees).

4.1.2. Diphosphine ligands

Complexes containing two phosphine ligands and complexes containing a chelating diphosphine often show different reactivities.[139] As chelate ligands, diphosphines such as $Ph_2P(CH_2)_nPPh_2$ generally adopt a cis arrangement around the metal, and are much less easily displaced from the complexes than monodentate ligands for entropic reasons, a property known as the chelate effect.[129] However, in those complexes, the length of the methylene chain (n), and thereby the "bite" of the chelates, critically affects their chelating ability and reactivity. For instance, the ability of the series of ligands $Ph_2P(CH_2)_nPPh_2$ to coordinate Ni(0) decreases in the order: $Ph_2P(CH_2)_3PPh_2 \sim Ph_2P(CH_2)_2PPh_2 > Ph_2P(CH_2)_4PPh_2 \gg Ph_2PCH_2PPh_2$, the latter displaying probably inadequate bite.[130] Therefore, bis-diphenylphosphinomethane prefers to form binuclear complexes in which the two metal centers are bridged by the diphosphines thus avoiding formation of the strained four-membered metallacyclic chelates.[139]

In the same way as cone angle for monophosphines is a parameter influencing the ligands reactivity, the notion of cone angle was also introduced for diphosphine chelate ligands as well as the bite angle, defined as the P-M-P angle, to rationalize the influence of diphosphine chelating ligands on

the reactivity of organometallic complexes (depicted in Figure 35 A).[129,130] Later, Casey and Whiteker introduced the notion of natural bite angle, which only considers the preferred chelation angle as dictated by the backbone constraints, and abstracts other constraints introduced by metal valence angle. Thus, the natural bite angle is determined by steric considerations solely. They also determined values of natural bite angles for a series of chelating diphosphine ligands by molecular mechanics calculations.[139] Several diphosphines have been designed to adopt cis coordination sites in square planar and octahedral metal complexes, such as DIPHOS, bis-diphenylphosphinoethane, which forms cis-chelated complexes, with a bite angle close to 90° due to the rigid 5-membered metallacycle. On the other hand, diphosphines with a large bite angle have been designed with the aim of obtaining better selectivities and rates for a number of reactions. Therefore, Ito and co-workers have developed the trans-chelating chiral diphosphine ligand TRAP 2, 2´´-bis[1-(diphenylphosphino)ethyl]-1,1´´-biferrocene, efficient for asymmetric Michael addition of α-cyano-carboxylates catalysed by ruthenium (see Figure 35C),[140] and Van Leeuwen and co-workers developed and studied a series of ligands structurally close to Xantphos, some of them displaying increased rates and selectivities in reactions such as rhodium-catalysed hydroformylation or nickel catalysed hydrocyanation, and explained the rates and selectivities with steric and electronic arguments.[141]

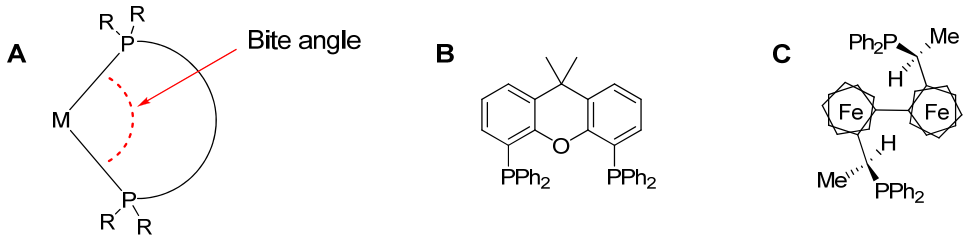


Figure 35: Bite angles of diphosphine ligands. A: Schematic definition of the bite angle for a chelate ligand.[130] B: an example of diphosphine ligand with a large bite angle: Xantphos (natural bite angle: 111.4 °).[141] C: other example of diphosphine ligand with a large bite angle: TRAP (bite angle: 164.4 °).[140]

Indeed, as for monodentate phosphorus ligands, steric and electronic effects affect ligands properties and consequently determine the related complexes reactivities. The PMP bite angle has been shown to play a role in the rates and selectivities of several reactions, and is related to both the "steric bite angle effects", involving interactions between ligands or between ligands and substrates, and the "electronic bite angle effects", relating to electronic changes emerging when the bite angle is modified. The latter can be described as an orbital effect, in the sense that the bite angle influences the metal hybridisation, thereby influencing the metal orbitals energies and reactivities. Therefore different transition states can be stabilised or destabilised, which impacts the catalytic reaction outcome in terms of reactivity and selectivity.[141]

A variety of diphosphine ligands displaying particular structural properties such as wide bite angle, chirality at the phosphorus center, chiral backbones, or planar chirality, has been synthesised and

showed attractive catalytic properties. Two examples are particularly worth mentioning, that illustrate the works of William S. Knowles and Ryoji Noyori, rewarded by the Nobel Prize in 2001 *"for their work on chirally catalysed hydrogenation reactions"*.[142] Both researchers developed chiral ligands that effectively and efficiently introduced asymmetry in hydrogentation reactions. Knowles and Sabacky developed the first asymmetric hydrogenation using a rhodium complex and a chiral phosphine ligand, achieving modest enantiomeric excess, but which was a proof of principle that enantioselectivity could be achieved in reactions catalysed by organometallic complexes (see Figure 36 A).[143,144] Further chiral monophosphines and diphosphines ligands have been developed until the synthesis of the diphosphine ligand DIPAMP (ethane-1,2-diylbis[(2-methoxyphenyl)phenylphosphane]), achieving excellent enantioselectivity, and its industrial use in the synthesis of L-DOPA (L-3,4-dihydroxyphenylalanine). The stereoselective hydrogenation step performed on the L-DOPA precursor is presented in Figure 36 B.[144,145]

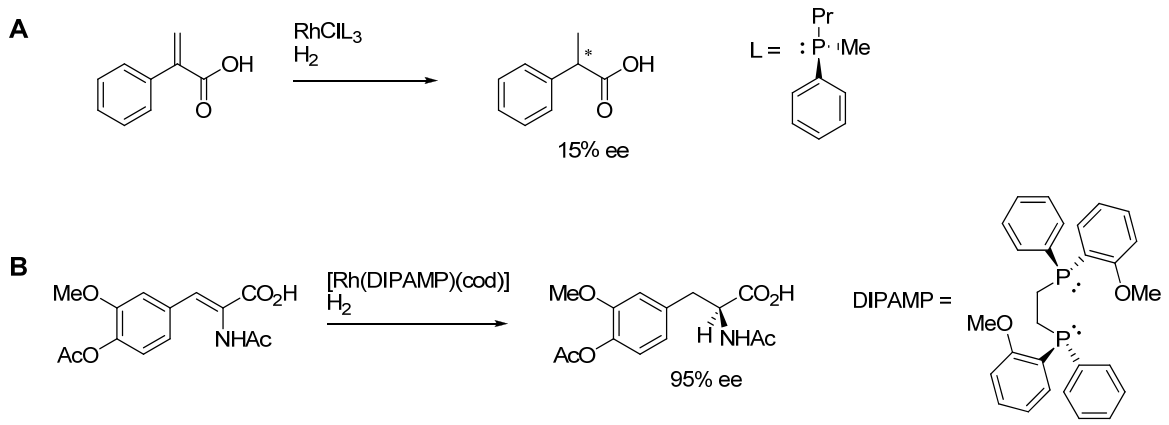


Figure 36: Enantioselective hydrogenations developed by Knowles. A: First example of enantioselective hydrogenation catalysed by Rh complex displaying chiral phosphorus ligand.[143,144] B: More efficient asymmetric catalysis of olefin hydrogenation by a Ru complex with DIPAMP ligand.[144,145]

On the other hand, Noyori developed the asymmetric reduction of ketones catalysed by Ru-complex. His original work focused on the reduction of β-keto-esters, for which he found that BINAP-Ru dihalide was a very efficient catalyst for the reaction (see Figure 37 A).[146] Later, he extended the scope of the catalytic reduction to "simple ketones" (i.e. ketones with no requirements on the β-position) modifying his complex to a BINAP/diamine – ruthenium complex, this complex can reduce aromatic, heteroaromatic, and olefinic ketones enantioselectively (see Figure 37 B).[147]

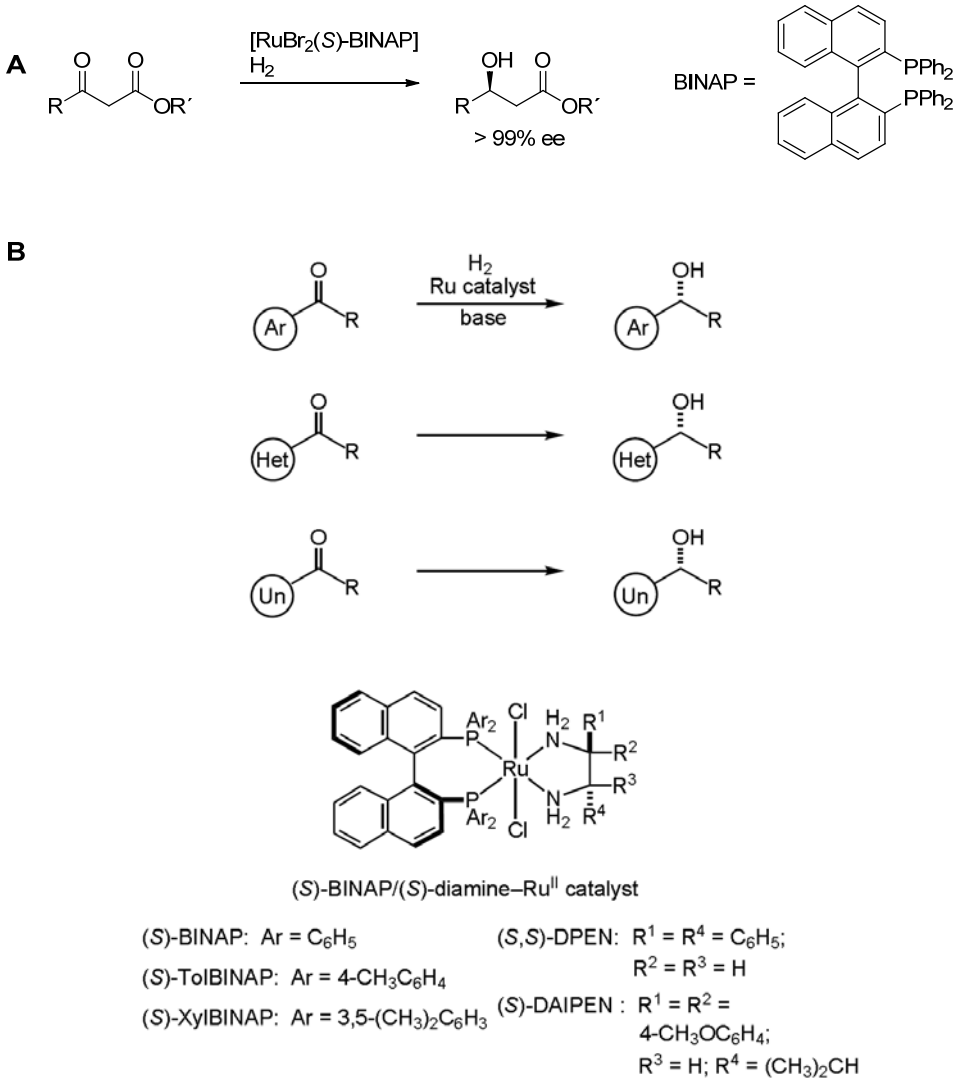


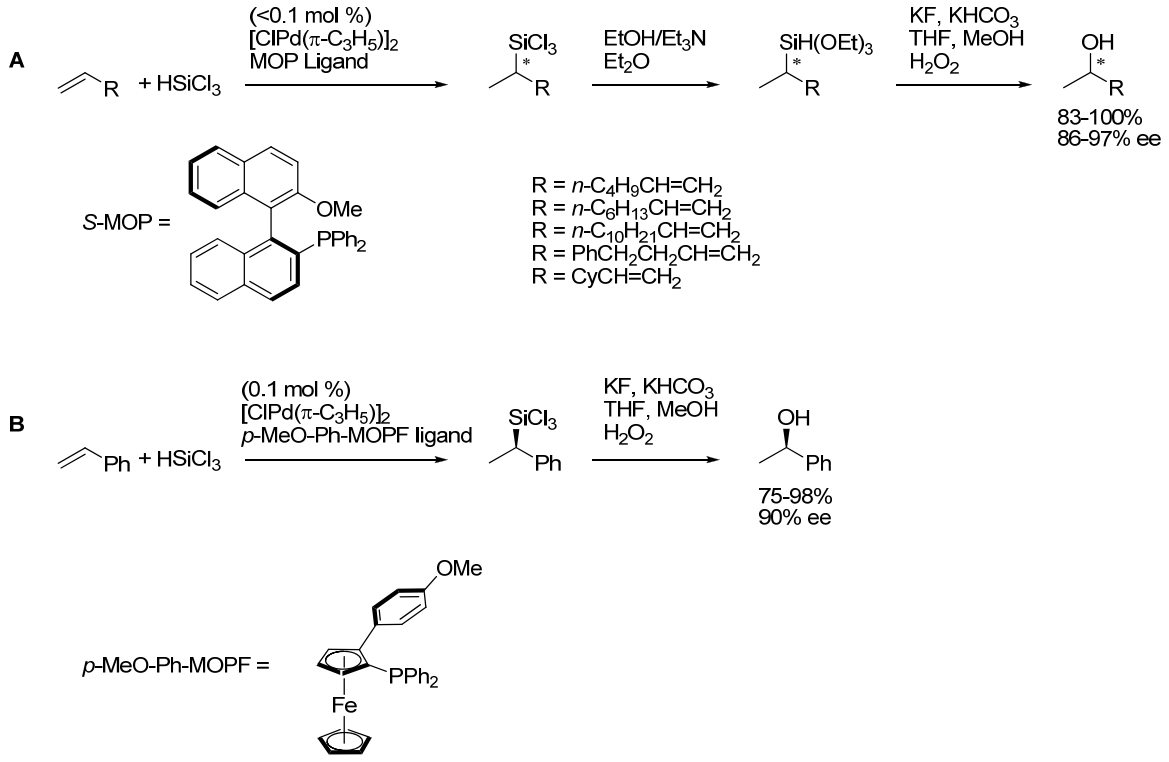
Figure 37: Enantioselective reduction of ketones catalysed by Ru complexes developed by Noyori. A: asymmetric reduction of β-keto-esters catalysed by BINAP-Ru-complex.[146] B: enlargement of the scope of the reaction by BINAP-diamine-Ru complex.[147]

4.1.3. Ligands symmetry and enantioselective reactions

If the importance of chiral diphosphine ligands in enantioselective reactions was already obvious in 1968 with Knowles and Sabacky´s work[143] and despite the early results of the application of monophosphines by e.g. Knowles, the latter gained popularity as alternatives to diphosphines in some enantioselective reactions only later, a milestone for this being the synthesis of the chiral monodentate phosphine ligands MOPs and their application in the synthesis of chiral alcohols published by Uozumi and Hayashi in 1991. In this work, chirality is introduced upon hydrosilylation reaction of prochiral terminal olefins with Markovnikov selectivity catalysed by palladium complexes, followed by Tamao-Fleming oxidation of the resulting chiral organosilane with retention of configuration to afford the alcohols in excellent yields and ee, as illustrated in

Scheme 24.[148] Further chiral phosphine ligands based on a planar chiral ferrocene scaffold have been synthesised and investigated for the same process on styrene, achieving good stereoselectivity.[149]

Scheme 24: Enantioselective hydrosilylation of prochiral alkenes followed by oxidation. A: As described by Uozumi and Hayashi,[148] B: As described by Pedersen and Johannsen.[149]



Other examples of atropisomery have been used to induce chirality on phosphorus ligands, as in the C_2-symmetric BINAP ligand, mentioned in paragraph 4.1.2. In this case, consideration of symmetry is also important. Indeed, C_2 symmetry reduces the number of transition states and/or intermediates in the catalytic reaction, and thereby increases the chance of a stereoselective outcome for the reaction. By definition, in the case of C_2 symmetry, each rotation of the ligand 180° about the C_2 rotation axis results in an identical situation, while the two situations would be diastereomeric in the absence of symmetry. Along those lines, C_3-symmetric ligands have gained interest, since C_3-symmetry results in identical situation for each 120° rotation. Thus, both achiral and chiral tripodal C_3 symmetrical phosphines, shown in Figure 38A and B, have been synthesised that form complexes with metal ions in various oxidation states.[150] Monophosphines having threefold rotational axis have also been synthesised with the aim to introduce a chirality axis that would be the same as the rotational axis. The design strategies were based on introducing aryl substituents on the phosphorus, taking advantage of non-bonding repulsive interactions between the rings, hoping to obtain propeller-like triarylphosphines and thereby introduce a helical chirality. However, low interconversion barrier and rapid racemisation, observed for the less complex ligand shown in Figure 38C, have limited its application. Thus, extremely encumbered substituents around the phosphorus (see Figure 38D) or more recently more complex designs, including peptidic bridging of aryl substituents as shown in Figure 38E were necessary to resolve such ligands.

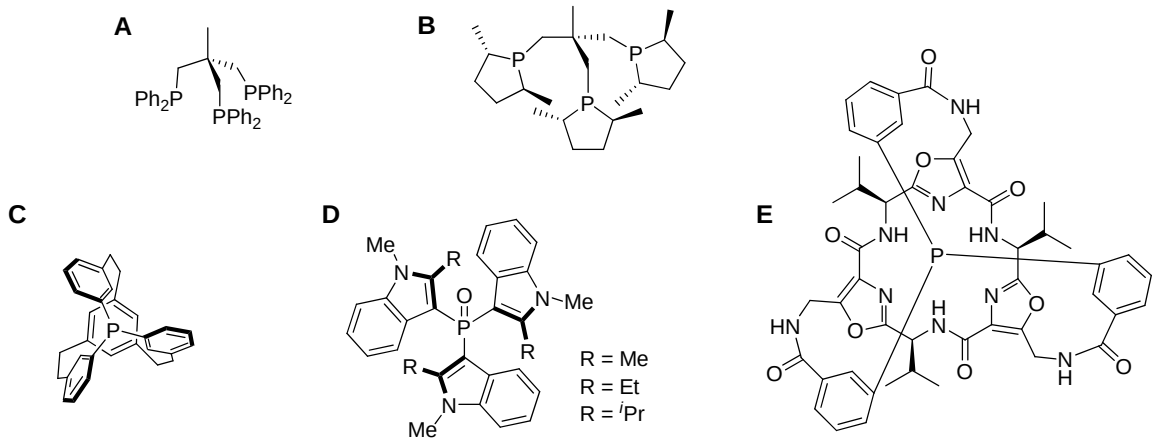


Figure 38: C_3 symmetric phosphine ligands. A: Achiral tripodal trisphosphine;[150] B: Chiral tripodal trisphosphine;[150] C: Unresolved C_3 symmetric phospha[2.2.2]cyclophane;[151] D: First resolved stable C_3-symmetric propeller-shaped triarylphosphine oxides; [152] E: Propeller-shaped triphenylphosphine stabilized by a chiral scaffold based on a cyclic peptide.[153]

More examples, depicted in Figure 39, show attempts to obtain stable chiral propeller shaped phosphine ligands based on bulky aryl substituents on the phosphorus, but X-ray analysis of those compounds illustrate the difficulty to obtain a true propeller-like triarylphosphine.[154,155,156]

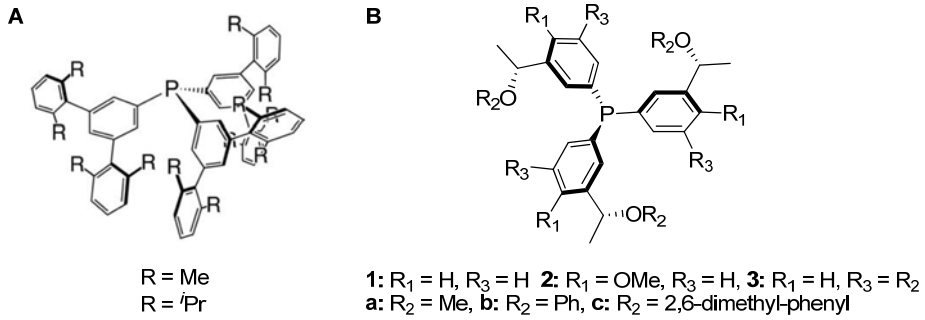


Figure 39: Examples of ligands that do not adopt perfect C_3-symmetric conformation.[154,155,156]

Ligands shown in Figure 39, even though they do not adopt a perfect C_3-symmetric conformation, have been tested in asymmetric allylation reactions, and showed some degree of enantioselectivity, however, the observed ee were very substrate-dependant.[156]

4.2. Design of a new C_3-symmetric phosphine ligand

A first attempt to synthesize a novel C_3-symmetric phosphacyclophane for enantioselective reactions was carried out by MSc student Louise Thorstholm.[157] In the original design, shown in Figure 40A, the phosphine oxide did not carry any chiral center, but it was hoped that the bridging alkyl chains in the system would render it rigid enough to display a proper propeller shape and chirality around the phosphorus atom. However, the interconversion barrier was too low, and the compound could not be resolved. Nonetheless, the phosphine oxide was reduced *in situ*, tested as ligand in the palladium-catalysed hydrosilylation of styrene and shown to form an active complex for the reaction. Therefore, we decided to continue the project, introducing a chiral center in each of the six benzylic positions, aiming at increasing the energy barrier for interconversion and thereby achieving a stable propeller shaped triarylphosphine. Furthermore, the two propellers will be diastereoisomers due to the presence of the chiral centres, increasing the likelihood that one propeller will be energetically favoured over the other. The structure of our target phosphine ligand is shown in Figure 40 B.

If the route for the synthesis of the first generation ligand was already a challenge, the introduction of the chiral centers changed the reactivity of the compounds, and none of the original procedures proved to be efficient for the synthesis of the latest phospine ligand.

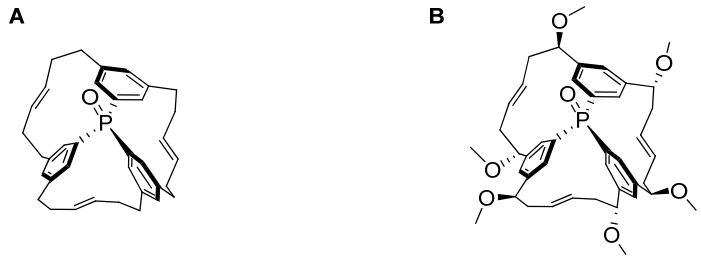


Figure 40: A: First generation phosphacyclophane synthesised by MSc student Louise Thorstholm;[157] B: Second generation phosphacyclophane targeted in this project. The additional stereocenters are aimed at enhancing the structure rigidity through a relay of chirality from the remote stereocenters.

4.3. Synthesis of the cyclophane

3D structure of the target phosphacyclophane is shown in Figure 41 (A), along with a "flattened" structure (B) that should ultimately help the reader picturing the target compound. We wished to synthesised the phosphacyclophane by ring closing metathesis (RCM) of tris-(3,5-di((*R*)-1-methoxybut-3-enyl)phenyl)-phosphine oxide. That step was envisioned to be the most challenging of the reactions sequence: three consecutive RCM should be performed on a system that becomes more and more strained when the rings are being closed. Moreover, phosphine oxides are known ligands for ruthenium, which might hamper the RCM. Tris-(3,5-di((*R*)-1-methoxybut-3-enyl)phenyl)-phosphine oxide can be synthesised from 1-bromo-3,5-di((*R*)-1-methoxybut-3-

enyl)benzene and an electrophilic phosphorus reagent such as PCl_3, or $P(OPh)_3$. Finally, the phenyl derivative can be synthesised from dimethyl 5-bromoisophthalate.

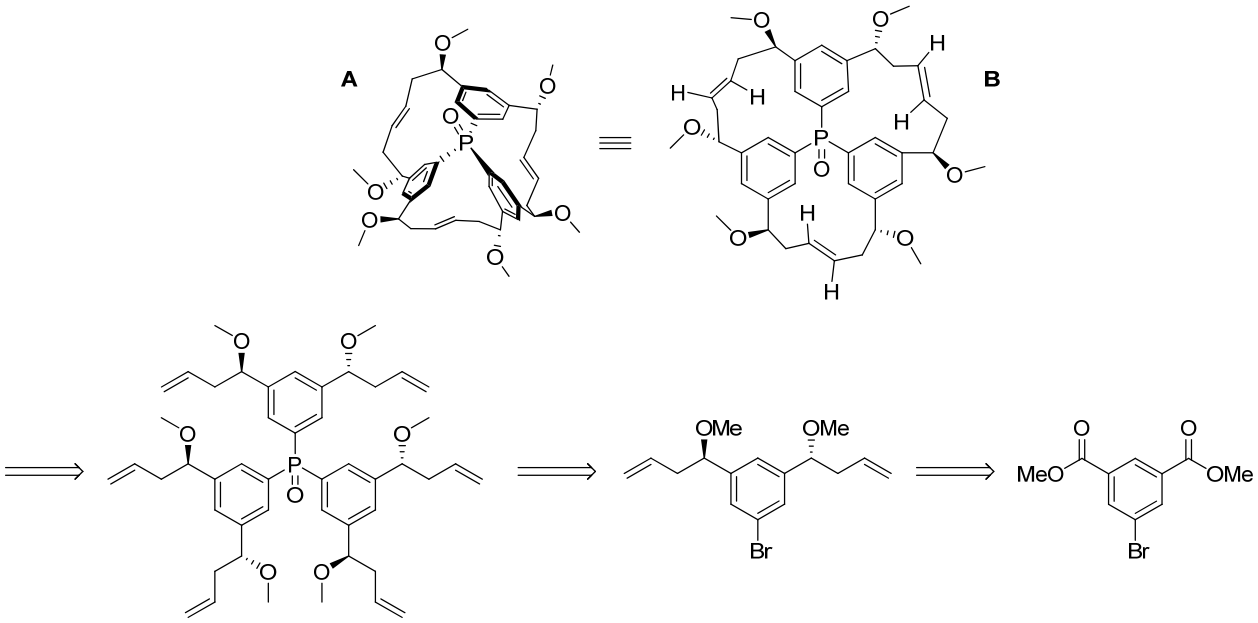
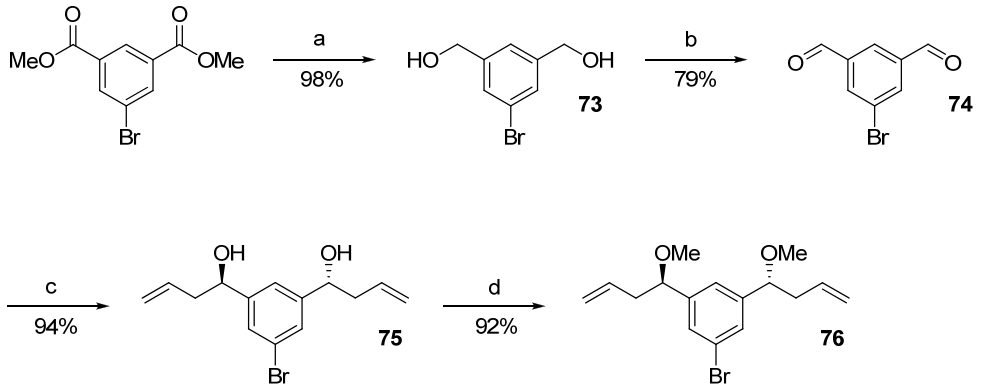


Figure 41: Retrosynthetic analysis for the desired C_3 symmetric phosphacyclophane.

Selective reduction of esters to aldehydes can be achieved in different ways,[158] the use of a controlled amount of DIBAL-H at low temperature,[159,160] being the most widely used reagent for partial reduction of esters. Therefore, dimethyl 5-bromoisophthalate was attempted reduced to the dialdehyde **74** directly using 2 to 2.2 equivalents of DIBAL-H at -78 °C. However, only little conversion was observed by TLC at that temperature. The same reactions were also attempted at -65 °C, but in that case only complex mixtures of the desired compound along with monoaldehyde, and products of reduction of the esters to the alchohols were obtained.[i] In the same way, Le *et al.*[161] reported a relatively low yield (45-55%) for the reduction of methyl benzoate to benzaldehyde using DIBAL-H, hence their decision to synthesise the aldehyde in a two steps-procedure, *via* full reduction to the alcohol followed by oxidation to the aldehyde, which route afforded the desired product in 84% yield over the two steps. Thus, to circumvent the issue of several reduction products, we decided to synthesise **74** *via* an intermediate, and dimethyl 5-bromoisophthalate was first reduced to the diol **73** with $LiAlH_4$, as described by Brunner *et al.*,[162] and oxidized to the dialdehyde via Swern oxidation[43] performed in CH_2Cl_2/DMSO for solubility reasons in a second step, to afford 3,5-diformylbromobenzene in high purity and good yield (77% over the two steps). A selective reduction of dimethyl isophthalate using sodium bis(2-mothoxyethoxy)aluminium hydride (SMEAH) and *N*-methylpiperazine in toluene has also been reported to give isophthalaldehyde in 66% yield,[158] but the two-step synthesis was preferred due to its high yields and simple procedures.

[i] work carried out by MSc. stud. Jakob F. Kure

Scheme 25: Synthesis of 1-bromo-3,5-bis((R)-1-methoxybut-3-enyl)benzene 76.[a]



[a] Reagents: (a): $LiAlH_4$, THF; (b): i) $(ClCO)_2$, DMSO, DMSO/CH_2Cl_2, ii)Et_3N; (c): i) (+)-Ipc_2BAll, THF, ii) MeOH, 3N NaOH, 30% H_2O_2; (d): NaH, MeI, DMF.

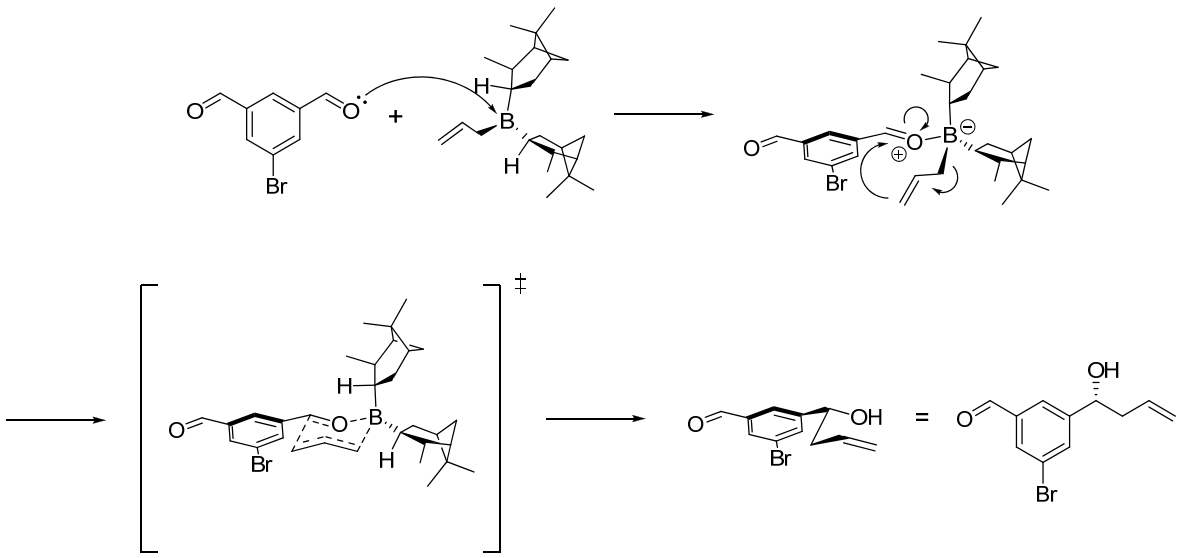
A general method for asymmetric allylboration of dialdehydes with (-)-*B*-allyldiisopinocampheylborane ((-)-Ipc_2BAll) for the synthesis of aromatic C_2-symmetric diols was reported by Brown and coworkers in 1997, that provided (1*S*,1'*S*)-1,1'-(1,3-phenylene)dibut-3-en-1-ol from 1,3-benzenedialdehyde in 89% yield (88% de, ≥98% ee).[163] Based on those reports, we chose to synthesise (1*R*,1'*R*)-1,1'-(5-bromo-1,3-phenylene)dibut-3-en-1-ol **75** via asymmetric allylboration of **74** with (+)-Ipc_2BAll, inspired by procedures reported by Brown and co-workers,[163,164] which led to the preparation of **75** in excellent yield and with a de of 85% and ee of 95-99% (determined by chiral HPLC). We found that for our substrate, carrying out the reaction at -78 °C in THF instead of -100 °C, as described in the original literature[163,164] still gave excellent ee, and we decided to perform the reaction at that temperature for convenience. Finally, methylation of the diol **75** afforded **76** in excellent yield.

This reaction sequence (from 5-bromoisophthalate to **76**) could be scaled up and performed on relatively large scales (20 g) without affecting the yields, and the products were stable when stored at 4 °C.

The stereoselectivity can be explained by the interactions and steric repulsions in the transition state. Indeed, allylboration reactions have been reported to occur *via* a six-centred transition state to form rearranged homoallylic alcohols.[165] The observed selectivity can be supported by the Traxler-Zimmerman transition state model[166] (see Scheme 26), however, it is difficult to understand why the attack of the allylborane occurs from the Re face of the aldehyde in this extent (98% ee was measured) and barely occurs from the Si face of the aldehyde. Nevertheless, the same observation was done when Brown and co-workers synthesised (1*S*,1'*S*)-1,1'-(1,3-phenylene)dibut-3-en-1-ol with ≥98% ee using (-)-Ipc_2BAll,[163] but to our knowledge, no mechanistic or transition state study has been reported to explain the outcome of these allylboration using (+)- nor (-)-Ipc_2BAll. Brown and co-workers have mainly carried out studies of allylboration of benzaldehyde derivatives using

the (-)-Ipc_2BAll to obtain (*S*)-homoallylic alcohols.[163,164,167,168] Yet, (1*R*)-1-phenylbut-3-en-1-ol has been synthesised by treatment of benzaldehyde with (+)-Ipc_2BAll at -100 °C in Et_2O,[169] which supports our hypothesis that (1*R*,1'*R*)-1,1'-(5-bromo-1,3-phenylene)dibut-3-en-1-ol would be obtained by asymmetric allylboration of 5-bromoisophthalaldehyde with (+)-Ipc_2BAll. Moreover, a study carried out on reactions of heteroaromatic aldehydes with (+)-Ipc_2BAll has also shown that (*R*)-homoallyllic alcohols were obtained as major products ,[170] even though the yields and ee were not as high as described in the study of the reaction of (-)-Ipc_2BAll with aromatic and heteroaromatic compounds described by Brown and co-workers,[163] this being possibly explained by the basic pyridyl nitrogen atom, which complexes a boron atom easily, and thus reduces the reactivity of the allylborane.[170]

Scheme 26: Mechanism and stereoselectivity of allylboration



In order to verify that the dialdehyde **74** followed the trend of literature substrate to afford the (*R,R)*-product **75** upon reaction with (+)-Ipc_2Ball, Mosher ester analysis was carried out.

Mosher and co-workers developed a method to determine the ee and the absolute configuration of chiral compounds with a stereo center bearing a hydroxyl (or amino) group,[171,172] which consists in coupling the chiral alcohol to a chiral carboxylic acid derivative in order to obtain diastereomeric esters. α-Methoxy-α-trifluoromethylphenylacetate (MTPA) esters were identified as derivatives that would allow determination of absolute configuration and ee, and this is probably the most widely used reagents for this purpose.[173,174] The esters of both (*R*)- and (*S*)-MTPA have to be synthesised, which can be done by rather mild and fast reaction with the commercially available acid chlorides. It is worth mentioning that the configuration of the MTPA-Cl is inverted upon esterification of the chiral alcohol (*i.e.* the acid chloride of R configuration (*R*)-MTPA-Cl is converted to the MTPA ester of *S* configuration, according to the Cahn-Ingold-Prelog rules.[175] The empirical determination

of the absolute configuration consists in measuring $\Delta\delta_H=\delta_S-\delta_R$: the chemical shift difference of any similar set of protons from the (*S*)- and (*R*)-MTPA ester respectively. The sign of the difference indicates the position of the considered set of protons relative to the MTPA phenyl and methoxy substituents.[171,172,176] The argument assumes that the esters predominately adopt the most stable conformation shown in Figure 42, it follows that the chemical shift of the substituent protons on the carbinol[i] syn to the phenyl ring are shifted upfield compared to the similar protons syn to the methoxy group. In other words, any group of protons with a higher chemical shift in the (*S*)-MTPA ester compared to the (R)-MTBA ester (positive $\Delta\delta_H$) can be assigned to R^2, and groups of protons with negative $\Delta\delta_H$ can be assigned to R^1.

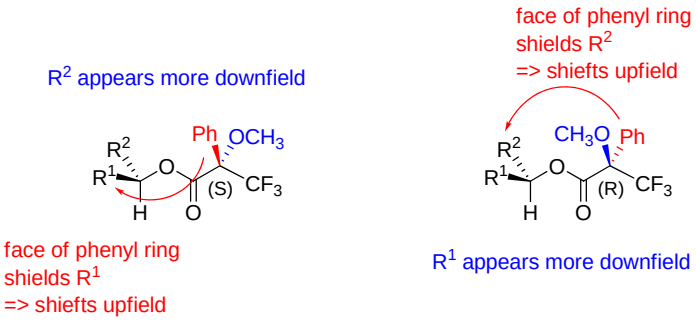


Figure 42: Interactions with Mosher ester substituents and consequent NMR shift behaviour.[171,174]

However, the ^1H region of interest might be crowded and the spectra difficult to interpret. Especially, signals of the two diastereoisomers might overlap, hampering ee measurements. Thus, ^{19}F NMR has also been explored as a possible tool for absolute stereochemistry determination and ee measurement. A trend has been observed, which was rationalised by the deshielding of the CF_3 substituent when the latter is coplanar with the ester carbonyl group group (see Figure 43). This is however highly dependent on sterical interactions, and is only observed for marked differences in the sizes of the substituents R^1 and R^2 (see Figure 43).[176] As a consequence, ^{19}F NMR analysis has shown to be less reliable for determining absolute configuration compared to the ^1H NMR Mosher ester method.[172]

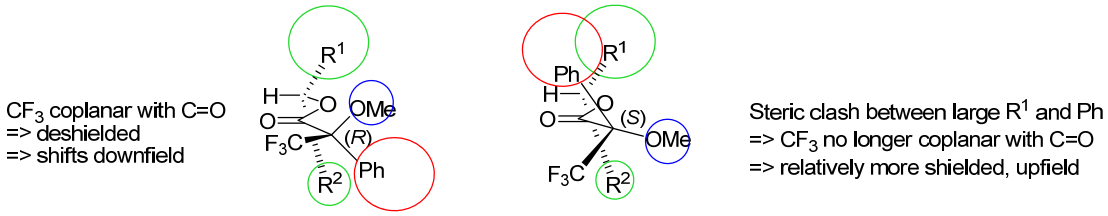
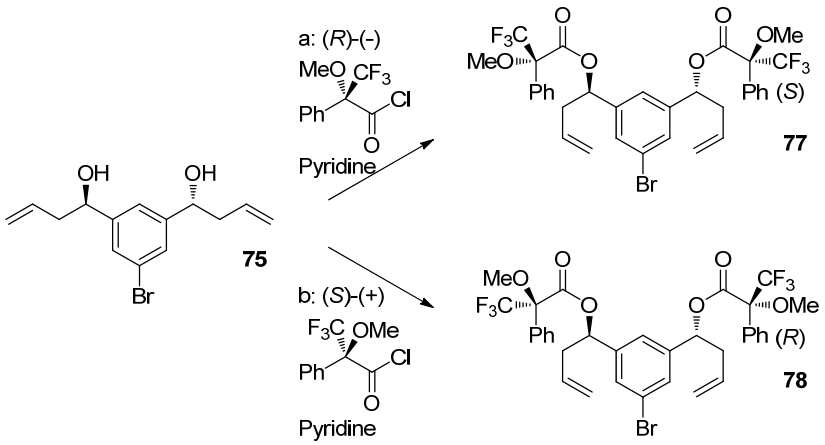


Figure 43: Configuration model for ^{19}F NMR.[176] Bond lengths have been exaggerated for the sake of clarity.

[i] Denomination used in the original literature.[171,173,176]

Therefore, **75** was reacted with (*R*)-(-)MTPA-Cl and (*S*)-(+)-MTPA-Cl in pyridine to obtain its Mosher ester derivatives **77** and **78** (see Scheme 27), for which both the crudes and the purified products were analysed by ^{1}H NMR and ^{19}F NMR. From the crude ^{1}H NMR, and ^{19}F the ee is at least 95%. For that purpose, the integrals of the methoxy signals were considered in the ^{1}H NMR, due to signal crowding in the other regions of interest.

Scheme 27: Synthesis of the Mosher ester derivatives of 75 for configuration analysis.



In order to determine the absolute stereochemistry, NMR data from the purified products **77** and **78** were analysed. As described above, the substituent shielded by the phenyl ring of the Mosher ester is shifted upfield, while the one on the same side as the methoxy group appears more downfield. In the ^{1}H NMR spectra of **77** and **78**, no change in chemical shift was observed for the methylene group, but the trend could be observed for the aromatic protons as well as for the vinylic protons (see Figure 44).

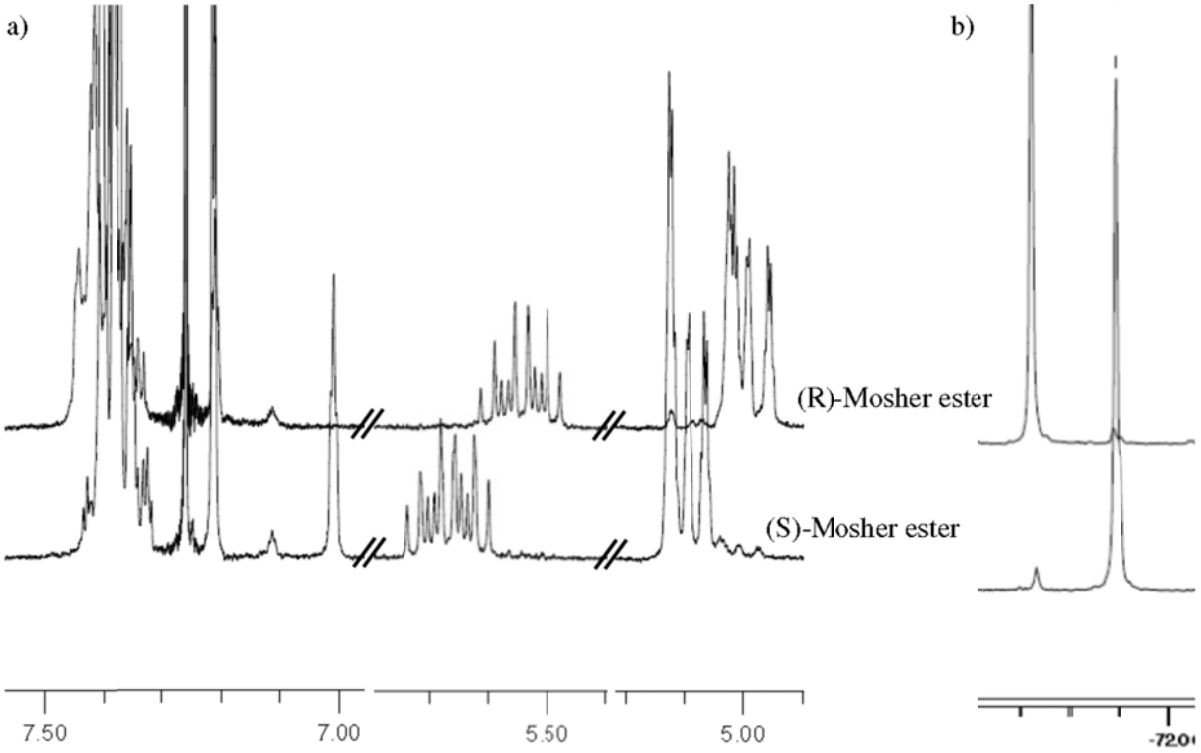


Figure 44: Assignment of absolute configuration of 75 from Mosher ester derivatives. a) ^{1}HNMR of the phenyl and vinylic protons of 78 ((R)-Mosher ester) and 77 ((S)-Mosher ester). b) ^{19}F NMR signal of the CF_3 Mosher ester substituent of 78 and 77.

It is evident from the partial ^{1}H NMR data shown in Figure 44 that the aromatic protons are shifted relatively downfield in the (*R*)-Mosher ester (negative $\Delta\delta_H$) and for the vinylic protons it is even more apparent that the $\Delta\delta_H$ is positive.

Taking the nomenclature from Figure 42, the chemical shift of protons on substituent R^1 is relatively upfield in the (*S*)-Mosher ester compared to the (*R*)-MTPA ester. From the NMR data in Figure 44, we can assign R^1 to the phenyl ring, and R^2, which is relatively downfield in (*S*)-Mosher ester compared to the (*R*)-MTPA ester, to the allyl substituent. Thus, we can assign the stereochemistry by replacing R^1 and R^2 with the aryl and the propene chain respectively and we confirm the expected (*R*) configuration (see Figure 45).

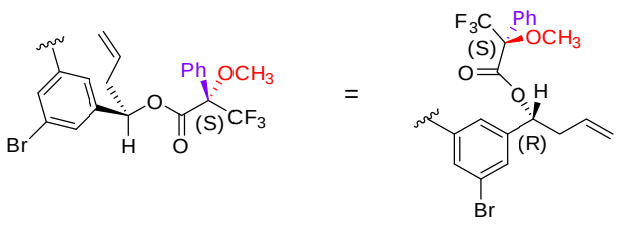
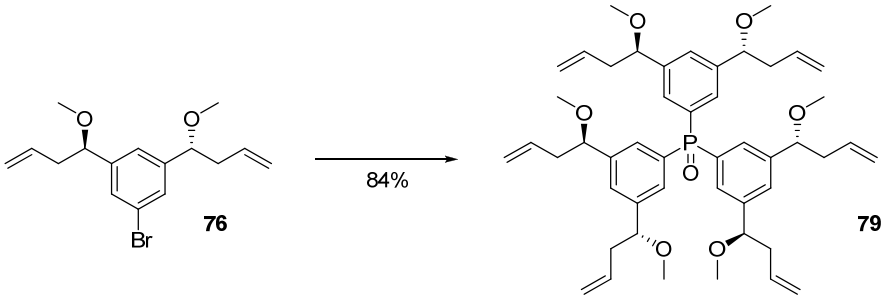


Figure 45: Absolute stereochemistry assignment.

^{19}F NMR of the two MTPA esters show a difference in chemical shift for the CF_3 groups of about 0.4 ppm, with the (*S*)-Mosher ester shifted upfield. This observation indicates that the CF_3 group is rotated out-of-plane with the carbonyl group of the (*S*)-MTPA ester (see Figure 43) and thus that the large aromatic group can be assigned to R^1, which supports the assignment of the stereocenter in **75** as (*R*)-configured.

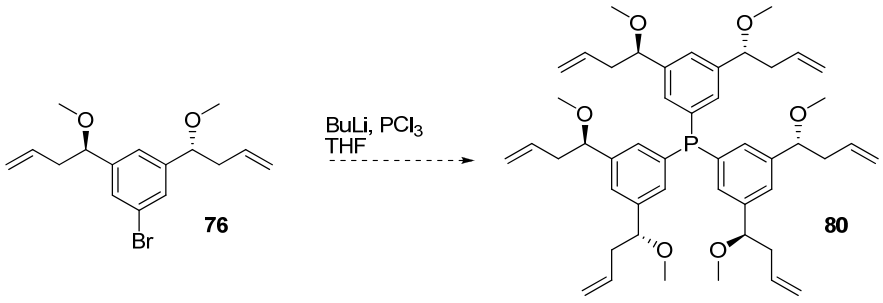
Scheme 28: Synthesis of the phosphine oxide 79.[a]



[a] Reagents: i) Mg, THF, ii) PCl_3, iii) ^{t}BuOOH.

Synthesis of the phosphine oxide **79** was challenging than expected and numerous experiments have been conducted before finding optimal conditions. We first sought to isolate the phosphine, but from the first attempts where traces of the compound were isolated, it was clear that the phosphine **80** (see Scheme 29) was extremely sensitive, and readily oxidized to the phosphine oxide during work-up, the latter being isolated in larger amounts than the phosphine. Thus, very fast in the test experiments, work up was preceded by treatment with ^{t}BuOOH, to fully oxidize the phosphine to its oxide, so only the phosphine oxide would have to be isolated.

Scheme 29: Attempted synthesis of tri-(3,5-bis((R)-1-methoxybut-3-enyl)phenyl)-phosphine 80.



Working on test reactions and as a consequence on rather small scales, we hoped to turn **76** to a nucleophile *via* lithium-halogen exchange, and react the resulting lithium compound with an

electrophilic phosphorus derivative such as PCl_3, such examples having been reported in the literature.[177,178] This was successful once using ^{t}BuLi, PCl_3 in THF at -78 °C, where **79** was isolated in 60% yield, but unfortunately this result could not be reproduced. Several attempts have been carried out to reproduce this result, and fine tuning of the conditions has been tried. Since mainly an unidentified by-product was isolated when the exchange was carried out at -78 °C, attempts have been carried out with reaction temperatures ranging from -90 °C to 0 °C, even though halogen-metal exchange reactions are usually carried out at low temperatures (-60 °C to -120 °C). According to Burgess and co-workers in their synthesis of triarylphosphines with a similar strategy, strict control of the temperature and slow addition of the reagents had a dramatic influence on the yield of this step. However, the authors do not comment on the side reaction occurring in the case of poor temperature control. In the reported procedure, halogen-lithium exchange is carried out at -78 °C, and PCl_3 addition is performed at temperatures ranging from -78 °C to -30 °C, depending on the substrate, to afford the desired phosphines in yields ranging from 40% to 64%.[156] The same conditions have been attempted but were not satisfactory for our substrates, and less than 20% yield was obtained. This reaction presumably requires specific and time consuming optimization for each aryl bromine considered, since the same group had already reported the synthesis of some of their triarylphosphines three years earlier, performing the halogen-lithium exchange as well as PCl_3 addition at -30 °C, for yields of 30-50%.[155] Lithium-halogen exchange is generally an extremely fast reaction, the rate of lithium-halogen exchange exceeding sometimes the rate of proton transfer.[179] However, on account of the poor yields attributed to a potential limited formation of the aryl lithium specie, reaction times for lithium-halogen exchange have been varied from one minute to one hour, without observed improvement. Burgess and co-workers report reaction times between 30 min to 1 h for the lithium-halogen exchange.[155,156] Performing extensive degassing by the freeze-pump-thaw method has also been performed, but only yields below 20% were obtained in all those test reactions. One observation was interesting: TLC analysis already showed several products before addition of PCl_3, and depending on the temperature and reaction time, the mixture would have different colours after ^{t}BuLi addition, ranging from clear yellow to dark red or green. It was also attempted to perform similar tests of reaction time and reaction temperature in Et_2O, but the observations were quite similar in both solvents. ^{n}BuLi was also tested for the lithium-bromine exchange, but it appeared to be less reactive and very little product was isolated when using ^{n}BuLi. The effect of adding TMEDA to the reactions with ^{n}BuLi and ^{t}BuLi was also tested, but did not increase the yield of the reaction. Different electrophilic phosphorus compounds were also considered: similar reactions have been carried out using $P(OPh)_3$ and PBr_3 instead of PCl_3, but with no improvement on the yield.

A Grignard reaction[55] was then attempted for the formation of the triarylphosphine oxide, inspired by literature examples to form a phosphorus-aryl bond,[180] but several attempts were necessary to find optimal conditions to initiate the reaction and intensive activation of the magnesium turnings was required. Magnesium turning were dried by regular heating under high vacuum, and subjected to mechanical activation,[181] by stirring the magnesium turnings with an olive-shaped stirring-bar overnight, which resulted in the magnesium being crushed into fine powder. The resulting finely grinded magnesium powder was covered with anhydrous THF and a crystal of iodine followed by

neat aryl bromide **76** were added to the magnesium powder in order to obtain a high concentration of **76** in close proximity to the magnesium. More dilute solutions of **76** in THF failed to initiate the reaction. Regular mild heating was then applied to the unstirred mixture until the reaction was started. Sometimes, addition of a small quantity of MeI was still necessary to initiate the reaction. When the reaction was started, the mixture was stirred gently, more uniform heating was applied by immersing the reaction flask into a warm oil bath (70 °C) and a solution of **76** in THF was added dropwise. Activation by ultrasound was also considered,[182] but failed to initiate the reaction. When the reaction could be initiated, **79** was obtained in very good and reproducible yields (typically about 80 % yield with best results of 84% for the formation of three bonds). This is a clear improvement over the lithium-halogen exchange, which seems to be extremely sensitive, not reproducible in our hands and was furthermore reported to afford a maximum of 40% yield on a similar substrate.[156]

The final three ring closing metatheses (RCM) necessary for the synthesis of our target compounds **82** were expected to be the most challenging step of the sequence (see Scheme 30).

Many catalysts have been developed for RCM reactions, but none of them can be qualified of best or most efficient for all RCM reactions.[183] For simple substrates, the most important factor is the catalyst activity, but for more challenging reactions, stability becomes an increasingly important factor. Standard catalysts performances have been compared, with regard to their reaction rates and stability. Even though the study was carried on simpler systems (formation of di- tri- and tetra-substituted olefins in cyclopentenes), the study showed that although Grubbs' catalysts 1st and 2nd generations as well as Hoveyda-Grubbs' catalysts 1st and 2nd generations (see Figure 46) all were effective in catalyzing the reactions to completion for di- and tri-substituted cyclopentenes, the 1st generation catalysts required significantly longer reaction times for the synthesis of tri-substituted olefins mainly due to their lower reactivity. Finally, the more challenging formation of tetra-substituted olefins could only be catalysed by the 2nd generation catalysts, Grubbs' catalyst 2nd generation being the most efficient, yet low yields were reported in this study.[183] Those catalysts appeared as a good starting point for the formation of the di-substituted olefins we intended to form, where sterics should not be an issue, but the strain in the rings system of the target compound probably slows the reaction and requires more stable catalysts.

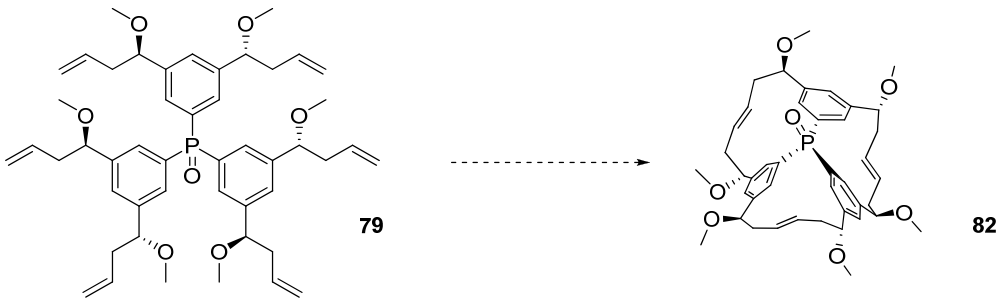
Large 13-membered rings, size of the rings we intended to form, have been accessed by RCM reaction using Grubbs' catalysts 1st and 2nd generation in good yields (63 to 81 %) by Rao *et al*..[184] Depending on the substrates, various ratios of *E* and *Z* isomers were obtained, independently of the catalyst used, but in this study, remote heteroatoms have been described to interact with ruthenium, thereby influencing the transition state geometries and the stereochemical outcome of the RCM.[184] Unfortunately, no phosphorus heterocycle was considered in this study. However, the RCM reaction catalysed by Hoveyda-Grubbs' catalyst 2nd generation and Grubbs' catalyst 1st and 2nd generation tolerates a wide range of functional groups,[185] and can be performed on highly and densely functionalised compounds such as carbohydrates. [186] Also, although Grubbs' catalyst 1st generation was ineffective for the ring closure of diallyl phenyl phosphine, it was on the contrary effective for RCM of diallyl phenyl phosphine oxide and other phosphine oxide derivatives,[187] and

Grubbs' catalyst 2nd generation has been used for high yielding RCM of compounds containing phosphorus, such as phosphonates[188] and phosphates.[189] However, it is worth noting that treatment with triphenylphosphine oxide has been described as an efficient method for the removal of residual ruthenium from olefin metathesis products.[190] It might follow that our substrates (in the open and/or in the cyclised form) forms a complex with Ru, and with the Ru-byproducts of the reaction, which would obviously hamper the isolation of the desired cyclised phosphine oxide.

Macrocyclization, although commonly used,[185] is often problematic because of the competition between the desired intramolecular reaction and intermolecular reactions giving rise to polymers. Therefore, high dilutions are generally required for the formation of large rings. Moreover, elevated temperatures and extended reaction times are required for difficult RCM of highly substituted or electron-deficient olefins, in which cases catalyst stability becomes a major issue.[191] Except for these general considerations, it appears that numerous parameters influence metathesis reactions and no guideline for general conditions will guarantee the success of the process:[185] each substrate requires fine tuning of the conditions.

Because the precursor **76** could not be observed by LCMS, the first sets of reactions carried out was being followed by TLC. The starting material **79** has a distinct R_f from the products, but many by-products were also formed during the reaction having similar R_f to the products of the RCM. The mixture were analysed by HPLC-MS, and luckily, the starting material **79** as well as the products of RCM could be observed by LC-MS. Thus, rapidly, test reactions were followed by LC-MS.

Scheme 30: Synthesis of 82



Initial test conditions included a small screening of solvent (toluene, CH_2Cl_2 and benzene), temperature effect (from 20 °C to reflux of the above-mentioned solvents), catalyst (Grubbs' catalyst 1st and 2nd generation,[192,193] and Hoveyda-Grubbs' catalyst 1st and 2nd generation (see Figure 46),[194,195] as well as catalyst loading (4 mol % to 20 mol %) and concentration of the starting material **79** (0.01M and 0.001M).

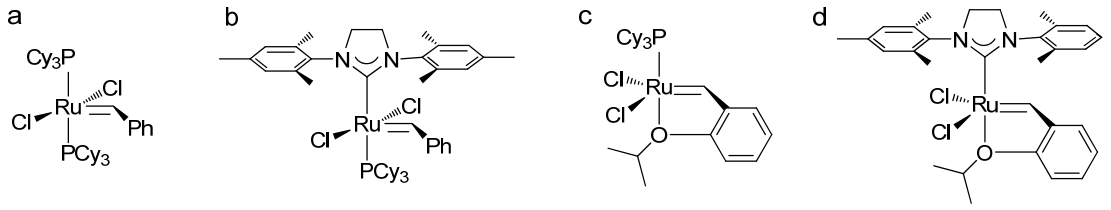
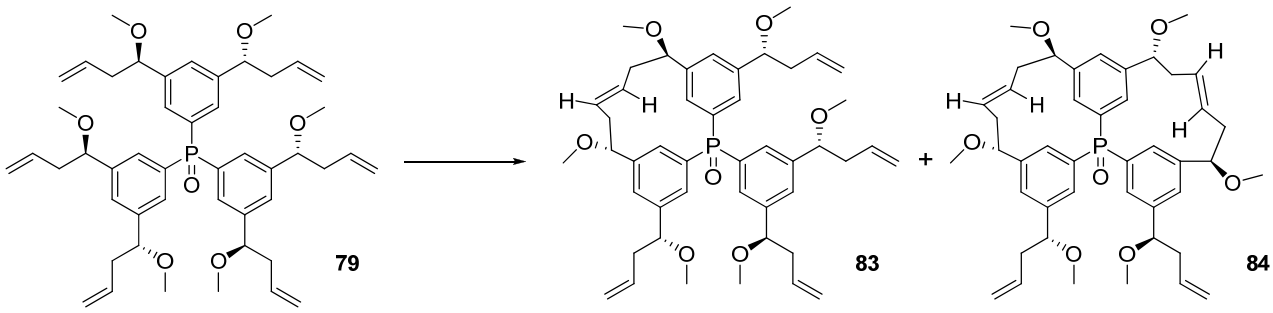


Figure 46: Catalysts considered in the preliminary experiments. a) Grubbs' catalyst 1st generation; b) Grubbs' catalyst 2nd generation, c) Hoveyda-Grubbs'catalyst 1st generation; d) Hoveyda-Grubbs' catalyst 2nd generation.

From those first test experiments, it seemed that the Grubbs' catalyst 2nd generation gave better results: more side products could be observed by TLC with the Hoveyda-Grubbs' catalysts, and for equal reaction times, **79** was fully reacted with the 2nd generations of the Grubbs' and Hoveyda-Grubbs' catalysts, but not for the 1st generation catalysts. However, even in the best cases, mixtures of one RCM and some two RCM products, compounds **83** and **84** respectively (see Scheme 31) were observed by LC-MS.

Scheme 31: One RCM (83) and two RCM (84) products observed in the test reactions.



A concentration of 0.01M of **79** was also chosen for the subsequent reactions, since the reaction was faster with that concentration, and it did not seem that cross-metathesis was efficiently avoided by the 10 times dilution.

For the solvents, more by-products were formed (observed by TLC) in toluene, and benzene and CH_2Cl_2 seemed better choices.

Interestingly, two peaks of m/z 727 (corresponding to the molecular weight of **84**) were observed in LC-MS traces. This led us to think that conformational strain might already give rise to two conformers of **84**, and that higher temperatures might be required for the last two alkene tethers to reach each other and thereby to allow the last RCM to occur. Thus, microwave heating was considered for the following reactions. Indeed, microwave heating under sealed vessel conditions, so-called "microwave flash heating", provides an extremely fast and more homogeneous heating than more traditional external heat source (as oil bath used in the preliminary test reactions).[196] This

rather modern heating method (the first reports of microwave heating to accelerate the rate of organic reactions were reported by Gedye *et al.* and by Giguere *et al.* in 1986[197,198]) has been successfully applied in RCM reactions and ene-yne metathesis, for which otherwise sluggish reactions were completed within minutes using microwave heating.[199,200] The improved rates of RCM reactions were assigned to the rapid and uniform heating of the reaction mixture, as well as to the increased lifetime of the catalyst due to elimination of the wall effects.[199,200] Even though CH_2Cl_2 has a low loss factor ($\tan\delta = 0.042$), it is not microwave transparent and heats up in microwave reactors. Finally, CH_2Cl_2 has been used effectively for RCM reactions at 100 °C under microwave hating.[196,200]

Further attempts of optimization were thus performed under microwave heating. The reactions were monitored by LC-MS, for which the UV trace combined with the MS-data gave a clear picture of the reaction outcomes, such as whether the starting material was fully reacted as well as whether products with the expected mass were formed, but no quantitative data could be extracted.

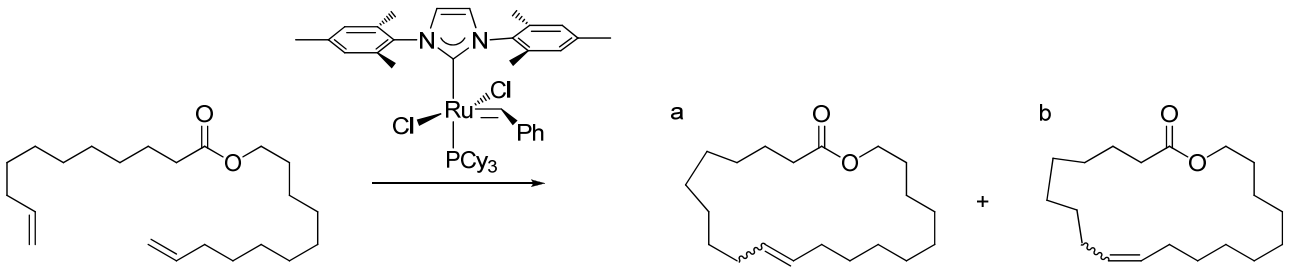
We first studied solvents effects, with benzene and CH_2Cl_2 (low microwave absorbing solvents), 1,2-dichloroethane and chlorobenzene (two medium microwave absorbing solvents):[196] it was expected that the difference in polarity of these solvents would have an effect on their heating using microwaves. Nevertheless, all four solvents could be heated to 120 °C, and the reactions were performed using Grubbs' catalyst 2nd generation (2.5 mol %) for 30 min. Benzene and chlorobenzene gave the poorest results, where the starting material was not fully converted, **83** was the major peak and the peak corresponding **84** was also significant. Reactions performed in 1,2-dichloroethane and CH_2Cl_2 had very similar outcomes, with a little less by-products in CH_2Cl_2. Gratifyingly, in those experiments, a peak for the formation of the three RCM (**82**, see Scheme 30) product was also observed yet along with a complex mixture containing the starting material, **83**, **84** and unidentified by-products. Thus, CH_2Cl_2 was chosen for the next experiments.

Reaction time was briefly investigated: increasing the reaction time to 1h did not give better conversion, but decreasing the reaction time to 15 min gave similar conversion to the RCM products and less by-products. Further decreasing the reaction time to 5 min resulted in the formation of **84** mainly, in a cleaner reaction. This result interested us: we thought it could be easier to isolate **84** and try to perform the last RCM on the pure **84**. Indeed, products of degradation of the catalyst might hamper its reactivity, which could be one reason why the last RCM seemed to be so difficult to carry out. We thus hoped to be able to form the desired product **82** from **84** having only the fresh batch of catalyst in the reaction vessel.

Due to the equipment limitations, scaling up was not possible and the latest reaction (2.5 % equiv. Grubbs' catalyst 2nd generation in CH_2Cl_2 at 120 °C for 5 min under microwave heating) was performed five times in order obtain enough material and try to isolate **84**. From those reactions, performed with the exact same conditions and on the same batch of precursor and catalyst, it appeared that the microwave experiments were hardly reproducible: the UV traces were similar, but not identical, especially, for one of those reactions, the starting material had apparently fully reacted, a result that had not been observed before, and could not be reproduced The crude was

flashed repeatedly with different eluent systems (EtOAc and toluene/MeOH 8:2), but this was not sufficient to purify the desired product. Recrystallization was attempted, but with no success (also due to the small amounts isolated after the first purification by flash chromatography: about 50 mg). Furthermore, a by-product with m/z = 713 (m/z (**84**) -14) having a retention time close to **84** was observed and could not be separated by flash chromatography. This mass corresponds to a loss of methylene unit, such a by-product can be attributed to an isomerisation of the terminal olefin followed by a constructive RCM. Indeed, Grubbs' catalysts 2nd generation has been reported to promote extensive olefin isomerisation[201,202] and isomerisation followed by metathesis promoted by Grubbs' catalyst 2nd generation was reported and studied.[201] Moreover, by-products of isomerisation followed by RCM have been isolated with the unsaturated derivative of Grubbs' catalyst 2nd generation (see Scheme 32). It has been reported that under RCM reaction conditions, isomerisation occurs when attempting to form large ring systems or when the precursors have conformations that are not germane to cyclisation, resulting in slow metathesis ring-closure events, which is the case with our substrate.

Scheme 32 Example of olefin isomerisation followed by RCM with Ru catalyst. a) desired 21-membered ring; b) by-product resulting from the olefin isomerisation prior to RCM.[203]



Reducing the temperature to 100 °C, 80 °C or 40 °C under microwave heating resulted in less by-product, but did not suppress its formation, accordingly, isomerisations have been reported to occur readily at temperatures of 50-60 °C.

However, Grubbs and co-workers have reported that high temperatures increase the formation of the isomerisation by-product, and suggest that benzoquinones can prevent isomerisation.[204] Therefore, we decided to pursue the investigation at 40 °C, and tested the efficiency of three quinones, namely 1,2-benzoquinone, 2,6-dichloro-1,4-benzoquinone and tetrafluoro-1,4-benzoquinone, in the suppression of the by-product formation. Indeed, the two latter were reported to be the most effective inhibitors for the isomerisation side reaction.[204] However, due to the scale limitation of the microwave equipment, and because we chose to decrease the reaction temperature to avoid isomerisation by-products, we chose to work on larger scales under conventional heating.

Thus, the next set of test experiments were carried out in CH_2Cl_2 at reflux (with oil-bath heating), adding two portions of benzoquinone (2 × 5 mol %) followed by the catalyst (2 × 2.5 mol % Grubbs' 2nd generation) over 2 h. Formation of the by-product was fully suppressed by 2,6-

dichloro-1,4-benzoquinone as well as by tetrafluoro-1,4-benzoquinone. Moreover, after addition of the second batch of catalyst, **82** had been formed to some extent (LC-MS), the same reaction performed on **84**, previously purified in some extent, did not lead to dramatically improved formation of **82**. Consequently, the idea of isolating **84** in a first stage was abandoned, and we tried instead to push the reaction toward the desired final product **82**.

Therefore, regular addition of 2,6-dichloro-1,4-benzoquinone (20 mol %) followed by Grubbs' catalyst 2nd generation (10 mol %) was performed every hour, and the reaction mixture analysed by LC-MS before and after each addition. After 4 h, the starting material was fully converted, and only traces of the product of one RCM **83** was observed, the desired compound **82** being the major product.

One last screening of catalyst was performed under these reaction conditions: From the previous work, it seemed that the second generation of Grubbs' catalyst and Hoveyda-Grubbs' catalyst were fully converting the starting material in similar reaction times, even though Hoveyda-Grubbs' catalyst seemed to give more side products. We tested both catalysts again under the last improved reaction conditions, and tested also two other catalysts very close in structure to the two previous ones, but a little less sterically hindered: dichloro[1,3-bis(2-methylphenyl)-2-imidazolidinylidene](benzylidene)(tricyclohexylphosphine)ruthenium(II) and dichloro[1,3-bis(2-methylphenyl)-2-imidazolidinylidene](2-isopropoxyphenylmethylene)ruthenium(II) (see Figure 47). Five additions of 2,6-dichloro-1,4-benzoquinone (20 mol %) followed by Ru-catalyst (10 mol %) was performed every hour, and the reaction mixture analysed by LC-MS before and after each addition in CH_2Cl_2 at reflux.

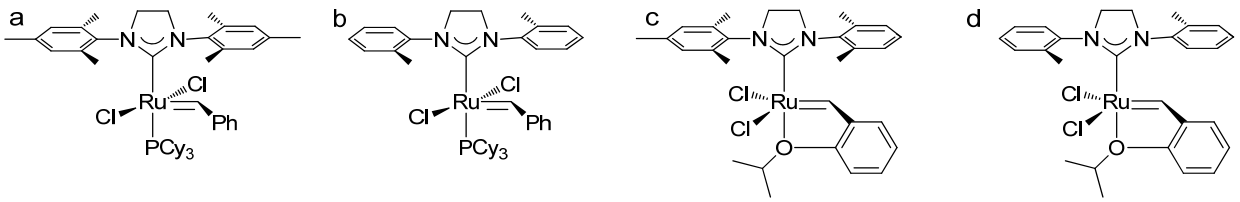


Figure 47: Catalysts tested in the latest experiments. a) Grubbs' catalyst 2nd generation; b) Dichloro[1,3-bis(2-methylphenyl)-2-imidazolidinylidene](benzylidene)(tricyclohexylphosphine)ruthenium(II), c) Hoveyda-Grubbs' 2nd generation; d) Dichloro[1,3-bis(2-methylphenyl)-2-imidazolidinylidene](2-isopropoxyphenylmethylene) ruthenium(II).

From those experiments, better conversion to **82** were obtained with Grubbs' catalyst 2nd generation and with Hoveyda-Grubbs' catalyst 2nd generation. Thus the two latest experiments were performed on larger scales (500 mg of starting material **79**). After the five additions, very little **83** was still present in the mixture and **84** was the major product, and the reaction was continued at reflux overnight, after which **83** had fully reacted, some **84** was left and **82** was formed in significant amounts (see Figure 48). The UV-trace obtained from the reaction performed with Grubbs' catalyst

2nd generation shows a shoulder on the peak of the desired product and it has not been identified whether the main peak is the desired compound **82** or a by-product.

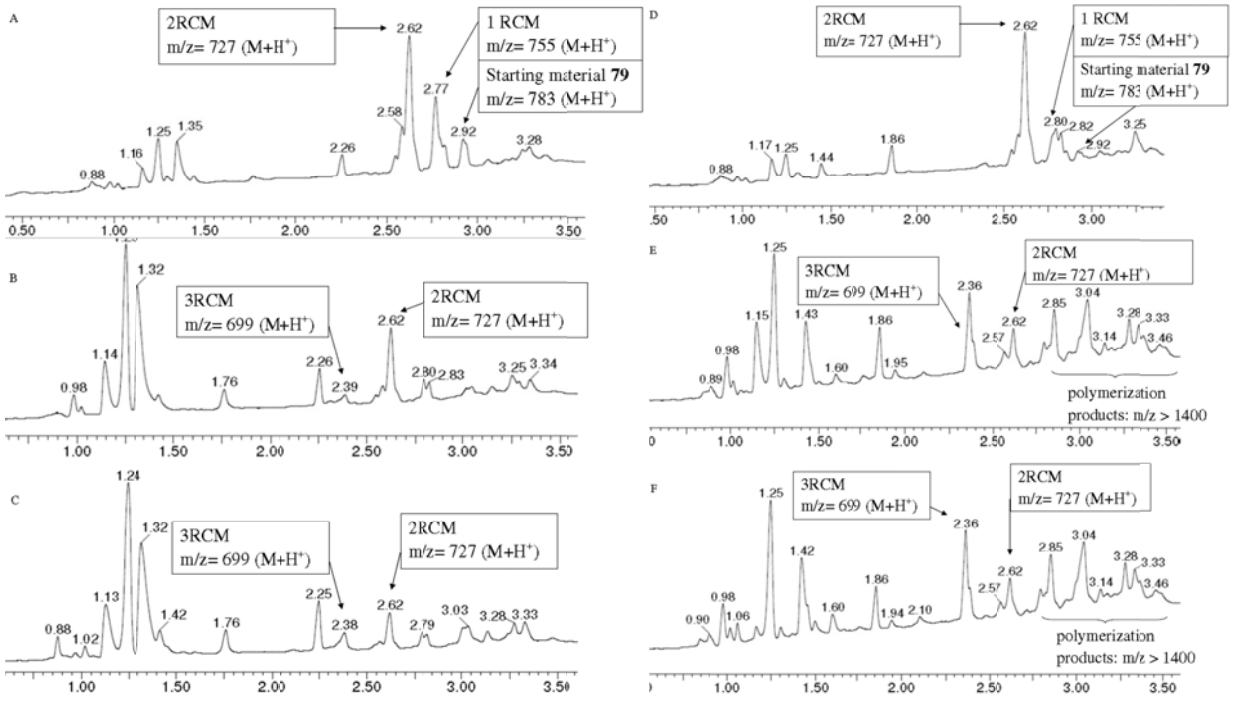


Figure 48: UV-traces from LC-MS analysis of the experiments performed with Hoveyda-Grubbs' catalyst 2nd generation [A: after 1h, B: after 5h and C: after 19 h.] and Grubbs' catalyst 2nd generation [D: after 1h, E: after 5h and F: after 19 h.].

Purification also revealed challenging for compound **82**, Figure 48 gives an idea of the mixture the desired compound had to be separated from. Cyclophane **82** could not be purified by direct preparative HPLC because of the complex mixture. Moreover, it seemed that the compound was behaving very differently on flash chromatography compared to TLC, and **82** could not be isolated after flash chromatography: even polar eluents (toluene/MeOH 80:20 and toluene/MeOH/H_2O 80:19.5:0.5) still resulted in high retention on the silica, and extensive tailing of the compound, such that only traces of **82** were isolated and yet still contaminated with by-products. Instead, the compound was purified by successive preparative TLC, using different eluent systems: toluene/methanol (80:20) followed by EtOAc/methanol (95:5) resulted in very different separation of the by-products as can be seen from Figure 49, and this permitted the isolation of **82** in reasonable purity. Final purification was performed by preparative HPLC.

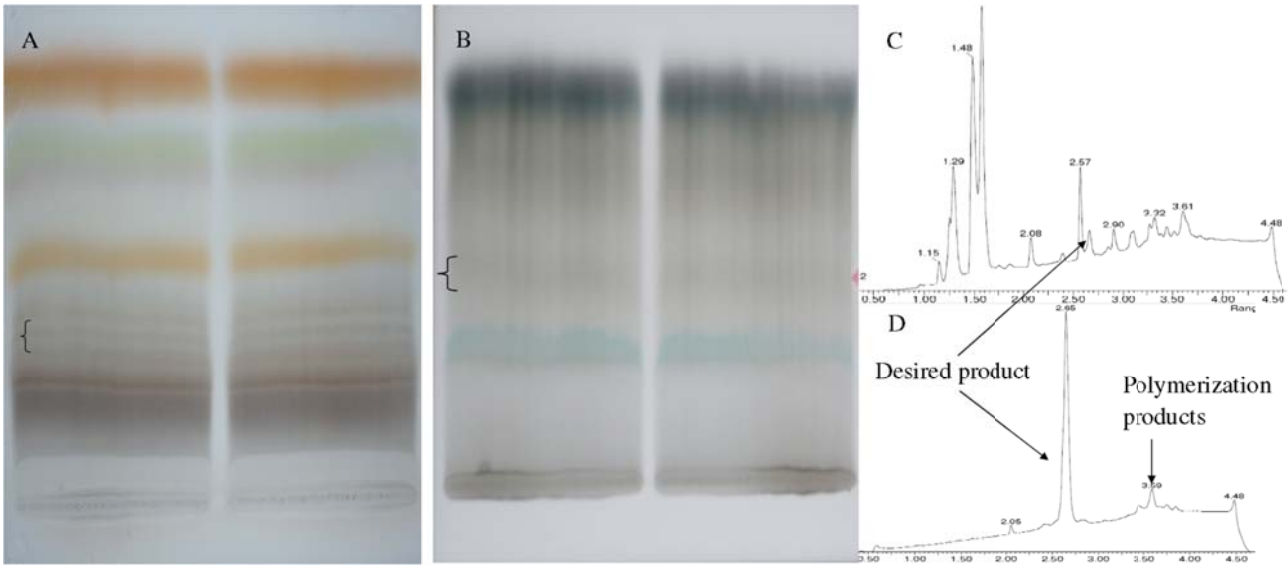


Figure 49: Successive preparative TLC and LC-MS analysis: A) first preparative TLC performed on the crude mixture (eluent: toluene/MeOH: 8:2); B) Preparative TLC performed on the residue obtained after the 1st preparative TLC (eluent: EtOAc/MeOH 95:5). The brackets show the band containing the desired compound (determined by LC-MS analysis); C) LCMS of the experiments performed with Hoveyda-Grubbs' catalyst 2nd generation before purification by preparative TLC; D) LCMS of the experiments performed with Hoveyda-Grubbs' catalyst 2nd generation after purification by preparative TLC

Two last experiments with 1,4-dichlorobenzoquinone have been performed using microwave heating to compare with the earlier reactions. In the first reaction, two batches of 20 mol % 1,4-dichlorobenzoquinone followed by 10% mol Grubbs' catalyst 2nd generation have been added and the mixture heated for 5 min at 100 °C each time, then one more batch of 40 mol % 1,4-dichlorobenzoquinone followed by 20 mol % Grubbs' catalyst 2nd generation have been added and the mixture heated for 20 min at 100 °C (samples were taken for HPLC analysis after each sequence and can be seen in Figure 50 A, B and C). In the second reaction, 80 mol % 1,4-dichlorobenzoquinone followed by 40 mol % Grubbs' catalyst 2nd generation have been added in one batch and heated at 100 °C for 20 min. As for regular heating experiments, the quinone effectively suppressed formation of the by-product with m/z=713, but mainly **84** was formed (see Figure 50 D). As seen from HPLC chromatograms (Figure 50), those experiments show a cleaner conversion to **84**, but the conversion to the desired compound **82** remains challenging. A better conversion to **82** was observed upon fractioned addition of the quinone and catalyst, and the compound was isolated with similar yield (2%) as when heating to 40 °C under regular heating, after the same purification procedures as described earlier.

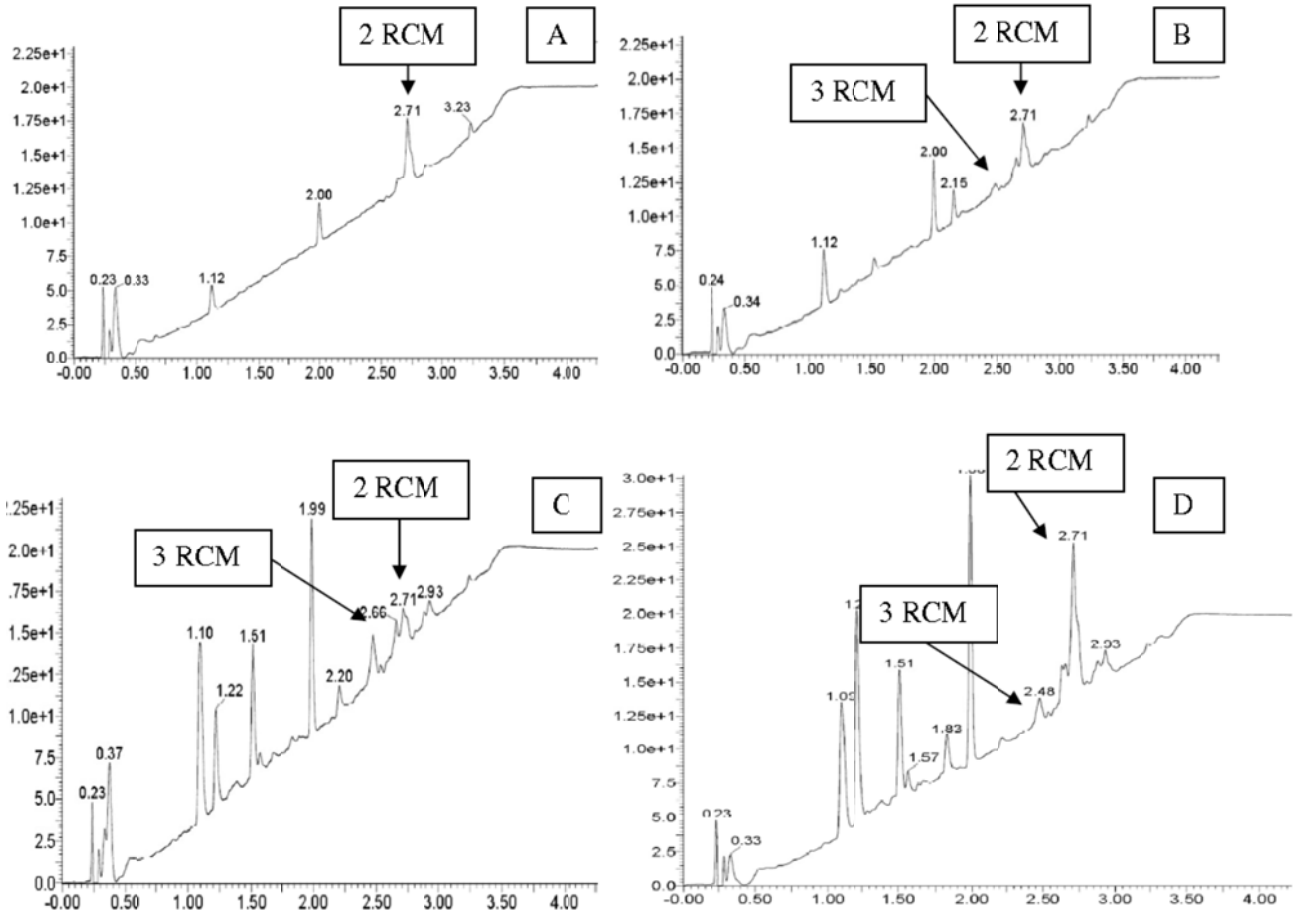


Figure 50: HPLC-MS analysis of the last two microwave reactions. A: First reaction after 5 min reaction at 100 °C with 20 mol % 1,4-dichlorobenzoquinone and 10 mol % Grubbs' catalyst 2nd generation. B: After second addition 20 mol % 1,4-dichlorobenzoquinone and 10 mol % Grubbs' catalyst 2nd generation and additional 5 min reaction at 100 °C. D: After addition 40 mol % 1,4-dichlorobenzoquinone and 20 mol %. Grubbs' catalyst 2nd generation, additional 20 min reaction at 100 °C. D: Second reaction, after 20 min reaction at 100 °C with 80 mol % 1,4-dichlorobenzoquinone and 40 mol % Grubbs' catalyst 2nd generation.

4.4. Structural studies

Compound **82** was characterized by NMR analysis, configuration around the alkene was determined by J coupling constant ($J(H_5\text{-}H_6)$ = 17.1 Hz) read from COSY NMR spectrum, since the ^{1}H NMR spectrum was too congested in the olefin region.

Compound **82** is potentially C_3 symmetric, if it is locked in a propeller-shaped with a high barrier to rotation and all three aryl rings are turning the same way, or alternatively, if there is rapid rotation around all three C-P bonds. In that scenario, it will display three sets of equivalent atoms, i.e. the structure can be fully described by a the set of atoms of a phenyl ring along with the atoms that link it to the next phenyl ring. It can be seen from the ^{1}H NMR data that this is indeed the case. Furthermore, the two sides of an aromatic ring are not equivalent; indeed, two different signals are observed in ^{1}H NMR for protons H_{10} and H_{11} for instance (see numbering from Figure 51).

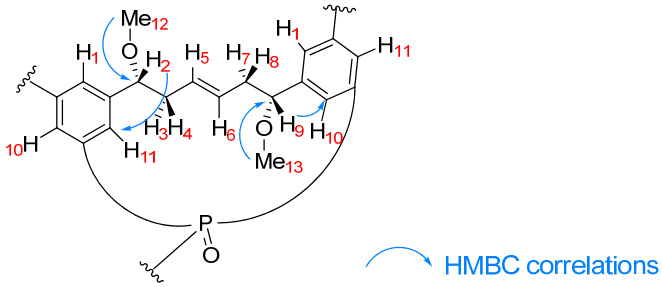


Figure 51: HMBC coupling pattern for 82.

This non-equivalence results from the propeller shape taken by the aromatic rings: one side of the phenyl ring is above the surface created by the ring system, on the same side as the P=O bond, while the other side is below the surface, and the same is true for all set of protons. Taking for reference Figure 52, protons and methoxy-groups on the same side as the P=O bond are on the β-face, and are pointing upwards (shown with a solid line), while the protons and methoxy groups on the face opposite to the P=O bond are pointing towards the cavity, which will be called the α-face (see Figure 52). The two ortho protons are not equivalent: one of them is on the α-face and the other on the β-face and have distinct chemical shifts. It is not possible with certainty to assign the signals from the protons on the the α- or on the β-face from the ^{1}H NMR data, since both protons will be in the anisotropic shielding cone of the neighboring phenyl ring. The protons on the β-face will feel the shielding effect of the P=O bond, which the α-protons will not. Differential shielding can also arise from proximity to the the alkene π-system in the bridging carbon chains.

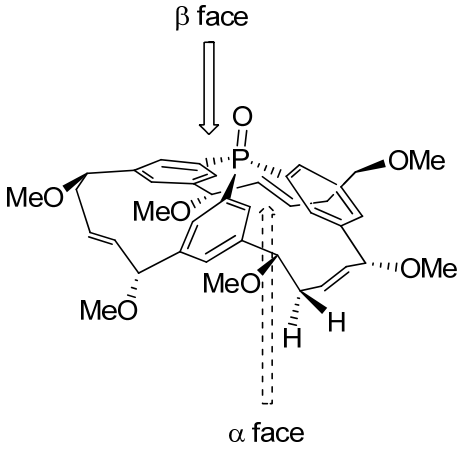


Figure 52: Definition of α-face and β-face.

In order to shed light on whether the phosphine oxide **82** was indeed locked in a preferred and stable propeller conformation, it was analysed by variable temperature ^{1}H-NMR and circular dichroism (CD). ^{1}H-NMR in dmso-d6 up to 150 °C did not show any significant changes in the

spectra, indicating that either the structure is flexible at room temperature or the barrier to interconversion between the two diastereomeric conformations is very high. We then proceeded to low-temperature NMR, which was obtained by cooling a sample of **82** in CD_2Cl_2 to -100 °C and heating it gradually to 25 °C. The only region of the spectrum that displayed any dependence on the temperature is in the aromatic region and this can be seen in Figure 53. While the signals at 7.6 and 7.5 ppm do not change appreciably, the last aromatic signal resonate at 7.0 ppm at room temperature, but moves upfield upon cooling, reaching 6.3 ppm at -100 °C. There is significant signal broadening at intermediate temperatures, which indicates a dynamic process that is slowed down upon cooling. One possible explanation for the observed temperature dependence is that at room temperature there is significant flexibility and rapid rotation of the C-P bonds or movement of the bridging carbon chains, and the shielding is averaged out somewhat by this movement. Upon cooling, this process is slowed down (leading to signal broadening) and upon reaching -100 °C (where the signals are sharp again), **82** is locked in a rigid conformation where the anisotropic shielding is maximized, leading to a chemical shift at 6.3 ppm. However, it cannot be ruled out that what is observed at room temperature is an average of the two diastereomeric conformations (a left-hand propeller and a right-hand propeller), which would arise if there was rapid equilibration between them. But, considering the significant chemical shift difference (0.5 ppm) between the two ortho protons, it does appear unlikely, since their local environment would be very similar in the two propellers, where the only difference would be in the remote stereocenters.

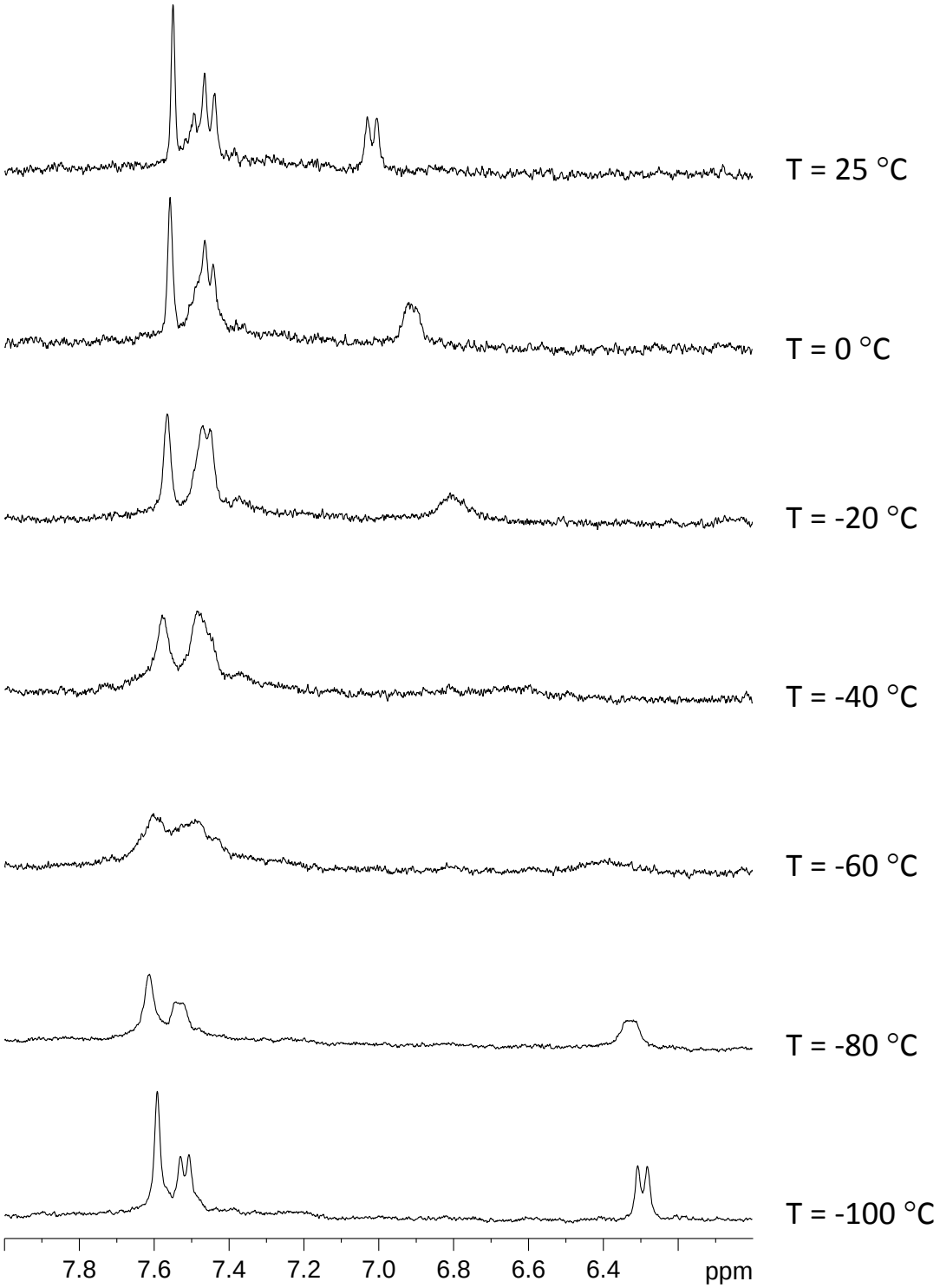


Figure 53: Variable-temperature 1H-NMR of 82 in CD_2Cl_2. The aromatic region is shown.

If one propeller conformation dominates at room temperature, one would expect this to be reflected in the CD spectrum, when this is compared to similar not-rigid molecules. Therefore, we obtained the CD spectra of **82** and the two precursors **76** (having only the two chiral centers) and **79** (a

triarylphosphine oxide expected to have a high degree of flexibility). The results can be seen in Figure 54 and the CD data does not support a rigid, single-propeller conformation for **82**.

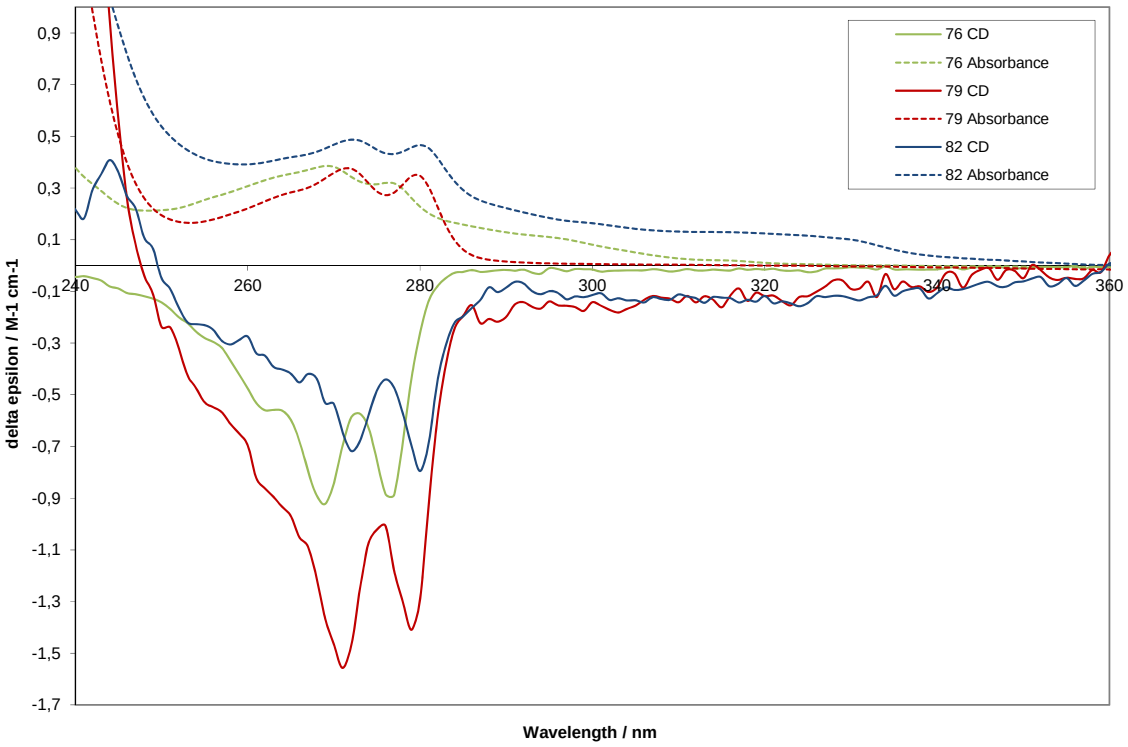


Figure 54: Absorbance and CD spectra for compounds 76, 79 and 82 in acetonitrile solution. It can be expected that a rigid, propeller conformation for 82 would lead to a shift in the CD spectrum compared to the precursors 76 and 79 and as can be seen, this is not the case.

4.5. Conclusion

A C_3-symmetric triarylphosphine oxide was designed in order to investigate whether central chiral centers could act as a relay and stabilise a single diastereomeric propeller conformation of the molecule **82**. The synthesis was executed after extensive optimisation of both the step to form the triarylphoshine **79** and the final one-pot three-RCM reaction, which eventually led to **82**, which could be isolated in 2% yield, due to formation of several byproducts and great difficulties in separating the desired compound from these and ruthenium compounds.

Initial structural studies of the solution conformation of **82** were performed using CD and variable temperature NMR. The results are inconclusive and further investigations will be needed to establish whether the relay strategy is successful.

5. Experimentals

5.1. General experimental

Starting materials, reagents, and solvents were purchased from Sigma-Aldrich Chemical Co. and used without further purification. CH_2Cl_2 was dried over 4 Å molecular sieves and THF was dried over sodium/benzophenone and distilled before use. Evaporation of solvents was done under reduced pressure (*in vacuo*). Thin layer chromatography (TLC) was performed on Merck aluminum sheets precoated with silica gel 60 F254. Compounds were visualized by UV irradiation at 254 nm and/or by charring after dipping in a solution of 6.25 g of $(NH_4)_6Mo_7O_{24}$ and 1.5 g of $Ce(SO_4)_2$ in 250 mL of 10% aqueous H_2SO_4, in an ethanolic solution of phosphomolybdenic acid (48 g/L) or in a solution of 10 mL of *p*-anisaldehyde, 10 mL of a concentrated aqueous solution of H_2SO_4 and 250 mL EtOH. Column chromatography was performed using Matrex 60 Å silica gel. The purity of the tested compounds was found to be >95% by HPLC. Normal-phase HPLC was performed on a Waters Alliance HPLC equipped with a diode array detector, using a LiChrospher Si 60 column and eluting with water/isopropanol/hexane mixtures or for chiral analysis a Chiralcel AS-H or OD-H column and eluting with hexane/2-propanol mixtures. RP-HPLC was obtained using a Shimadzu LC-2010C analytical HPLC by employing a XTerra RP8 5μm (4.6*150mm) column and eluting with water/acetonitrile mixtures containing 0.1% TFA. Analytical UPLC/MS (ESI) analysis was performed on a Waters AQUITY RP-UPLC system equipped with a diode array detector using an AQUITY UPLC BEH C-18 column (*d* 1.7 μm, 2.1 x 50 mm; column temp: 65 °C; flow: 0.6 mL/min). Eluents A (0.1% HCO_2H in water) and B (0.1% HCO_2H in acetonitrile) were used in a linear gradient (5% B to 100% B) in a total run time of 5.2 min. The LC system was coupled to a SQD mass spectrometer. The LC system was coupled to a Micromass LCT orthogonal time-of-flight mass spectrometer equipped with a Lock Mass probe operating in positive electrospray mode. NMR spectra were recorded using a Bruker AC 200 MHz spectrometer, a Varian Mercury 300 MHz spectrometer, a Bruker Ascend 400 MHz spectrometer, a Varian Unity Inova 500 MHz spectrometer or a Bruker Ascend 500 MHz spectrometer. Chemical shifts were measured in ppm and coupling constants in Hz, and the field is indicated in each case. IR analysis was carried out on a Bruker Alpha FT-IR spectrometer and optical rotations were measured with a Perkin-Elmer 341 polarimeter, units for $[\propto]_{589}^{20}$ are 10^{-1} deg cm^2 g^{-1}. CD was performed on a Jasco J-815 CD spectrometer equipped with a Jasco CDF-426 S/15 controller and a Julabo F12 temperature controller. HRMS was recorded on an Ionspec Ultima Fourier transform mass spectrometer. Melting points were measured by a Buch & Holm melting point apparatus and given in degrees Celsius (°C) uncorrected.

5.2. Procedures for biophysical and biological characterisation

Liposome preparation, particle size determination and main phase transition temperature measurement

The phospholipids were dissolved in $CHCl_3$ in a test tube and dried under vacuum for 15 h to form a thin film. The (phospho)lipids (2 mM) were solubilized by addition of aqueous buffer (HEPES: 0.15 M KCl, 30 μM $CaCl_2$, 10 μM EDTA, 10 mM HEPES, pH 7.5 or PBS: 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 2.7 mM potassium chloride, 0.137 M sodium chloride, pH 7.4) and vortexed periodically over 1 h at 20 °C. Subsequently, the solutions were extruded through a 100 nm polycarbonate cutoff membrane (about 20 times) using a Hamilton syringe extruder (Avanti Polar Lipids, Birmingham, AL). The particle size distribution of the formulated lipids was measured by DLS. The DLS measurements were obtained using a Zetasizer Nano Particle Analyzer (ZS ZEN3600, Malvern Instrument, Westborough, MA) or a ZetaPALS zeta potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY). DSC was performed from 10 to 70 °C using a MicroCal MC-2 (Northhampton, MA) on samples at a scan rate of 10 °C /h. A DSC scan was also performed on the HEPES buffer solution and was used to subtract a baseline from the thermograms of the measured samples.

$sPLA_2$ activity measurements monitored by MALDI-TOF-MS

The formulated phospholipids (0.40 mL, 2 mM) were diluted in an aqueous buffer (2.1 mL, 0.15 M KCl, 30 μM $CaCl_2$, 10 μM EDTA, 10 mM HEPES, pH 7.5) and the mixture was stirred at 37 °C in a test tube protected from light. The catalytic reaction was initiated by addition of human $sPLA_2$ IIA (40 μL tear fluid). Sampling was done after 0, 1, 2, 4, 8, 24 and 48 and 96 h by collecting 100 μL of the reaction mixture and rapidly mixing it with a solution of $CHCl_3$/MeOH/H_2O/AcOH 4:8:1:1 (0.5 mL) in order to stop the reaction. The mixture was washed with water (0.5 mL) and the organic phase (80 μL) was isolated by extraction and then concentrated *in vacuo*. For the MALDI-TOF-MS analysis the extract was mixed with 9 μL of DHB matrix (0.5 M DHB, 2 mM CF_3COONa, 1 mg/mL DMPG in MeOH), and 0.5 μL of this mixture was used for the MS analysis. Tear fluid was used as the source for human $sPLA_2$ IIA. Tear fluid was collected from healthy subject exposed to onion vapor. Tear fluid has a high concentration of $sPLA_2$ IIA and is the only prevalent $sPLA_2$ species found in tears. The $sPLA_2$ IIA content in tears in healthy subjects (54.5 μg/ml) is one of the highest amounts of $sPLA_2$ IIA content reported in human secretions.[77,78,79]

The CuAAC reaction on liposomes

Lipids were dissolved in CH_3Cl/MeOH (9:1) and mixed in the ratio 95:5 POPC/**56** or POPC/**57**. The solvent was removed under a stream of nitrogen and the films placed under vacuum overnight to remove remaining traces of organic solvent. The obtained films were hydrated in a PBS buffer, at room temperature for 1 h by periodical vortexing, followed by extrusion at room temperature through a 100 nm polycarbonate filter using an Avanti Polar Lipids mini-extruder. Preformed functionalized liposomes (25 mM, 0.25 mL, 1 equiv.) were mixed with the corresponding coupling partner (fluorescein-azide for the liposomes containing **56** and fluorescein-alkyne for liposomes with **57**) (0.95 mM; 107 μL, 0.65 equiv.), sodium ascorbate in PBS (5.6 mM, 50 μL, 1.67 equiv.), either ligand in PBS (2.24 mM, 50 μL, 0.66 equiv.) or PBS (50 μL) and copper sulfate (1.12 mM; 50 μL, 0.33 equiv.) The reactions were shaken at room temperature and aliquots were taken for HPLC analysis after 0 (before addition of copper sulfate), 120, 480 and 1440 minutes (45 μL solution was removed, mixed with 45 μL PBS and 30 μL of this was injected for HPLC). A linear gradient was used from 100% A (water containing 0.1% TFA) to 100% B (MeCN containing 0.1% TFA) over 20 min with a flow rate of 1 mL/min. The AUC for the fluorescein derivatives was measured at 280 nm to monitor the conjugation efficiency.

Reaction between propargyl amine functionalised liposomes and FITC

Lipids were dissolved in CH_3Cl/MeOH (9:1) and mixed in the ratio 99:1 POPC/**59**. The solvent was removed under a stream of nitrogen and the films placed under vacuum overnight to remove remaining traces of organic solvent. The obtained films were hydrated in a PBS buffer, at room temperature for 1 h; followed by 5 freeze−thaw cycles and extrusion at room temperature through a 100 nm polycarbonate filter using an Avanti Polar Lipids mini-extruder. The size distribution of the liposomes was analyzed using dynamic light scattering, before and after the incubation with FITC. Preformed functionalized liposomes (25 mM, 0.25 mL, 1 equiv.) were mixed with the corresponding fluorescein isothiocyanate (0.15 mM, 107 μL, 0.5 equiv.) dissolved in PBS, then PBS was added to a final volume of 0.5 mL. The reactions were shaken (not stirred; to avoid foaming) at room temperature and aliquots (40 μL) removed for analysis by analytical HPLC. A linear gradient was used from 70% A (aqueous solution containing 5% MeCN and 0.1% TFA) to 100% B (MeCN containing 0.1% TFA) over 20 min with a flow rate of 1 mL/min. The AUC for the free FITC was compared to the AUC of the phospholipid coupled products at 254 nm to monitor the conjugation efficiency.

Experimental data for compounds

10-Phenyldecanal (2)

In a flame-dried 100 mL three-necked round-bottomed flask, anhydrous Me_2SO (14.1 mmol, 1 mL) in anhydrous CH_2Cl_2 (32 mL) under argon was cooled to -78 °C. A solution of $(COCl)_2$ (7.05 mmol, 597 µL) in anhydrous CH_2Cl_2 (1.5 mL) was added dropwise in order to keep the reaction temperature below -60 °C, and the misture was stirred at -78 °C for 10 min, after what a solution of 10-phenyldecanol (6.41 mmol, 1.5 g) in CH_2Cl_2 (6 mL) was added dropwise (maintain the reaction temperature below -60 °C) and the reaction mixture was stirred for 45 min. Et_3N (32.05 mmol, 4.5 mL) was added dropwise at -78 °C, the mixture was stirred for 15 min at that temperature then allowed to reach 20 °C slowly. Water (40 mL) was added, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 40 mL). The organic phases were combined, washed with aq. 1% HCl (100 mL), water (100 mL), aq. 5% Na_2CO_3 (100 mL) and water (100 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give 1.485 mg of a clear oil. The crude product was used without further purification. R*f* = 0.22 (hexane/EtOAC 19:1). ^{1}H NMR (300 MHz,$CDCl_3$): δ 9.76 (t, *J* = 1.9 Hz, 1H, C<u>H</u>O), 7.30-7.25 (m, 2H, Ph-<u>meta</u>), 7.18-7.15 (m, 3H, Ph-<u>ortho</u> and -<u>para</u>), 2.60 (t, *J* =7.5 Hz, 2H, C<u>H$_2$</u>-Ph), 2.41 (td, *J* = 7.3, 1.9 Hz, 2H, C<u>H$_2$</u>-CHO), 1.60 (m, 4H, C<u>H$_2$</u>-CH_2-CHO and C<u>H$_2$</u>-CH_2-Ph), 1.29 (m, 10H, CH_2-(C<u>H$_2$</u>)$_5$-CH_2).[214] ^{13}C NMR (75 MHz, $CDCl_3$): δ 203.0, 142.9, 128.3 (2C), 128.2 (2C), 125.5, 45.6, 43.9, 36.0, 31.5, 29.4, 29.3, 29.2, 29.1, 22.0.

Methyl *(E)*-12-phenyldodec-2-enoate (3)

In a flame-dried 100 mL round bottom flask, NaH ((60 % w/w) in mineral oil, 10.26 mmol, 410 mg) was suspended in freshly distilled DME (25 mL) under argon, and methyl diethylphosphonoacetate (9.36 mmol, 1.7 mL) was added dropwise. The solution was stirred at 45 °C for 1 h, **2** (6.41 mmol, 1.49 g) was added and the solution was stirred for 2 h at 50 °C. The crude mixture was then poured into ice-water, the organic layer was separated, and the aqueous layer was extracted with EtOAc (3 × 25 mL). The organic phases were combined, washed with ice-water (100 mL), brine (100 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a yellow oil. Purification of the crude oil by flash chromatography (heptane/EtOAc 29:1) yielded **3** as a colourless oil (1.37 g, 74%). R_f = 0.33 (toluene/hexane 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.27 (m, 2H, aromatic-*meta*), 7.20-7.18 (m, 3H, aromatic-*ortho* and -*para*), 6.99 (dt, *J* = 15.6, 7.1 Hz, 1H C<u>H</u>=CH-COOMe), 5.83 (dt, *J* = 15.6, 1.5 Hz, 1H, CH=C<u>H</u>-COOMe), 3.73 (s, 3H, O-C<u>H$_3$</u>), 2.61 (t, *J* = 7.9 Hz, 2H, C<u>H$_2$</u>-Ph), 2.2 (qd, *J* = 7.1, 1.5 Hz, 2H, C<u>H$_2$</u>-CH=CH-COOMe), 1.67-1.57 (m, 2H, C<u>H$_2$</u>-CH_2-Ph), 1.48-1.40 (m, 2H, Ph-CH_2-CH_2-(C<u>H$_2$</u>)$_6$-CH_2-CH=CH=COOMe), 1.34-1.27 (m, 10 H, Ph-CH_2-CH_2-(C<u>H$_2$</u>)$_6$-CH_2-CH=CH=COOMe). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.1, 149.7, 142.8, 128.3 (2C), 128.2 (2C), 125.5, 120.8, 51.3, 35.9, 32.1, 31.5, 29.4 (2C), 29.3, 29.2, 29.0, 28.0. IR (neat): 3025, 2924, 2853, 1723, 1656, 1434, 1267, 1131, 1030, 979, 698 cm^{-1}. HRMS (ESI +) $C_{19}H_{28}O_2$, m/z [M+H$^+$] 289.2168, found 289.2165.

_(E)_-12-Phenyldodec-2-enoic acid (4)

In a 50 mL round-bottom flask, a solution of the ester **3** (2.98 mmol, 860 mg) and aq. 10% (w/w) NaOH (3.7 mL) in EtOH (16 mL) was stirred at 20 °C. After 2 h, 1% aq. HCl was added at 0 °C to reach pH≈4. A white precipitate was formed during HCl addition. The mixture was stored at 4 °C for 17 h, and the white precipitate was filtered off and recrystallised from EtOH/H_2O to afford 750 mg of **4** as white crystals (92 %). R*f* = 0.18 (toluene/EtOAc 9:1). Mp.: 37.5 °C (lit. 40 °C).[215] ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.8-9.5 (br s, 1H, COO<u>H</u>), 7.30-7.27 (m, 2H, aromatic -*meta*), 7.20-7.18 (m, 3H, aromatic -*orhto* -*para*), 7.09 (dt, *J* = 15.5, 7.0 Hz, 1H, C<u>H</u>=CH-COOH), 5.83 (dt, *J* = 15.5, 1.5 Hz, 1H, CH=C<u>H</u>-COOH), 2.61 (m, 2H, C<u>H$_2$</u>-Ph), 2.23 (qd, *J* = 7.0, 1.5 Hz, 2H, C<u>H$_2$</u>-CH=CH), 1.65-1.57 (m, 2H, C<u>H$_2$</u>-CH$_2$-Ph), 1.49-1.42 (m, 2H, (C<u>H$_2$</u>)$_6$-CH$_2$-CH=CH), 1.36-1.28 (m, 10H, (C<u>H$_2$</u>)$_6$-CH$_2$-CH=CH).[215] ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.0, 152.4, 142.3, 128.4 (2C), 128.2 (2C), 125.5, 120.7, 36.0, 32.3, 31.5, 29.4 (2C), 29.3 (2C), 29.1, 27.8. IR (neat): 3027, 2917, 2849, 1691, 1648, 1496, 1468, 1420, 1289, 1219, 931, 710, 691 cm^{-1}. HRMS (ESI +) $C_{18}H_{26}O_2$, m/z [M+H$^+$] 275.2011, found 275.2014.

Methyl (_Z_)-12-phenyldodec-2-enoate (5)

In a flame-dried 100 mL round bottom flask, NaH ((60 % w/w) in mineral oil, 1.83 mmol, 44 mg) was suspended in freshly distilled DME (35 mL) and anhydrous THF (7.5 mL) under argon, and the resulting suspension was cooled to - 78 °C. Methyl *P,P*-bis(2,2,2-trifluoroethyl)phosphonoacetate (1.77 mmol, 374 µL) was added dropwise at - 78 °C, and the reaction mixture was stirred at that temperature. After 30 min, freshly synthesized **2** (1.22 mmol, 283 mg) was added and the solution was stirred for 3 h at - 78 °C, then at 20 °C for 65 h. Sat. aq. NH_4Cl was added at 0 °C, the organic layer was separated, and the aqueous layer was extracted with Et_2O (3 × 40 mL). The organic phases were combined, dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give a yellow oil. Purification of the crude oil by flash chromatography (toluene/hexane 1:1) yielded **5** as a colourless oil (250 mg, 71%). R$_f$ = 0.35 (toluene/hexane 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31 - 7.16 (m, 5H), 6.24 (dt, *J* = 11.5, 7.6 Hz, 1H, C<u>H</u>=CH-COOMe), 5.78 (dt, *J* = 11.5, 1.7 Hz, 1H, CH=C<u>H</u>-COOMe), 3.70 (s, 3H, O-C<u>H$_3$</u>), 2.70 – 2.58 (m, 4H, C<u>H$_2$</u>-Ph and C<u>H$_2$</u>-CH=CH-COOMe), 1.64 - 1.57 (m, 2H, C<u>H$_2$</u>-CH$_2$-Ph), 1.47-1.30 (m, 12H, Ph-CH$_2$-CH$_2$-(C<u>H$_2$</u>)$_6$-CH$_2$-CH=CH=COOMe). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.8, 151.0, 142.9, 128.3 (2C), 128.1 (2C), 125.5, 119.0, 50.9, 35.9, 31.5, 29.4 (2C), 29.3 (2C), 29.2, 28.9 (2C). IR (neat): 3027, 2924, 2853, 1723, 1644, 1437, 1171 cm^{-1}. HRMS (ESI +) $C_{19}H_{28}O_2$, m/z [M+H$^+$] 289.2168, found 289.2167.

_(Z)_-12-Phenyldodec-2-enoic acid (6)

In a 25 mL round-bottom flask, a solution of the ester **5** (0.69 mmol, 200 mg) and aq. 10% (w/w) NaOH (840 µL) in ^{t}BuOH 3.5 mL) was stirred at 20 °C. After 5 days, 1% aq. HCl was added at 0 °C to reach pH≈4. A white precipitate was formed during HCl addition. The mixture was stored at 4 °C for 17 h, and the white precipitate was filtered off, washed with H_2O and recrystallised from

EtOH/H_2O to afford 164 mg of **6** as white crystals (87 %). R*f* = 0.26 (toluene/EtOAc 1:1). Mp.: 39 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.15 (m, 5H, $C_6\underline{H}_5$), 6.36 (dt, *J* = 11.5, 7.6 Hz, 1H, C<u>H</u>=CH-COOH), 5.79 (dt, *J* = 11.5, 1.7 Hz, 1H, CH=C<u>H</u>-COOH), 2.70-2.58 (m, 4H, $C\underline{H}_2$-Ph and $C\underline{H}_2$-CH=CH), 1.66-1.58 (m, 2H, $C\underline{H}_2$-CH_2-Ph), 1.47-1.29 (m, 12H, $(C\underline{H}_2)_6$-CH_2-CH=CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.2, 153.8, 143.1, 128.5 (2C), 128.3 (2C), 125.7, 119.1, 36.1, 31.7, 29.6 (2C), 29.5, 29.46, 29.40, 29.36, 29.08. IR (neat): 3056, 2923, 2849, 1692, 1631, 1435, 1231, 903, 708 cm^{-1}. HRMS (ESI +) $C_{18}H_{26}O_2$, m/z [M+H$^+$] 275.2011, found 275.2006.

Ethyl (*E*)-2-methyl-12-phenyldodec-2-enoate (7)

To a suspension of NaH ((60 % w/w) in mineral oil, 6.03 mmol, 241 mg) in freshly distilled DME (8.5 mL) under argon, was added dropwise a solution of triethyl-2-phosphonopropionate (6.03 mmol, 1.3 mL) in freshly distilled DME (1 mL). The mixture was stirred at 50 °C for 30 min, and a solution of freshly synthesized **2** (3.45 mmol, 800 mg) in freshly distilled DME was added and the solution was stirred for 18 h at 70 °C. The crude mixture was then poured in ice-water (10 mL), the organic layer was separated, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The organic phases were combined, washed with brine (100 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude oil was purified by flash chromatography (toluene/hexane 1:1) to give **7** as a colourless oil in 59 % isolated yield (641 mg). The isomer ethyl (*Z*)-2-methyl-12-phenyldodec-2-enoate (**9**) was also isolated (89 mg, 8 %). R$_f$ = 0.16 (toluene/hexane 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.14 (m, 5H, $C_6\underline{H}_5$), 6.76 (tq, *J* = 7.5, 1.3 Hz, 1H, C<u>H</u>=C(CH_3)-COOEt), 4.19 (q, *J* = 7.1 Hz, 2H, COO-$C\underline{H}_2$-CH_3), 2.62-2.59 (m, 2H, $C\underline{H}_2$-Ph), 2.15 (q, *J* = 7.5 Hz, 2H, $C\underline{H}_2$-CH=C(CH_3)-COOEt), 1.83 (d, *J* = 1.3 Hz, 3H, CH=C($C\underline{H}_3$)-COOEt), 1.63-1.56 (m, 2H, $C\underline{H}_2$-CH_2-Ph), 1.45-1.27 (m, 15 H, Ph-CH_2-CH_2-$C_6\underline{H}_{12}$-CH_2-CH_2 and CH_2-$C\underline{H}_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.5, 143.0, 142.6, 128.5 (2C), 128.3 (2C), 127.8, 125.7, 60.5, 36.1, 31.6, 29.6 (3C), 29.51, 29.4, 28.3, 28.7, 14.4, 12.47. IR (neat): 3026, 2980, 2924, 2853, 1708, 1453, 1258 cm^{-1}. HRMS (ESI +) $C_{21}H_{32}O_2$, m/z [M+H$^+$] 317.2481, found 317.2482.

(*E*)-2-Methyl-12-phenyldodec-2-enoic acid (8)

In a 50 mL round-bottom flask, a solution of the ester **7** (1.93 mmol, 590 mg) and aq. 10% (w/w) NaOH (2.5 mL) in EtOH (10 mL) was stirred at 20 °C. After 40 h, 1% aq. HCl was added at 0 °C to reach pH≈4. A white precipitate was formed during HCl addition. The mixture was stored at 4 °C for 17 h, and the white precipitate was filtered off, washed with water and recrystallised from EtOH/H_2O to afford 494 mg of **8** as white crystals (89 %). R$_f$ = 0.51 (toluene/EtOAc 3:1). Mp.: 41 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.14 (m, 5H, $C_6\underline{H}_5$), 6.92 (t, *J* = 7.4 Hz, 1H, C<u>H</u>=C(CH_3)-COOH), 2.60 (m, 2H, $C\underline{H}_2$-Ph), 2.19 (q, *J* = 7.4 Hz, 2H, $C\underline{H}_2$-CH=CH), 1.83 (s, CH=C($C\underline{H}_3$)COO), 1.66-1.56 (m, 2H, $C\underline{H}_2$-CH_2-Ph), 1.47-1.29 (m, 12H, $(C\underline{H}_2)_6$-CH_2-CH=C(CH_3)). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.6, 145.7, 143.1, 128.6 (2C), 128.4 (2C), 127.1, 125.7, 36.2, 31.8, 29.7 (2C), 29.6 (2C), 29.5, 29.14, 28.6, 12.2. IR (neat): 2917, 2848, 2651, 2540, 1680, 1632, 1467, 1421, 1287 cm^{-1}. HRMS (ESI +) $C_{19}H_{28}O_2$, m/z [M+Na$^+$] 311.1987, found 311.1989.

Ethyl (*Z*)-2-methyl-12-phenyldodec-2-enoate (9)

To a suspension of NaH ((60 % w/w) in mineral oil, 3.88 mmol, 155 mg) in freshly distilled DME (70 mL) and anhydrous THF (10 mL) cooled to -78 °C under argon, was added slowly ethyl-2-[bis(2,2,2-trifluoroethyl)phosphono]propionate (3.75 mmol, 1.30 g), and the mixture was stirred at -78 °C for 30 min. After that, freshly synthesized **2** (2.58 mmol, 600 mg) was added and the solution was stirred for 3 h at -78 °C and then at 20 °C for 18 h. The crude mixture was then poured in ice-water (150 mL), the organic layer was separated, and the aqueous layer was extracted with EtOAc (3 × 100 mL). The organic phases were combined, washed with brine (100 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude oil was purified by flash chromatography (toluene/hexane 1:1) to give **9** as colourless oil in 67 % isolated yield (550 mg). R_f = 0.22 (toluene/hexane 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.15 (m, 5H, $C_6\underline{H}_5$), 5.92 (t, *J* = 7.5 Hz, 1H, C<u>H</u>=C(CH_3)-COOEt), 4.20 (q, *J* = 7.1 Hz, 2H, COO-C<u>H_2</u>-CH_3), 2.62-2.57 (m, 2H, C<u>H_2</u>-Ph), 2.43 (q, *J* = 7.5 Hz, 2H, C<u>H_2</u>-CH=C(CH_3)-COOEt), 1.89 (s, 3H, CH=C(C<u>H_3</u>)-COOEt), 1.66-1.58 (m, 2H, C<u>H_2</u>-CH_2-Ph), 1.41-1.28 (m, 15 H, Ph-CH_2-CH_2-$C_6\underline{H}_{12}$-CH_2-CH_2 and $COOCH_2$-C<u>H_3</u>). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.2, 143.1, 142.9, 128.4 (2C), 128.2 (2C), 127.0, 125.5, 60.0, 36.0, 31.5, 29.6, 29.5 (3C), 29.4, 28.3 (2C), 20.7, 14.3. IR (neat): 3025, 2978, 2924, 2853, 1713, 1454, 1229 cm $^{-1}$. HRMS (ESI +) $C_{21}H_{32}O_2$, m/z [M+Na^+] 339.2300, found 339.2311.

(*Z*)-2-Methyl-12-phenyldodec-2-enoic acid (10)

In a 50 mL round-bottom flask, a solution of the ester **9** (2.09 mmol, 662 mg) and aq. 10% (w/w) NaOH (2.5 mL) in EtOH (11 mL) was stirred at 20 °C. After 7 days, 1% aq. HCl was added at 0 °C to reach pH≈4. A white precipitate was formed during HCl addition. The mixture was stored at 4 °C for 17 h, and the white precipitate was filtered off, washed with water and recrystallised from EtOH/H_2O to afford 345 mg of **10** as white crystals (83 %). R_f = 0.55 (toluene/EtOAc 3:1). Mp.: 60.9-61.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31-7.14 (m, 5H, $C_6\underline{H}_5$), 6.10 (tq, *J* = 7.5, 1.4 Hz, 1H, C<u>H</u>=C(CH_3)-COOH), 2.63-2.58 (m, 2H, C<u>H_2</u>-Ph), 2.52 (qq, *J* = 7.5, 1.4 Hz, 2H, C<u>H_2</u>-CH=C(CH_3)), 1.92 (q, *J* = 1.4 Hz, 3H, CH=C(C<u>H_3</u>)COO), 1.66-1.56 (m, 2H, C<u>H_2</u>-CH_2-Ph), 1.43-1.29 (m, 12H, $(C\underline{H}_2)_6$-CH_2-CH=C(CH_3)). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.6, 147.1, 142.9, 128.4 (2C), 128.2 (2C), 125.9, 125.5, 36.0, 31.5, 29.8, 29.5 (2C), 29.4 (2C), 29.3(2C), 20.5. IR (neat): 2922, 2849, 1687, 1669, 1625, 1460, 1354, 1253 cm^{-1}. HRMS (ESI +) $C_{19}H_{28}O_2$, m/z [M+Na^+] 311.1987, found 311.1978.

Methyl (*E*)-3-methyl-12-phenyldodec-2-enoate (11)

In a flame-dried 25 mL flask, NaH ((60 % w/w in mineral oil), 3.90 mmol, 170 mg) was suspended in freshly distilled DME (10 mL) under argon, and methyl diethylphosphonoacetate (3.80 mmol, 690 µL) was added dropwise. The solution was stirred at 45 °C for 30 min, ketone **16** (1.30 mmol, 320 mg) was added rapidly and the solution was stirred for 18 h at 65 °C. The solution was poured into ice-water (20 mL), the organic phase was separated, and the aqueous layer was washed with EtOAc (3 × 15 mL). The organic phases were combined, washed with ice-water, brine, dried over

Na_2SO_4 and concentrated *in vacuo* to give a yellow oil. The desired product **11** was isolated after purification by flash chromatography (toluene/hexane 1:1) as colourless oil (218 mg, 56 %). The other isomer, methyl (*Z)*-3-methyl-12-phenyldodec-2-enoat (**13**) was also isolated (62 mg, 16 %). R*f* = 0.13 (toluene/heptane 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.14 (m, 5H, $C_6\underline{H}_5$), 5.67-5.66 (m, 1H, $C(CH_3)$=C<u>H</u>-COO), 3.69 (s, 3H, COO-$C\underline{H}_3$), 2.63-2.58 (m, 2H, CH_2-Ph), 2.16-2.10 (m, 5H, $C\underline{H}_2$-$C(C\underline{H}_3)$=CH-COO), 1.66-1.56 (m, 2H, $C\underline{H}_2$-CH_2-Ph), 1.50-1.43 (m, 2H, $C\underline{H}_2$-CH_2-$C(CH_3)$=CH-COO), 1.31-1.27 (m, 10H, Ph-CH_2-CH_2-$C_5\underline{H}_{10}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.3, 160.8, 142.9, 128.4 (2C), 128.2 (2C), 125.5, 114.9, 50.8, 40.9, 36.0, 31.5, 29.4 (3C), 29.3, 29.1, 27.3, 18.7. IR (neat): 3025, 2925, 2853, 1718, 1649, 1433, 1356, 1220, 1145, 1030, 865, 697 cm^{-1}. HRMS (ESI +) $C_{20}H_{30}O_2$, m/z [M+H$^+$] 303.2324, found 303.2317.

(*E*)-3-Methyl-12-phenyldodec-2-enoic acid (12)

To a solution of **11** (3.34 mmol, 1.01 g) in anhydrous Et_2O (15 mL) under argon, was added TMSOK (10.03 mmol, 1.29 g) and the resulting mixture was stirred at 20 °C for 25 h. After that time, TLC showed full conversion of the starting material, the mixture was cooled to 0 °C and 1M HCl was added to reach pH≈3. The organic phase was separated, the aqueous phase was extracted with EtOAc (3 × 10 mL), the combined organic phases were washed with brine and dried over anhydrous $MgSO_4$. **12** was isolated in 71 % yield (684 mg) as white crystals after concentration *in vacuo* and purification by flash chromatography (toluene/EtOAc 99:1). The isomer, acid **14** was also isolated (55 mg, 6 % yield). R_f = 0.3 (toluene/EtOAc 4:1). Mp.: 40.5-41 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.72 (br. s, 1H, COO<u>H</u>), 7.31-7.14 (m, 5H, $C_6\underline{H}_5$), 5.69 (q, *J* = 1.1 Hz, 1H, $C(CH_3)$=C<u>H</u>-COO), 2.63-2.58 (m, 2H, $C\underline{H}_2$-Ph), 2.18-2.13 (m, 5H, $C\underline{H}_2$-$C(C\underline{H}_3)$=CH-COO), 1.66-1.57 (m, 2H, $C\underline{H}_2$-CH_2-Ph), 1.52- 1.45 (m, 2H, $C\underline{H}_2$-CH_2-C(CH_3)=C), 1.35-1.28 (m, 10H, Ph-CH_2-CH_2-$C_5\underline{H}_{10}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.1, 163.7, 142.9, 128.4 (2C), 128.2 (2C), 125.5, 114.9, 41.2, 36.0, 31.5, 29.4 (3C), 29.3, 29.1, 27.3, 19.1. IR (neat): 3023, 2919, 2850, 1686, 1635, 1495, 1469, 1253, 1162, 938 cm^{-1}. HRMS (ESI +) $C_{19}H_{28}O_2$, m/z [M+Na$^+$] 311.1987, found 311.1984.

Methyl (*Z*)-3-methyl-12-phenyldodec-2-enoate (13)

In a flame-dried 250 mL flask, NaH ((60 % w/w in mineral oil), 15.88 mmol, 635 mg) was suspended in freshly distilled DME (160 mL) under argon, and methyl-2-[bis(2,2,2-trifluoroethyl)phosphono]acetate (11.68 mmol, 3.716 g) was added dropwise. The solution was stirred at 45 °C for 30 min, **16** (5.90 mmol, 1.45 g) was added and the solution was stirred for 18 h at 65 °C. The solution was poured into ice-water (150 mL), the organic phase was separated, and the aqueous layer was washed with EtOAc (3 × 100 mL). The organic phases were combined, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give a yellow oil. The desired product **13** was obtained after purification by flash chromatography (toluene/heptane 1:1) as colourless oil (480 mg, 27 %). The other isomer, methyl (*E)*-3-methyl-12-phenyldodec-2-enoat (**11**)

was also isolated (525 mg, 30 %). R*f* = 0.19 (toluene/heptane 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.14 (m, 5H, $C_6\underline{H}_5$), 5.65 (m, 1H, $C(CH_3)$=C<u>H</u>-COO), 3.67 (s, 3H, COO-$C\underline{H}_3$), 2.64-2.57 (m, 4H, $C\underline{H}_2$-Ph and $C\underline{H}_2$-$C(CH_3)$=CH-COO), 1.88 (d, *J* = 1.4 Hz, 3H, CH_2-$C(C\underline{H}_3)$=CH-COO), 1.64-1.56 (m, 2H, $C\underline{H}_2$-CH_2-Ph), 1.48-1.41 (m, 2H, $C\underline{H}_2$-CH_2-$C(CH_3)$=CH-COO), 1.30 (m, 10H, Ph-CH_2-CH_2-$C_5\underline{H}_{10}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.8, 161.3, 142.9, 128.4 (2C), 128.2 (2C), 125.5, 115.4, 50.1, 36.0, 33.4 31.5, 29.7, 29.5 (3C), 29.3, 28.2, 25.2. IR (neat): 3025, 2925, 2853, 1718, 1649, 1433, 1356, 1220, 1145, 1030, 865, 697 cm^{-1}. HRMS (ESI +) $C_{20}H_{30}O_2$, m/z [$M+H^+$] 303.2324, found 303.2313.

(*Z*)-3-Methyl-12-phenyldodec-2-enoic acid (14)

To a solution of **13** (2.46 mmol, 744 mg) in anhydrous Et_2O (10 mL) under argon, was added TMSOK (7.39 mmol, 948 mg) and the resulting mixture was stirred at 20 °C. After 23 h, another portion of TMSOK (1.95 mmol, 250 mg) was added and the mixture was stirred at 20 °C for additional 4 h. The mixture was then cooled to 0 °C and 1M HCl was added to reach pH≈3. The organic phase was separated, the aqueous phase was extracted with EtOAc (3 × 10 mL), the combined organic phases were washed with brine and dried over anhydrous $MgSO_4$. **14** was isolated in 62 % yield (435 mg) as white crystals after concentration *in vacuo* and purification by flash chromatography (toluene/EtOAc 99:1). The isomer, acid **12** was also isolated (50 mg, 7 % yield). R_f = 0.38 (toluene/EtOAc 4:1). Mp.: 61.5-62.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.32 (br s, 1H, COO<u>H</u>), 7.30-7.14 (m, 5H, $C_6\underline{H}_5$), 5.68-5.67 (m, 1H, $C(CH_3)$=C<u>H</u>-COO), 2.65-2.57 (m, 4H, $C\underline{H}_2$-Ph and $C\underline{H}_2$-$C(CH_3)$=CH-COO), 1.91 (d, *J* = 1.3 Hz, 3H, CH_2-$C(C\underline{H}_3)$=CH-COO), 1.66-1.56 (m, 2H, $C\underline{H}_2$-CH_2-Ph), 1.48-1.41 (m, 2H, $C\underline{H}_2$-CH_2-$C(CH_3)$=C), 1.33-1.28 (m, 10H, Ph-CH_2-CH_2-$C_5\underline{H}_{10}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.1, 164.2, 142.9, 128.4 (2C), 128.2 (2C), 125.5, 115.2, 36.0, 33.5, 31.5, 29.6, 29.5 (2 C), 29.4, 29.3, 28.1, 25.5. IR (neat): 3026, 2924, 2853, 1690, 1639, 1453, 1417, 1248 cm^{-1}. HRMS (ESI +) $C_{19}H_{28}O_2$, m/z [$M+H^+$] 289.2168, found 289.2169.

11-Phenylundecan-2-ol (15)

In a flame-dried 250 mL 3-neck round bottom flask equipped with a condenser, were suspended Mg turnings (12.89 mmol, 313 mg) in anhydrous Et_2O (38 mL) under argon, and MeI (11.17 mmol, 696 µL) was added dropwise. The resulting mixture was stirred at 20 °C for 2.5 h, after what the magnesium turnings had fully reacted and a solution of freshly synthesized **2** (8.59 mmol, 1.99 g) in Et_2O (10 mL) was added dropwise and the resulting mixture was stirred at 20 °C. After 2.5 h, the mixture was cooled to 0 °C, sat. aq. NH_4Cl (25 mL) was added, the organic phase was separated, and the aqueous phase was washed with Et_2O (3 × 25 mL). The combined organic layers were washed with water and with brine, dried over $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography (hexane/EtOAc 6:1) to afford 1.58 g of **15** as a clear oil (79 %). R_f = 0.44 (hexane/EtOAc 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40-7.32 (m, 2H, C_5H_6-*meta*), 7.27-7.22 (m, 3H, C_6H_5 –*ortho* and -*para*), 3.86-3.84 (m, 1H, C<u>H</u>-OH), 2.67 (t, *J* = 7.6 Hz, $C\underline{H}_2$-Ph, 2H), 1.73-

1.63 (m, 2H, C$\underline{H_2}$-CH_2-Ph), 1.54-1.34 (m, 14H, $C_7\underline{H_{14}}$-CH-OH), 1.25 (d, *J* = 6.2 Hz, 3H, CH-C$\underline{H_3}$).[56] ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.9, 128.4 (2C), 128.2 (2C), 125.5, 68.2, 39.3, 35.9, 31.5, 29.6 (2C), 29.5 (2C), 29.3, 25.7, 23.5. IR (neat): 3349, 3026, 2923, 2852, 1603, 1453, 1123, 1029, 477, 696 cm^{-1}. HRMS (ESI +) $C_{17}H_{28}O$, m/z [M+Na$^+$] 271.2038, found 271.2027.

11-Phenylundecan-2-one (16)

In a flame-dried 250 mL three-necked flask, a mixture of Me_2SO (29.38 mmol, 2.1 mL) in anhydrous CH_2Cl_2 (66 mL) was cooled to -78 °C. A mixture of $(COCl)_2$ (14.69 mmol, 1.2 mL) in anhydrous CH_2Cl_2 (3.5 mL) was added slowly in order to keep the reaction temperature below -60 °C, and the mixture was stirred at -78 °C. After 30 min, a solution of **15** (13.35 mmol, 3.31 g) in CH_2Cl_2 (14 mL) was added dropwise in order to keep the reaction temperature below -60 °C and the reaction mixture was stirred at -78 °C for 1 h. Et_3N (66.75 mmol, 9.3 mL) was added dropwise, the mixture stirred for additional 15 min at -78 °C and was then allowed to reach 20 °C. Water (80 mL) was added, the organic layer was separated and the aqueous layer was washed with CH_2Cl_2 (3 × 50 mL). The organic phases were combined, washed with aq. 1% HCl (100 mL), water (100 mL), aq. 5% Na_2CO_3 (100 mL) and brine (100 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give a clear oil, yielding 93 % (3.04 g) of **16** as a clear oil after flash chromatography (heptane/EtOAc 19:1). R_f = 0.16 (heptane/EtOAc 19:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31-7.14 (m, 5, $C_6\underline{H_5}$), 2.62-2.57 (m, 2H, C$\underline{H_2}$-Ph), 2.4 (t, *J* = 7.4 Hz, 2H, C$\underline{H_2}$-C=O), 2.13 (s, 3H,C=O-C$\underline{H_3}$), 1.66-1.52 (m, 4H, C$\underline{H_2}$-CH_2-Ph and C$\underline{H_2}$-CH_2-C=O), 1.32-1.27 (m, 10H, Ph-CH_2-CH_2-$C_5\underline{H_{10}}$-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 209.3, 142.9, 128.4 (2C), 128.2 (2C), 125.5, 43.8, 35.9, 31.5, 29.8, 29.4 (3C), 29.3, 29.1, 23.8. IR (neat): 3026, 2925, 2853, 1714, 1495, 1453, 1365, 1164, 745, 698 cm^{-1}. HRMS (ESI +) $C_{17}H_{26}O$, m/z [M+H$^+$] 247.2062, found 247.2059.

1,2-*O*-Isopropylidene-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (17)

PMBOH (7.25 mmol, 1 g) was dissolved in concentrated HCl (2.5 mL) and the solution was stirred at 20 °C. After 20 h, the solution had turned yellow, and TLC showed full conversion of the starting material. Water (2.5 mL) was then added, the mixture was extracted with CH_2Cl_2 (3 × 5 mL), dried over $CaCl_2$ and concentrated *in vacuo* to give 1.08 g of a pink oil (crude yield = 95 %). The crude was analyzed by NMR, and used without further purification. R_f = 0.58 (hexane/EtOAc 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 and 6.89 (AA'XX' pattern, *J* = 8.7 Hz, 4H, aromatic), 4.57 (s, 2H, CH_2-Cl), 3.81 (s, 3H, Ph-CH_3).[60] ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.6, 130.0 (2C), 129.7, 114.1 (2C), 55.3, 46.3.

In a 25 mL flame-dried flask, was prepared a suspension of NaH (60 % (w/w) in mineral oil, 6.7 mmol, 268 mg) in anhydrous DMF (3 mL) cooled to 0 °C under argon, to which was added 1,2-*O*-isopropylidene-sn-glycerol (4.19 mmol, 553 mg) dropwise at 0 °C and the resulting mixture was stirred at 20 °C. After 1 h, the resulting green mixture was cooled to 0 °C, the freshly synthesised PMBCl (6.07 mmol, 950 mg) was added dropwise and the mixture was stirred at 20 °C for 20 h.

The reaction was quenched by addition of water (10 mL) at 0 °C, the organic phase was separated, and the aqueous phase was extracted with Et_2O (3 × 10 mL). The organic phases were combined and washed with water (30 mL), brine (30 mL), dried over Na_2SO4, and concentrated *in vacuo*. The crude oil thereby obtained was purified by flash chromatography (hexane/EtOAc 6:1) to afford the desired acetonide as a colourless oil in 79 % yield (830 mg). R_f = 0.15 (hexane/EtOAc 6:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 and 6.89 (AA'XX' pattern, *J* = 8.7 Hz, 4H, aromatic), 4.53 (d, *J* = 11.7 Hz, 1H, C$\underline{H_2}$-Ph), 4.47 (d, *J* = 11.7 Hz, 1H, C$\underline{H_2}$-Ph), 4.28 (qnt, *J* = 6.0 Hz, 1H, CH_2-C$\underline{H}$-CH_2-OPMB), 4.04 (dd, *J* = 8.2, 6.0 Hz, 1H, C$\underline{H_2}$-CH-CH_2-OPMB), 3.80 (s, 3H, O-C$\underline{H_3}$), 3.72 (dd, *J* = 8.2, 6.0 Hz, 1H, C$\underline{H_2}$-CH-CH_2-OPMB), 3.52 (dd, *J* = 9.8, 6.0 Hz, 1H, CH_2-CH-C$\underline{H_2}$-OPMB), 3.43 (dd, *J* = 9.8, 6.0 Hz, 1H, CH_2-CH-C$\underline{H_2}$-OPMB), 1.42 (s, 3H, C-C$\underline{H_3}$), 1.36 (s, 3H, C-C$\underline{H_3}$).[60] ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.2, 129.4 (2C), 128.6, 113.8 (2C), 109.4, 74.4, 73.1, 70.7, 66.9, 55.3, 26.6, 26.4.

3-*O*-(*para*-Methoxybenzyl)-*sn*-glycerol (18)

In a 25 mL flask, **17** (3.29 mmol, 830 mg) was dissolved in methanol (1.2 mL) and 1M HCl (0.7 mL), and the mixture was stirred at 20 °C. After 3 h, $NaHCO_3$ was added (2 mL), and the mixture was extracted with EtOAc (3 × 3 mL). The combined organic phases were washed with H_2O and brine, dried over $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography (CH_2Cl_2/MeOH 10:1). The desired product was isolated in 68% yield (475 mg) as white powder. R_f = 0.39 (CH_2Cl_2/MeOH 9:1). Mp.: 42-43 °C. $[\propto]_D^{20}$= -1.55° (c = 6.9, $CHCl_3$). (lit. $[\propto]_D^{20}$= -1.4° (c = 6.9, $CHCl_3$)[39]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 and 6.80 (AA'XX' pattern, *J* = 8.7 Hz, 4H, aromatic), 4.45 (s, 2H, C$\underline{H_2}$-Ph), 3.88-3.82 (m, 1 H, CH_2-C$\underline{H}$-CH_2), 3.79 (s, 3H, O-C$\underline{H_3}$), 3.65 (dd, *J* = 11.5, 3.2 Hz, 1H, OH-C$\underline{H_2}$-CH-CH_2), 3.56 (dd, *J* = 11.5, 5.8 Hz, 1H, OH-C$\underline{H_2}$-CH-CH_2), 3.51-3.42 (m, 2H, OH-CH_2-CH-C$\underline{H_2}$), 2.3 (br s, 2H, 2×O$\underline{H}$).[39] ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.3, 129.7, 129.4 (2C), 113.8 (2C), 73.1, 71.3, 70.7, 64.0, 55.2.[39] IR (neat): 3512, 2915, 2848, 1737, 1712, 1613, 1514, 1366, 1171, 1116, 1078, 1031, 812, 718 cm^{-1}. HRMS (ESI +) $C_{11}H_{16}O_4$, m/z [M+Na^+] 235.0946, found 235.0956.

1-Stearoyl-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (19)

In a 250 mL flask, stearic acid (8.26 mmol, 2.34 g) was dissolved in anhydrous CH_2Cl_2 (80 mL). DCC (8.66 mmol, 1.78 g) and DMAP (2.36 mmol, 288 mg) were added, and the resulting mixture was stirred at 20 °C. After 30 min, the mixture was cooled to 0 °C and a solution of the **18** (7.87 mmol, 1.67 g) in anhydrous CH_2Cl_2 (80 mL) was added slowly. The mixture was stirred at 0 °C for 2.5 h, and then allowed to reach 20 °C. The mixture was concentrated *in vacuo*, the product was purified by flash chromatography (hexane/EtOAc 5:1) and obtained as a white solid in 70% yield (2.62 g). R_f = 0.15 (hexane/EtOAc 5:1). Mp.: 49.5-50 °C. $[\propto]_D^{20}$= + 0.23° (c = 5.9, $CHCl_3$). (lit. $[\propto]_D^{20}$= + 0.1° (c = 5.9, $CHCl_3$).[39] ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 and 6.80 (AA'XX' pattern, *J* = 8.7 Hz, 4H, aromatic), 4.49 (s, 2H, C$\underline{H_2}$-Ph), 4.20-4.09 (m, 2H, $C_{18}H_{35}$-COO-C$\underline{H_2}$-C$\underline{H}$-CH_2),

4.05-3.98 (m, 1H, $C_{18}H_{35}$-COO-C$\underline{H_2}$-CH-CH_2), 3.81 (s, 3H, O-C$\underline{H_3}$), 3.52 (dd, *J* = 9.6, 4.3 Hz, 1H, $C_{18}H_{35}$-COO-CH_2-CH-C$\underline{H_2}$), 3.45 (dd, *J* = 9.6, 6.1 Hz, 1H, $C_{18}H_{35}$-COO-CH_2-CH-C$\underline{H_2}$), 2.50 (d, *J* = 4.8 Hz, 1H, O$\underline{H}$), 2.31 (t, *J* = 7.5 Hz, 2H, C$\underline{H_2}$COO), 1.63-1.60 (m, 2H, C$\underline{H_2}$-CH_2-COO), 1.32-1.25 (m, 28H, CH_3-$C_{14}\underline{H_{28}}$-CH_2-CH_2-COO), 0.88 (t, *J* = 6.7 Hz, 3H, C$\underline{H_3}$).[39] ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.9, 159.3, 129.7, 129.4 (2C), 113.8 (2C), 73.2, 70.5, 68.9, 65.3, 55.3, 34.1, 31.9, 29.7 (7C), 29.6, 29.4, 29.3, 29.2, 29.1, 24.9, 22.7, 14.1.[39] IR (neat): 3265, 2973, 2939, 2862, 1610, 1584, 1461, 1241, 1206, 1175, 1020, 812, 711 cm^{-1}. HRMS (ESI +) $C_{29}H_{50}O_6$, m/z [M+Na^+] 501.3556, found 501.3563.

1-Stearoyl-2-((*E*)-12-phenyldodec-2-enoyl)-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (20)

In a 50 mL flask, **4** (0.547 mmol, 150 mg) and **19** (0.61 mmol, 290 mg) were dissolved in anhydrous CH_2Cl_2 (10 mL), DCC (0.852 mmol, 175 mg) and DMAP (0.547 mmol, 67 mg) were added, and the mixture was stirred at 20 °C. After 2 h, a second portion of **4** (0.547 mmol, 150 mg), DCC (0.852 mmol, 175 mg) and DMAP (0.547 mmol, 67 mg) was added and the solution was stirred for 1 h. The mixture was concentrated *in vacuo* and the product was purified by flash chromatography (hexane/EtOAc 10:1) and isolated as a white solid in 88% yield (392 mg). R_f = 1.6 (hexane/EtOAc 10:1). Mp.: 51.9-52.1 °C. $[\propto]_D^{20}$= +5.91° (c = 1.6, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29-7.17 (m, 7H, $C_6\underline{H_5}$ and C$\underline{H}$-CH-C-OMe), 7.0 (dt, *J* = 15.6, 6.9 Hz, 1H, C$\underline{H}$=CH-COO), 6.89-6.85 (m, 2H, CH-C$\underline{H}$-C-OMe), 5.83 (dt, *J* = 15.6, 1.3 Hz, 1H, CH=C$\underline{H}$-COO), 5.30-5.23 (m, 1H, CH_2-C$\underline{H}$-CH_2), 4.50 (d, *J* = 11.7 Hz, 1H, C$\underline{H_2}$-Ph-OMe), 4.45 (d, *J* = 11.7 Hz, 1H, C$\underline{H_2}$-Ph-OMe), 4.34 (dd, *J* = 11.8, 3.9 Hz, 1H, $C_{17}H_{35}$-COOC$\underline{H_2}$), 4.25 (dd, *J* = 11.8, 6.3 Hz, 1H, $C_{17}H_{35}$-COOC$\underline{H_2}$), 3.80 (s, 3H, O-C$\underline{H_3}$), 3.59 (d, *J* = 5.9 Hz, 2H, C$\underline{H_2}$-OPMB), 2.61-2.59 (m, 2H, C_8H_{16}-C$\underline{H_2}$-Ph), 2.28 (t, *J* = 7.5 Hz, 2H, $C_{16}H_{33}$-C$\underline{H_2}$-COO), 2.19 (q, *J* = 6.9 Hz, 2H, C$\underline{H_2}$-CH=CH-COO), 1.63-1.53 (m, 4H, $C_{15}H_{31}$-C$\underline{H_2}$-CH_2-COO and C$\underline{H_2}$-CH_2-Ph), 1.45-1.25 (m,40 H, $C_6\underline{H_{12}}$-CH_2-CH=CH and CH_3-$C_{14}\underline{H_{28}}$-CH_2), 0.88 (t, *J* = 6.6 Hz, 3H, C$\underline{H_3}$-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.4, 165.9, 159.3, 150.6, 142.8, 129.8, 129.3 (2C), 128.3 (2C), 128.2 (2C), 125.5, 120.7, 113.8 (2C), 72.9, 70.0, 67.8, 62.7, 55.2, 35.9, 34.1, 32.3, 31.9, 31.5, 29.7 (10C), 29.5 (2C), 29.4, 29.3 (2C), 29.2, 29.1, 27.9, 24.9, 22.7, 14.1. IR (neat): 2957, 2914, 2848, 1725, 1511, 1366, 1204, 1170, 1108, 1082, 1029 cm^{-1}. HRMS (ESI +) $C_{47}H_{74}O_6$, m/z [M+Na^+] 757.5383, found 757.5384.

1-*O*-(*para*-Methoxybenzyl)-2,3-*O*-isopropylidene-*sn*-glycerol (21)

The synthesis was performed following the same procedure as for 1,2-*O*-isopropylidene-3-(*para*-methoxybenzyl)-*sn*-glycerol (**17**), starting from 2,3-*O*-isopropylidene-sn-glycerol (12.88 mmol, 1.7 g), NaH (60 % (w/w) in mineral oil, 20.6 mmol, 825 mg), freshly synthesized PMBCl (18.68 mmol, 2.92 g) and 30 mL DMF and affording 2.50 g of 1-*O*-(*para*-methoxybenzyl)-2,3-*O*-isopropylidene-*sn*-glycerol (**21**) (77 % yield). R_f = 0.15 (hexane/EtOAc 6:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 and 6.89 (AA'XX' pattern, *J* = 8.7 Hz, 4H, aromatic), 4.53 (d, *J* = 11.7 Hz, 1H, C$\underline{H_2}$-Ph), 4.47 (d, *J*

= 11.7 Hz, 1H, C$\underline{H_2}$-Ph), 4.28 (qnt, *J* = 6.0 Hz, 1H, CH_2-C$\underline{H}$-CH_2-OPMB), 4.04 (dd, *J* = 8.2, 6.0 Hz, 1H, C$\underline{H_2}$-CH-CH_2-OPMB), 3.80 (d, *J* = 1.9 Hz, 3H, O-C$\underline{H_3}$), 3.72 (dd, *J* = 8.2, 6.0 Hz, 1H, C$\underline{H_2}$-CH-CH_2-OPMB), 3.52 (dd, *J* = 9.8, 6.0 Hz, 1H, CH_2-CH-C$\underline{H_2}$-OPMB), 3.43 (dd, *J* = 9.8, 6.0 Hz, 1H, CH_2-CH-C$\underline{H_2}$-OPMB), 1.42 (s, 3H, C-C$\underline{H_3}$), 1.36 (s, 3H, C-C$\underline{H_3}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.2, 129.4 (2C), 128.6, 113.8 (2C), 109.4, 74.4, 73.1, 70.7, 66.9, 55.3, 26.6, 26.4.

1-*O*-*(para*-Methoxybenzyl)-*sn*-glycerol (22)

1-*O*-*(para*-Methoxybenzyl)-*sn*-glycerol was prepared by deprotection of 1-(para-methoxybenzyl)-2,3-*O*-isopropylidene-*sn*-glycerol (**21**) (9.92 mmol, 2.50 g), as described for **18**, in MeOH (3.5 mL) and HCl (2 mL), to afford 1.51 g of (**22**) as white powder (yield: 72 %). R_f = 0.39 (CH_2Cl_2/MeOH 9:1). Mp.: 44 °C. $[\propto]_D^{20}$= + 0.35° (c = 1.0, $CHCl_3$). (lit. $[\propto]_D^{20}$= + 1.2° (c = 3.5, $CHCl_3$))[39]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 and 6.80 (AA'XX' pattern, *J* = 8.7 Hz, 4H, aromatic), 4.45 (s, 2H, C$\underline{H_2}$-Ph), 3.88-3.82 (m, 1 H, CH_2-C$\underline{H}$-CH_2), 3.79 (s, 3H, O-C$\underline{H_3}$), 3.65 (dd, *J* = 11.5, 3.2 Hz, 1H, HO-C$\underline{H_2}$-CH-CH_2), 3.56 (dd, *J* = 11.5, 5.8 Hz, 1H, HO-C$\underline{H_2}$-CH-CH_2), 3.51-3.42 (m, 2H, HO-CH_2-CH-C$\underline{H_2}$), 2.3 (br s, 2H, 2×O$\underline{H}$).[39] ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.3, 129.7, 129.4 (2C), 113.8 (2C), 73.1, 71.3, 70.7, 64.0, 55.2.[39] IR (neat): 3512, 2915, 2848, 1737, 1712, 1613, 1514, 1366, 1171, 1116, 1078, 1031, 812, 718 cm^{-1}. HRMS (ESI +) $C_{11}H_{16}O_4$, m/z [M+Na^+] 235.0946, found 235.0942.

1-*O*-*(para*-Methoxybenzyl)-3-stearoyl-*sn*-glycerol (23)

1-*O*-*(para*-Methoxybenzyl)-3-stearoyl-*sn*-glycerol has been synthesized in 55 % yield (2.23 g), following the same procedure as for **19**, starting from stearic acid (8.92 mmol, 2.532 g) in anhydrous CH_2Cl_2 (85 mL), DCC (9.34 mmol, 1.92 g), and DMAP (2.55 mmol, 311 mg) and **22** (8.49 mmol, 1.77 g) in anhydrous CH_2Cl_2 (85 mL). R_f = 0.46 (hexane/EtOAc 1:1). Mp.: 50.1-50.9 °C. $[\propto]_D^{20}$= - 1.33° (c = 0.98, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 and 6.80 (AA'XX' pattern, *J* = 8.7 Hz, 4H, aromatic), 4.49 (s, 2H, C$\underline{H_2}$-Ph), 4.20-4.09 (m, 2H, $C_{18}H_{35}$-COO-C$\underline{H_2}$-C$\underline{H}$-CH_2), 4.05-3.98 (m, 1H, $C_{18}H_{35}$-COO-C$\underline{H_2}$-CH-CH_2), 3.81 (s, 3H, O-C$\underline{H_3}$), 3.52 (dd, *J* = 9.6, 4.3 Hz, 1H, $C_{18}H_{35}$-COO-CH_2-CH-C$\underline{H_2}$), 3.45 (dd, *J* = 9.6, 6.1 Hz, 1H, $C_{18}H_{35}$-COO-CH_2-CH-C$\underline{H_2}$), 2.50 (d, *J* = 4.8 Hz, 1H, O$\underline{H}$), 2.31 (t, *J* = 7.5 Hz, 2H, C$\underline{H_2}$COO), 1.62-1.60 (m, 2H, C$\underline{H_2}$-CH_2-COO), 1.32-1.23 (m, 28H, CH_3-$C_{14}\underline{H_{28}}$-CH_2-CH_2-COO), 0.88 (t, *J* = 6.7 Hz, 3H, C$\underline{H_3}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.9, 159.3, 129.7, 129.4 (2C), 113.8 (2C), 73.2, 70.5, 68.9, 65.3, 55.3, 34.1, 31.9, 29.7 (7C), 29.6, 29.4, 29.3, 29.2, 29.1, 24.9, 22.7, 14.1. IR (neat): 3265, 2973, 2939, 2862, 1610, 1584, 1461, 1241, 1206, 1175, 1020, 812, 711 cm^{-1}.

1-Stearoyl-2-((*Z)*-12-phenyldodec-2-enoyl)-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (24)

In a flame-dried 25 mL flask, a solution of Ph_3P (1.5 mmol, 394 mg) in anhydrous THF (8 mL) was cooled to 0 °C under argon and DIAD (0.75 mmol, 148 µL) was added, and the solution became white. After the mixture had been stirred for 10 min at 0 °C, **23** (0.63 mmol, 302 mg) was added (the mixture turned yellow), followed by **6** (0.75 mmol, 206 mg) (the mixture became clear again) and the mixture was stirred at 20 °C for 2 h. The mixture was cooled to 0 °C and a second portion of Ph_3P (1.5 mmol, 394 mg) and DIAD (0.75 mmol, 148 µL) was added and the resulting mixture was stirred for 10 min at 0 °C. Compound **6** (0.75 mmol, 206 mg) was then added and the mixture was stirred at 20 °C for 5 h, after which TLC (toluene/EtOAc 98:2) showed full conversion of the starting material (**23**). The mixture was concentrated *in vacuo* and the product was purified by flash chromatography (toluene/EtOAc 99:1) to afford 1-stearoyl-2-((*Z*)-12-phenyldodec-2- enoyl)-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (**24**) as white crystals in 93 % yield (428 mg). R_f = 0.17 (toluene/EtOAc 9:2). Mp: 32-33 °C. $[\propto]_D^{20}$= + 5.48° (c = 1.46, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29-7.17 (m, 7H, $C_6\underline{H}_5$ and 2×C<u>H</u>-CH-C-OMe), 6.89-6.84 (m, 2H, CH-C<u>H</u>-C-OMe), 6.27 (dt, *J* = 11.5, 7.5 Hz, 1H, C<u>H</u>=CH-COO), 5.78 (dt, *J* = 11.5, 1.7 Hz, 1H, CH=C<u>H</u>-COO), 5.29-5.22 (m, 1H, CH_2-C<u>H</u>-CH_2), 4.50 (d, *J* = 11.7 Hz, 1H, C<u>H</u>$_2$-Ph-OMe), 4.45 (d, *J* = 11.7 Hz, 1H, C<u>H</u>$_2$-Ph-OMe), 4.33 (dd, *J* = 11.8, 3.9 Hz, 1H, $C_{17}H_{35}$-COOC<u>H</u>$_2$), 4.23 (dd, *J* = 11.8, 6.3 Hz, 1H, $C_{17}H_{35}$-COOC<u>H</u>$_2$), 3.80 (s, 3H, O-C<u>H</u>$_3$), 3.59 (d, *J* = 5.3 Hz, 2H, C<u>H</u>$_2$-OPMB), 2.67-2.57 (m, 4H, C_8H_{16}-C<u>H</u>$_2$-Ph and C<u>H</u>$_2$-CH=CH-COO), 2.27 (t, *J* = 7.5 Hz, 2H, $C_{16}H_{33}$-C<u>H</u>$_2$-COO), 1.65-1.53 (m, 4H, $C_{15}H_{31}$-C<u>H</u>$_2$-CH_2-COO and C<u>H</u>$_2$-CH_2-Ph), 1.45-1.25 (m, 40 H, $C_6\underline{H}_{12}$-CH_2-CH=CH and CH_3-$C_{14}\underline{H}_{28}$-CH_2), 0.88 (t, *J* = 6.7 Hz, 3H, C<u>H</u>$_3$-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.4, 165.5, 159.2, 151.8, 142.9, 129.8, 129.3 (2C), 128.4 (2C), 128.2 (2C), 125.5, 119.1, 113.8 (2C), 72.9, 69.6, 67.9, 62.7, 55.2, 35.9, 34.1, 31.9, 31.5, 29.7 (9C), 29.6, 29.5 (3C), 29.4, 29.3 (2C), 29.2, 29.1, 29.0, 24.9, 22.7, 14.1. IR (neat): 3032, 2958, 2915, 2849, 1724, 1711, 1642, 1612, 1512, 1468, 1454, 1442, 1419, 1245, 1233, 1185, 1170, 1148, 1108, 1082, 1029 cm^{-1}. HRMS (ESI +) $C_{47}H_{74}O_6$, m/z [M+H$^+$] 735.5564, found 735.5556.

1-Stearoyl-2-((*E*)-2-methyl-12-phenyldodec-2-enoyl)-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (25)

Following the same procedure as for the synthesis of 1-stearoyl-2-((*Z*)-12-phenyldodec-2-enoyl)-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (**24**), and starting from **23** (0.32 mmol, 153 mg) in anhydrous THF (4 mL), Ph_3P (2 × 0.76 mmol, 2 × 200 mg), DIAD (2 × 0.38 mmol, 2 × 75 µL) and **8** (2 × 0.38 mmol, 2 × 110 mg), 1-stearoyl-2-((*E*)-2-methyl-12-phenyldodec-2-enoyl)-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (**25**) was obtained in 94 % yield (224 mg) as colourless oil. R_f = 0.17 (toluene). $[\propto]_D^{20}$= + 6.4° (c = 1.98, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.14 (m, 7H, $C_6\underline{H}_5$ and C<u>H</u>-CH-C-OMe), 6.89-6.85 (m, 2H, CH-C<u>H</u>-C-OMe), 6.78 (t, *J* = 7.5, 1.0 Hz, 1H, C<u>H</u>=C(CH_3)-COO), 5.30-5.23 (m, 1H, CH_2-C<u>H</u>-CH_2), 4.50 (d, *J* = 11.7 Hz, 1H, C<u>H</u>$_2$-Ph-OMe), 4.45 (d, *J* = 11.7 Hz, 1H, C<u>H</u>$_2$-Ph-OMe), 4.35 (dd, *J* = 11.8, 3.9 Hz, 1H, $C_{17}H_{35}$-COOC<u>H</u>$_2$), 4.26 (dd, *J* = 11.8, 6.3 Hz, 1H, $C_{17}H_{35}$-COOC<u>H</u>$_2$), 3.80 (s, 3H, O-C<u>H</u>$_3$), 3.62 (dd, *J* = 10.5, 5.1 Hz, 1H, C<u>H</u>$_2$-OPMB), 3.58 (dd, *J* = 10.5, 5.4 Hz, 1H, C<u>H</u>$_2$-OPMB), 2.62-2.57 (m, 2H, C_8H_{16}-C<u>H</u>$_2$-Ph), 2.27 (t, *J* = 7.5 Hz, 2H, $C_{16}H_{33}$-C<u>H</u>$_2$-COO), 2.16 (q, *J* = 7.5 Hz, 2H, C<u>H</u>$_2$-CH=C(CH_3)-COO), 1.82 (d, *J*

= 1.0 Hz, 3H, CH=C(C<u>H</u>$_3$)COO), 1.66-1.53 (m, 4H, $C_{15}H_{31}$-C<u>H</u>$_2$-CH_2-COO and C<u>H</u>$_2$-CH_2-Ph), 1.45-1.25 (m, 40 H, C_6<u>H</u>$_{12}$-CH_2-CH=CH and CH_3-C_{14}<u>H</u>$_{28}$-CH_2), 0.88 (t, *J* = 6.7 Hz, 3H, C<u>H</u>$_3$-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.4, 167.4, 159.2, 143.5, 142.9, 129.8, 129.2 (2C), 128.4 (2C), 128.2 (2C), 127.1, 125.5, 113.8 (2C), 72.9, 70.3, 67.8, 62.7, 55.2, 36.0, 34.2, 31.9, 31.5, 29.7 (8C), 29.6, 29.5 (2C), 29.4 (3C), 29.3 (2C), 29.1, 28.8, 28.5, 24.9, 22.7, 14.1, 12.4. IR (neat): 2922, 2852, 1741, 1713, 1612, 1513, 1463, 1455, 1246, 1172, 1093, 1036, 820, 744, 698 cm^{-1}. HRMS (ESI +) $C_{48}H_{76}O_6$, m/z [M+Na$^+$] 771.5540, found 771.5543.

1-Stearoyl-2-((*Z*)-2-methyl-12-phenyldodec-2-enoyl)-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (26)

Following the same procedure as for the synthesis of 1-stearoyl-2-((*Z*)-12-phenyldodec-2-enoyl)-3-*O*-(*para*- methoxybenzyl)-*sn*-glycerol (**24**), and starting from **23** (0.78 mmol, 371 mg) in anhydrous THF (10 mL), Ph_3P (2 × 1.85 mmol, 2 × 484 mg), DIAD (2 × 0.92 mmol, 2 × 181 µL) and **10** (2 × 0.92 mmol, 2 × 266 mg), 1-stearoyl-2-((*Z*)-2-methyl-12-phenyldodec-2-enoyl)-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (**26**) was obtained in 76 % yield (442 mg) as colourless oil. R_f = 0.1 (toluene/EtOAc 99:1). $[\alpha]_D^{20}$= + 6.8° (c = 2.21, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.14 (m, 7H, C_6<u>H</u>$_5$ and C<u>H</u>-CH-C-OMe), 6.88-6.85 (m, 2H, CH-C<u>H</u>-C-OMe), 5.92 (t, *J* = 7.4 Hz, 1H, C<u>H</u>=C(CH_3)-COO), 5.31-5.22 (m, 1H, CH_2-C<u>H</u>-CH_2), 4.50 (d, *J* = 11.7 Hz, 1H, C<u>H</u>$_2$-Ph-OMe), 4.45 (d, *J* = 11.7 Hz, 1H, C<u>H</u>$_2$-Ph-OMe), 4.36 (dd, *J* = 11.8, 3.8 Hz, 1H, $C_{17}H_{35}$-COOC<u>H</u>$_2$), 4.24 (dd, *J* = 11.8, 6.3 Hz, 1H, $C_{17}H_{35}$-COOC<u>H</u>$_2$), 3.80 (s, 3H, O-C<u>H</u>$_3$), 3.65-3.55 (m, 2H, C<u>H</u>$_2$-OPMB), 2.62-2.57 (m, 2H, C_8H_{16}-C<u>H</u>$_2$-Ph), 2.43 (q, *J* = 7.4 Hz, 2H, C<u>H</u>$_2$-CH=C(CH_3)-COO), 2.27 (t, *J* = 7.5 Hz, 2H, $C_{16}H_{33}$-C<u>H</u>$_2$-COO), 1.89-1.88 (s, 3H, CH=C(C<u>H</u>$_3$)COO), 1.63-1.57 (m, 4H, $C_{15}H_{31}$-C<u>H</u>$_2$-CH_2-COO and C<u>H</u>$_2$-CH_2-Ph), 1.40-1.25 (m, 40 H, C_6<u>H</u>$_{12}$-CH_2-CH=CH and CH_3-C_{14}<u>H</u>$_{28}$-CH_2), 0.88 (t, *J* = 6.3 Hz, 3H, C<u>H</u>$_3$-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.4, 167.2, 159.2, 144.3, 142.9, 129.8, 129.2 (2C), 128.4 (2C), 128.2 (2C), 126.5, 125.5, 113.8 (2C), 72.9, 70.0, 67.9, 62.7, 55.2, 36.0, 34.1, 31.9, 31.5, 29.7 (9C), 29.6, 29.5 (3C), 29.4(2C), 29.3 (2C), 29.1, 24.9, 22.7, 20.6, 14.1, 12.4. IR (neat): 2922, 2852, 1741, 1713, 1612, 1513, 1463, 1455, 1246, 1172, 1093, 1036, 820, 744, 698 cm^{-1}. HRMS (ESI +) $C_{48}H_{76}O_6$, m/z [M+H$^+$] 749.5720, found 749.5722.

1-Stearoyl-2-((*E*)-3-methyl-12-phenyldodec-2-enoyl)-3-*O*-(*para*-methoxybenzyl)-sn-glycerol (27)

In a flame-dried 25 mL flask, a solution of Ph_3P (1.74 mmol, 454 mg) in anhydrous THF (10 mL) was cooled to 0 °C under argon and DIAD (0.87 mmol, 171 µL) was added, and the solution became white. After the mixture had been stirred for 10 min at 0 °C, **23** (0.79 mmol, 377 mg) was added (the mixture turned yellow), followed by **12** (0.87 mmol, 250 mg) (the mixture became clear again) and the mixture was stirred at 20 °C for 2 h. The mixture was cooled to 0 °C and a second portion of Ph_3P (1.74 mmol, 454 mg) and DIAD (0.87 mmol, 171 µL) was added and the resulting mixture was stirred for 10 min at 0 °C. Carboxylic acid **12** (0.87 mmol, 250 mg) was then added

and the mixture was stirred at 20 °C for 1.5 h, after which TLC (toluene/EtOAc 98:2) showed full conversion of the starting material (**23**). The mixture was concentrated *in vacuo* and the product was purified by flash chromatography (toluene/EtOAc 99:1) to afford 1-stearoyl-2-((*E*)-3-methyl-12-phenyldodec-2-enoyl)-3-(*para*-methoxybenzyl)-*sn*-glycerol (**27**) as clear oil in 68 % yield (401 mg). R_f = 0.59 (toluene/EtOAc 9:1). $[\propto]_D^{20}$ = + 5.7° (c = 1.13, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.14 (m, 7H, $C_6\underline{H}_5$ and 2×C<u>H</u>-CH-C-OMe), 6.89-6.84 (m, 2H, CH-C<u>H</u>-C-OMe), 5.68 (q, *J* = 1.1 Hz, 1H, C(CH_3)=C<u>H</u>-COO), 5.28-5.21 (m, 1H, CH_2-C<u>H</u>-CH_2), 4.51 (d, *J* = 11.7 Hz, 1H, C<u>H_2</u>-Ph-OMe), 4.45 (d, *J* = 11.7 Hz, 1H, C<u>H_2</u>-Ph-OMe), 4.32 (dd, *J* = 11.8, 4.0 Hz, 1H, $C_{17}H_{35}$-COOC<u>H_2</u>), 4.23 (dd, *J* = 11.8, 6.2 Hz, 1H, $C_{17}H_{35}$-COOC<u>H_2</u>), 3.80 (s, 3H, O-C<u>H_3</u>), 3.60 (dd, *J* = 10.4, 5.1 Hz, 1H, C<u>H_2</u>-OPMB), 3.56 (dd, *J* = 10.4, 5.3 Hz, 1H, C<u>H_2</u>-OPMB), 2.63-2.57 (m, 2H, C_8H_{16}-C<u>H_2</u>-Ph), 2.27 (t, *J* = 7.5 Hz, 2H, $C_{16}H_{33}$-C<u>H_2</u>-COO), 2.15-2.10 (m, 5H, C(C<u>H_3</u>)=CH-COO and C<u>H_2</u>-C(CH_3)=CH-COO), 1.66-1.53 (m, 4H, $C_{15}H_{31}$-C<u>H_2</u>-CH_2-COO and C<u>H_2</u>-CH_2-Ph), 1.51-1.42 (m, 2H, C<u>H_2</u>-CH_2-C(CH_3)=C), 1.34-1.22 (m, 38 H, $C_5\underline{H}_{10}$-CH_2-C(CH_3)=CH and CH_3-$C_{14}\underline{H}_{28}$-CH_2), 0.88 (t, *J* = 6.7 Hz, 3H, C<u>H_3</u>-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.4, 165.8, 161.8, 159.2, 142.9, 129.8, 129.3 (2C), 128.4 (2C), 128.2 (2C), 125.5, 114.9, 113.8 (2C), 72.9, 69.2, 67.9, 62.8, 55.2, 41.1, 36.0, 34.1, 31.9, 29.7 (9C), 29.6, 29.5, 29.4 (3C), 29.3 (2C), 29.2, 29.1, 27.4, 24.9, 22.7, 18.9, 14.1. IR (neat): 2922, 2852, 1740, 1719, 1513, 1456, 1247, 1217, 1139, 1096 cm^{-1}. HRMS (ESI +) $C_{48}H_{76}O_6$, m/z [M+H^+] 749.5720, found 749.5722.

1-Stearoyl-2-((*Z*)-3-methyl-12-phenyldodec-2-enoyl)-3-*O*-(*para*-methoxybenzyl)-sn-glycerol (28)

In a flame-dried 25 mL flask, a solution of Ph_3P (1.39 mmol, 364 mg) in anhydrous THF (8 mL) was cooled to 0 °C under argon and DIAD (0.69 mmol, 137 µL) was added, and the solution became white. After the mixture had been stirred for 10 min at 0 °C, **23** (0.63 mmol, 301 mg) was added (the mixture turned yellow), followed by **14** (0.69 mmol, 200 mg) (the mixture became clear again) and the mixture was stirred at 20 °C for 2 h. The mixture had taken a yellow colour over the 2 h and was cooled to 0 °C again. A second portion of Ph_3P (1.39 mmol, 364 mg) and DIAD (0.69 mmol, 137 µL) was added and the resulting mixture was stirred for 10 min at 0 °C. Carboxylic acid **14** (0.69 mmol, 200 mg) was then added and the mixture was stirred at 20 °C for 1.5 h, after which TLC (toluene/EtOAc 98:2) showed full conversion of the starting material (**23**). The mixture was concentrated *in vacuo* and the product was purified by flash chromatography (toluene/EtOAc 99:1) to afford 1-stearoyl-2-((*Z*)-3-methyl-12-phenyldodec-2-enoyl)-3-(*para*-methoxybenzyl)-*sn*-glycerol (**28**) as clear oil in 74 % yield (351 mg). R_f = 0.71 (toluene/EtOAc 9:1). $[\propto]_D^{20}$ = + 6.0° (c = 1.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28-7.15 (m, 7H, $C_6\underline{H}_5$ and 2×C<u>H</u>-CH-C-OMe), 6.87-6.85 (m, 2H, CH-C<u>H</u>-C-OMe), 5.66 (m, 1H, C(CH_3)=C<u>H</u>-COO), 5.25-5.21 (m, 1H, CH_2-C<u>H</u>-CH_2), 4.49 (d, *J* = 11.7 Hz, 1H, C<u>H_2</u>-Ph-OMe), 4.44 (d, *J* = 11.7 Hz, 1H, C<u>H_2</u>-Ph-OMe), 4.31 (dd, *J* = 11.8, 3.9 Hz, 1H, $C_{17}H_{35}$-COOC<u>H_2</u>), 4.21 (dd, *J* = 11.8, 6.3 Hz, 1H, $C_{17}H_{35}$-COOC<u>H_2</u>), 3.79 (s, 3H, O-C<u>H_3</u>), 3.58 (dd, *J* = 10.5, 5.1 Hz, 1H, C<u>H_2</u>-OPMB), 3.56 (dd, *J* = 10.5, 5.2 Hz, 1H, C<u>H_2</u>-OPMB), 2.61-2.58 (m, 4H, C_8H_{16}-C<u>H_2</u>-Ph and C<u>H_2</u>-C(CH_3)=CH-COO), 2.27 (t, *J* = 7.6 Hz, 2H, $C_{16}H_{33}$-C<u>H_2</u>-COO), 1.88 (s, 3H, C(C<u>H_3</u>)=CH-COO), 1.63-1.55 (m, 4H, $C_{15}H_{31}$-C<u>H_2</u>-CH_2-COO and C<u>H_2</u>-CH_2-

Ph), 1.47-1.41 (m, 2H, C$\underline{H_2}$-CH_2-C(CH_3)=C), 1.30-1.23 (m, 38 H, $C_5\underline{H_{10}}$-CH_2-C(CH_3)=CH and CH_3-$C_{14}\underline{H_{28}}$-CH_2), 0.88 (t, *J* = 7.0 Hz, 3H, C$\underline{H_3}$-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.5, 165.3, 162.2, 159.2, 142.9, 129.8, 129.3 (2C), 128.4 (2C), 128.2 (2C), 125.5, 115.5, 113.7 (2C), 72.9, 69.2, 67.9, 62.8, 55.2, 36.0, 34.1, 33.6, 31.9, 31.5, 29.8, 29.7 (8C), 29.5 (4C), 29.4, 29.3 (2C), 29.1, 28.2, 25.3, 24.9, 22.7, 14.1. IR (neat): 2922, 2852, 1741, 1721, 1513, 1455, 1246, 1172, 1140, 1095, 1037 cm^{-1}. HRMS (ESI +) $C_{48}H_{76}O_6$, m/z [M+H$^+$] 749.5720, found 749.5727.

Allyl *para*-methoxybenzoate (29)[42]

Allyl alcohol (73.5 mmol, 5.0 mL), Et_3N (110.3 mmol, 15.4 mL) and DMAP (3.7 mmol, 0.45 g) were dissolved in CH_2Cl_2 (250 mL). *para*-Methoxybenzoyl chloride (88.2 mmol, 11.9 mL) was added dropwise and the reaction mixture was stirred at 20 °C for 2 h. After that H_2O (200 mL) was added, the organic layer was separated and the aqueous layer was washed with CH_2Cl_2 (3 × 300 mL), and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to afford 14.0 g (99%) of **29** as colourless oil. R*f* = 0.77 (heptane/EtOAc 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (d, *J* = 9.0 Hz, 2H, C$\underline{H}$-C-OMe), 6.93 (d, *J* = 9.0 Hz, 2H, C$\underline{H}$-C-COO), 6.05 (ddt, *J* = 17.2, 10.5, 5.6 Hz, 1H, C$\underline{H}$=CH_2), 5.41 (dd, *J* = 17.2, 1.6 Hz, 1H, CH=C$\underline{H_2}$), 5.31– 5.26 (m, 1H, CH=C$\underline{H_2}$), 4.81 (dt, *J* = 5.6, 1.4 Hz, 2H, C$\underline{H_2}$-CH=CH_2), 3.78 (s, 3H, O-C$\underline{H_3}$).

1-(*para*-Methoxybenzoyl)-2,3-di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycerol (30)[42]

$K_2OsO_4 \cdot 2H_2O$ (0.74 mmol, 271 mg), $(DHQD)_2PHAL$ (0.88 mmol, 687 mg), $K_3Fe(CN)_6$ (220.6 mmol, 72.6 g) and K_2CO_3 (220.6 mmol, 30.5 g) were dissolved in a mixture of ^{t}BuOH (370 mL) and H_2O (370 mL) and stirred at 20 °C for 15 min. **29** (73.5 mmol, 14.1 g) was then added, and the resulting mixture was stirred at 20 °C for 2 h and excess reagent was quenched by addition of Na_2SO_3 (0.88 mol, 111 g). The organic layer was separated and the aqueous layer was washed with EtOAc (3 × 500 mL). Toluene (200 mL) was added to the combined organic phases and concentration *in vacuo* afforded (*S*)-1-*O*-(*para*-methoxybenzoyl)-glycerol as a white solid, ^{1}H NMR was in agreement with literature data,[216] and estimated to be pure enough to proceed with the crude product. Enantiomeric excess (>97%) was determined by chiral HPLC. HPLC (chiral) Chiralpak AS-H at 20 °C, λ= 254 nm, hexane/2-propanol 75:25, retention times 17.8 min (*S*), 23.4 min *(R)* at 0.4 mL/min flow rate. The hplc separation of the enantiomers was verified by performing the reaction in the absence of a chiral ligand to obtain the racemic diol. The crude product of (*S*)-1-*O*-(*para*-methoxybenzoyl)-glycerol and imidazole (235.3 mmol, 16.0 g) were dissolved in DMF (78 mL) and heated to 60 °C, TBSCl (220.6 mmol, 33.2 g) was added and the mixture was stirred at that temperature. After 4 h another portion of TBSCl (36.5 mmol, 5.5 g) and imidazole (36.7 mmol, 2.5 g) were added, and the reaction mixture was stirred for additional 4h. H_2O (50 mL) and Et_2O (150 mL) were then added and the organic layer was separated, and the aqueous layer was washed with Et_2O (3 × 150 mL). The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$ (100 mL). Toluene (200 mL) was added to the combined organic layers and

concentration *in vacuo* gave a colourless oil that afforded **30** as clear oil in 99 % yield (32.6 g) after purification by flash chromatography (heptane/EtOAc 8:1). R_f = 0.28 (heptane/EtOAc 10:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01 (d, *J* = 8.8 Hz, 2H, C<u>H</u>-C-OMe), 6.93 (d, *J* = 8.8 Hz, 2H, C<u>H</u>-C-COO), 4.41 (dd, *J* = 11.3, 4.0 Hz, 1H, C<u>H$_2$</u>-O-C=O), 4.25 (dd, *J* = 11.3, 6.1 Hz, 1H, C<u>H$_2$</u>-O-C=O), 4.06-4.00 (m, 1H,C<u>H$_2$</u>-OTBS), 3.89 (s, 3H, O-C<u>H$_3$</u>), 3.65-3.63 (m, 2H, CH_2-C<u>H</u>-OTBSand C<u>H$_2$</u>-OTBS), 0.90 (s, 9H, Si-C-(C<u>H$_3$</u>)$_3$), 0.88 (s, 9H, Si-C-(C<u>H$_3$</u>)$_3$), 0.10-0.07 (m, 12H, 2×Si-(C<u>H$_3$</u>)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.4, 163.4, 131.8 (2C), 122.9, 113.7 (2C), 71.5, 66.5, 65.0, 55.5, 26.1 (3C), 25.9 (3C), 18.5, 18.2, -4.4, -4.6, -5.2, -5.3. IR (neat) 2955, 2857, 1718, 1607, 1512, 1472, 1256, 1102, 840, 774 cm^{-1}.

2,3-Di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycerol (31)[42]

To a solution of 1-(*para*-methoxybenzoyl)-2,3-di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycerol (**30**) (19.79 mmol, 9 g) in anhydrous CH_2Cl_2 (70 mL) cooled to -78 °C under argon, was added dropwise 1M in hexane DIBAL-H (40 mmol, 40 mL) over 25 min, and the resulting mixture was stirred at -78 °C. Two other portions of 1M in hexane DIBAL-H (20 mmol, 20 mL) were added dropwise at -78 °C after 2 h and after 3 h respectively, and after 4.5 h, MeOH (6 mL) was added dropwise at -78 °C to quench excess DIBAL-H, and the mixture was stirred at -78 °C for 15 min. A sat. aq. Solution of Rochelle's salt (20 mL) was then added at -78 °C, and the reaction mixture was allowed to reach 20 °C over 1 h. H_2O (100 mL) was added, the organic layer was extracted, and the aqueous layer was washed with EtOAc (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, concentrated *in vacuo* and the resulting crude oil was purified by flash chromatography (CH_2Cl_2) to afford the desired **31** as clear oil in 79 % yield (5 g). R_f = 0.45 (CH_2Cl_2). $[\propto]_D^{20}$= + 17.9 ° (c = 0.6, $CHCl_3$). (lit. $[\propto]_D^{20}$= + 18.5 ° (c = 0.6, $CHCl_3$)). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.80-3.73 (m, 1H, CH_2-C<u>H</u>-CH_2), 3.66-3.52 (m, 4H, C<u>H$_2$</u>-CH-C<u>H$_2$</u>), 2.13 (t, *J* = 6.1 Hz, 1H, O<u>H</u>), 0.89 (s, 18 H, Si-C-(C<u>H$_3$</u>)$_3$), 0.09 (s, 6H, Si-(C<u>H$_3$</u>)$_2$), 0.06 (s, 6H, Si-(C<u>H$_3$</u>)$_2$). ^{13}C: NMR (75 MHz, $CDCl_3$): δ 72.7, 65.0, 64.9, 26.0 (3C), 18.4, 18.2, -4.4, -4.7, -5.3, -5.4.

2,3-Di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycero-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (32)[42]

To a solution of 2,3-di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycerol (**31**) (2.82 mmol, 904 mg), and diisopropylethylamine (5.92 mmol, 765 mg) in anhydrous CH_2Cl_2 under argon, was added dropwise 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (4.22 mmol, 1 g), and the mixture was stirred at 20 °C for 1.5 h. EtOAc (20 mL) was then added, followed by sat. aq. $NaHCO_3$ (50 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL), the combined organic layers were dried over $MgSO_4$, concentrated *in vacuo* and the desired phosphoramidite **32** was obtained in 90 % yield (1.31 g) as a clear oil after purification by flash chromatography (EtOAc). R_f = 1.0 (EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$, two diastereoisomers): δ 3.89-3.44 (m, 7H, C<u>H$_2$</u>-C<u>H</u>-C<u>H$_2$</u> and C<u>H$_2$</u>-CH_2-CN), 2.62 (t, *J* = 6.7 Hz, 2H, C<u>H$_2$</u>-CN, main isomer,

2.63, t, J = 6.7 Hz, C$\underline{H_2}$-CN,2nd isomer), 1.19-1.16 (m (2 × d from the 2 isomers), 12H, 2×(C$\underline{H_3}$)$_2$-CH-N), 0.89 (s, 9H, Si-C-(C$\underline{H_3}$)$_3$), 0.88 (s, 9H, Si-C-(C$\underline{H_3}$)$_3$), 0.08-0.05 (m, 12H, 2×(Si-C$\underline{H_3}$)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, two diastereoisomers): δ 117.8 (0.5 C), 117.7 (0.5 C), 73.3 (d, J = 7.3 Hz, 0.5 C), 73.2 (d, J = 8.3 Hz, 0.5 C), 65.1 (d, J = 15.2 Hz, 0.5 C), 65.0 (0.5 C), 64.9 (0.5 C), 64.8 (d, J = 15.7 Hz, 0.5 C), 58.7 (d, J = 11.9 Hz, 0.5 C), 58.4 (d, J = 12.5 Hz, 0.5 C), 43.2 (d, J = 4.7 Hz), 43.1(d, J = 4.6 Hz), 26.1 (3C), 26.0 (3C), 24.8, 24.7 (2C), 24.6, 20.6 (0.5 C), 20.5 (0.5C), 18.5, 18.3, -4.3 (0.5 C), -4.4 (0.5 C), -4.5, -5.2, -5.3. ^{31}P NMR (202 MHz, $CDCl_3$): δ 149.0, 148.4.

1-Stearoyl-2-((*E*)-12-phenyldodec-2-enoyl)-*sn*-glycerol (33)

20 (0.71 mmol, 522 mg) was dissolved in a mixture of CH_2Cl_2 (10 mL) and phosphate buffer (pH=7) (0.5 mL), and DDQ (1.26 mmol, 284 mg) was added in two portions over 45 min. The two-phase mixture was stirred vigorously at 20 °C for 1.5 h, and ice-cold sat. aq. $NaHCO_3$ (15 mL) and CH_2Cl_2 (10 mL) were added at 0 °C. The organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL) and the combined organic layers were washed with cold brine (15 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The product was purified by flash chromatography (heptane/EtOAc 7:1) and obtained as clear oil in 80% yield (349 mg). R_f = 0.18 (heptane/EtOAc 3:1). $[\propto]_D^{20}$= -1.13 ° (c = 1.06, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.25 (m, 2H, Ph-*meta*), 7.19-7.16 (m, 3H, Ph -*ortho* and -*para*), 7.00 (dt, J = 15.6, 7.0 Hz, 1H, C$\underline{H}$=CH-COO), 5.84 (d, J = 15.6 Hz, 1H, CH=C$\underline{H}$-COO), 5.14-5.12 (m, 1H, CH_2-C$\underline{H}$-CH_2), 4.32-4.28 (m, 2H, $C_{17}H_{35}$COO-C$\underline{H_2}$), 3.78-3.74 (m, 2H, CH_2-CH-C$\underline{H_2}$-OH), 2.61-2.59 (m, C$\underline{H_2}$-Ph), 2.32 (t, J = 7.5 Hz, 2 H, $C_{16}H_{33}$-C$\underline{H_2}$-COO), 2.20 (q, J = 7.0 Hz, 2H, C$\underline{H_2}$-CH=CH-COO), 1.62-1.58 (m, 4H, $C_{15}H_{31}$-C$\underline{H_2}$-CH_2-COO and C$\underline{H_2}$-CH_2-Ph), 1.25 (m, 40H, CH_3-$C_{14}H_{28}$-CH_2-CH_2-COO and Ph-CH_2-CH_2-$C_6\underline{H_{12}}$-CH_2-CH=CH), 0.88 (t, J = 6.7 Hz, 3H, C$\underline{H_3}$-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.8, 166.2, 151.1, 142.9, 128.4 (2C), 128.2 (2C), 125.5, 120.4, 72.2, 62.0, 61.6, 35.9, 34.1, 32.3, 31.9, 31.5, 29.7 (8C), 29.6, 29.5, 29.4 (2C), 29.3 (2C), 29.2, 29.1, 27.9, 24.9, 22.7, 14.1. IR (neat): 3530 (br.), 2922, 2852, 1723, 1508, 14632, 1248, 1174, 1151, 1034, 827, 698 cm^{-1}.HRMS (ESI +) $C_{39}H_{66}O_5$, m/z [M+Na$^+$] 637.4808, found 637.4812.

1-Stearoyl-2-((*Z*)-12-phenyldodec-2-enoyl)-*sn*-glycerol (34)

Following the general procedure described for the synthesis of compound **33**, and starting from **24** (0.567 mmol, 416 mg) in CH_2Cl_2 (8 mL) and phosphate buffer (pH=7, 0.4 mL), and from DDQ (1.0 mmol, 226 mg), **34** was obtained in 77 % yield (268 mg) as white crystals. R_f = 0.09 (heptane/EtOAc 6:1). Mp.: 32.0-31.2 °C. $[\propto]_D^{20}$= -8.33 ° (c = 1.26, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29-7.13 (m, 5H, C_6H_5), 6.29 (dt, J = 11.5, 7.5 Hz, 1H, C$\underline{H}$=CH-COO), 5.79 (dt, J = 11.5, 1.7 Hz, 1H, CH=C$\underline{H}$-COO), 5.11 (m, 1H, CH_2-C$\underline{H}$-CH_2), 4.32 (dd, J = 11.2, 4.0 Hz, 1H, $C_{17}H_{35}$COO-C$\underline{H_2}$), 4.27 (dd, J = 11.2, 4.9 Hz, 1H, $C_{17}H_{35}$COO-C$\underline{H_2}$), 3.75 (dd, J = 6.3, 5.1 Hz, 2H, CH_2-CH-C$\underline{H_2}$-OH), 2.68-2.57 (m, 4H, C_8H_{16}-C$\underline{H_2}$-Ph and C$\underline{H_2}$-CH=CH-COO), 2.34-2.26 (m, 3 H, $C_{16}H_{33}$-C$\underline{H_2}$-COO and O$\underline{H}$), 1.63-1.56 (m, 4H, $C_{15}H_{31}$-C$\underline{H_2}$-CH_2-COO and C$\underline{H_2}$-CH_2-Ph), 1.46-1.26

(m, 40H, CH_3-$C_{14}H_{28}$-CH_2-CH_2-COO and Ph-CH_2-CH_2-C_6H_{12}-CH_2-CH=CH), 0.88 (t, *J* = 6.7 Hz, 3H, CH_3-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.8, 165.7, 152.4, 142.8, 128.3 (2C), 128.1 (2C), 125.5, 118.8, 71.7, 62.0, 61.5, 35.9, 34.1, 32.3, 31.9, 31.5, 29.7 (7C), 29.6 (2C), 29.4 (2C), 29.3 (3C), 29.2 (2C), 29.1, 28.9, 24.9, 22.7, 14.1. IR (neat): 3533 (br.), 2919, 2851, 1724, 1496, 1166, 1049 cm^{-1}. HRMS (ESI +) $C_{39}H_{66}O_5$, m/z [M+Na^+] 637.4808, found 637.4826.

1-Stearoyl-2-((*E*)-2-methyl-12-phenyldodec-2-enoyl)-*sn*-glycerol (35)

Following the same procedure as described for the synthesis of compound **33**, starting from **25** (0.36 mmol, 270 mg) in CH_2Cl_2 (5 mL) and phosphate buffer (pH = 7, 250 µL) and DDQ (2 × 0.32 mmol, 2 × 72 mg), to give **35** in 60 % yield (121 mg) as white solid. Mp.: 27.3-28.1 °C. R_f = 0.13 (hexane/EtOAc 6:1). $[\propto]_D^{20}$= + 3.07 ° (c = 1.79, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.14 (m, 5H, C_6H_5), 6.81 (tq, *J* = 7.5, 1.4 Hz, 1H, CH=C(CH_3)-COO), 5.12 (m, 1H, CH_2-CH-CH_2), 4.36-4.29 (m, 2H, $C_{17}H_{35}$COO-CH_2), 3.77 (t, *J* = 5.6 Hz, 2H, CH_2-CH-CH_2-OH), 2.60 (m, 2H, CH_2-Ph), 2.32 (t, *J* = 7.5 Hz, 2H, $C_{16}H_{33}$-CH_2-COO), 2.31 (br. s, 1H, OH), 2.17 (q, *J* = 7.5 Hz, 2H, CH_2-CH=C(CH_3)-COO), 1.83 (d, *J* = 1.4 Hz, 3H, CH=C(CH_3)COO), 1.63-1.56 (m, 4H, $C_{15}H_{31}$-CH_2-CH_2-COO and CH_2-CH_2-Ph), 1.45-1.26 (m, 40H, CH_3-$C_{14}H_{28}$-CH_2-CH_2-COO and Ph-CH_2-CH_2-C_6H_{12}-CH_2-CH=CH), 0.88 (t, *J* = 6.7 Hz, 3H, CH_3-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.8, 167.7, 144.0, 142.8, 128.3 (2C), 128.2 (2C), 127.0, 125.5, 72.5, 62.0, 61.7, 35.9, 34.1, 31.9, 31.5, 29.7 (8C), 29.6 (2C), 29.5, 29.4 (2C), 29.3 (2C), 29.2, 29.1, 28.8, 28.5, 24.9, 22.7, 14.1, 12.3. IR (neat): 3522 (br.), 2918, 2949, 1741, 1713, 1688, 1463, 1272, 1253, 1169, 1147, 1099, 1056 cm^{-1}. HRMS (ESI +) $C_{40}H_{68}O_5$, m/z [M+H^+] 629.5145, found 629.5154.

1-Stearoyl-2-((*Z*)-2-methyl-12-phenyldodec-2-enoyl)-*sn*-glycerol (36)

Following the same procedure as described for the synthesis of compound **33**, starting from **26** (0.58 mmol, 433 mg) in CH_2Cl_2 (8 mL) and phosphate buffer (pH = 7, 0.4 mL) and DDQ (2 × 0.51 mmol, 2 × 116 mg), to give **36** as white crystals in 80 % yield (290 mg). R_f = 0.13 (hexane/EtOAc 6:1). Mp.: 30.8-31.1 °C. $[\propto]_D^{20}$= -2.4 ° (c = 1.37, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31-7.14 (m, 5H, C_6H_5), 6.00 (tq, *J* = 7.5, 1.4 Hz, 1H, CH=C(CH_3)-COO), 5.15 (m, 1H, CH_2-CH-CH_2), 4.36 (dd, *J* = 11.9, 4.5 Hz, 1H, $C_{17}H_{35}$COO-CH_2), 4.30 (dd, *J* = 11.9, 5.5 Hz, 1H, $C_{17}H_{35}$COO-CH_2), 3.77 (dd, *J* = 6.3, 5.2 Hz, 2H, CH_2-CH-CH_2-OH), 2.63-2.58 (m, 2H, CH_2-Ph), 2.45 (q, *J* = 7.5 Hz, 2H, CH_2-CH=C(CH_3)-COO), 2.32 (t, *J* = 7.5 Hz, 3H, $C_{16}H_{33}$-CH_2-COO and OH), 1.90 (d, *J* = 1.4 Hz, 3H, CH=C(CH_3)COO), 1.64-1.57 (m, 4H, $C_{15}H_{31}$-CH_2-CH_2-COO and CH_2-CH_2-Ph), 1.43-1.26 (m, 40H, CH_3-$C_{14}H_{28}$-CH_2-CH_2-COO and Ph-CH_2-CH_2-C_6H_{12}-CH_2-CH=CH), 0.89 (t, *J* = 6.7 Hz, 3H, CH_3-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.7, 167.4, 144.9, 142.8, 128.3 (2C), 128.1 (2C), 126.2, 125.5, 72.1, 62.1, 61.5, 35.9, 34.1, 31.9, 31.5, 29.7 (9C), 29.6 (2C), 29.5 (2C), 29.4 (2C), 29.3 (2C), 29.2, 29.1, 24.9, 22.7, 20.57, 14.1. IR (neat): 3527 (br.), 2922, 2852, 1742, 1719, 1455, 1154, 1095, 1048 cm^{-1}. HRMS (ESI +) $C_{40}H_{68}O_5$, m/z [M+H^+] 629.5145, found 629.5138.

1-Stearoyl-2-((*E*)-3-methyl-12-phenyldodec-2-enoyl)-*sn*-glycerol (37)

Following the same procedure as described for the synthesis of compound **33**, starting from **27** (0.35 mmol, 260 mg) in CH_2Cl_2 (5 mL) and phosphate buffer (pH = 7, 270 µL) and DDQ (2 × 0.31 mmol, 2 × 69 mg), to give **37** as white solid in 60 % yield (130 mg). R_f = 0.39 (heptane/EtOAc 3:1). Mp.: 37.7-39.1 °C. $[\propto]_D^{20}$= -2.63 ° (c = 1.71, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.14 (m, 5H, $C_6\underline{H}_5$), 5.69 (q, *J* = 1.1 Hz, 1H, $C(CH_3)$=C<u>H</u>-COO), 5.13-5.07 (m, 1H, CH_2-C<u>H</u>-CH_2), 4.32 (dd, *J* = 12.0, 4.9 Hz, 1H, $C_{17}H_{35}COO$-C$\underline{H}_2$), 4.27 (dd, *J* = 12.0, 5.6 Hz, 1H, $C_{17}H_{35}COO$-C$\underline{H}_2$), 3.76-3.73 (m, 2H, CH_2-CH-C$\underline{H}_2$-OH), 2.63-2.57 (m, 2H, C$\underline{H}_2$-Ph), 2.32 (t, *J* = 7.5 Hz, 2H, $C_{16}H_{33}$-C$\underline{H}_2$-COO), 2.21-2.11 (m, 5H, C$\underline{H}_2$-C(C$\underline{H}_3$)=CH-COO), 1.64-1.56 (m, 4H, $C_{15}H_{31}$-C$\underline{H}_2$-CH_2-COO and C$\underline{H}_2$-CH_2-Ph), 1.50-1.42 (m, 2H, C$\underline{H}_2$-CH_2-C(CH_3)=CH), 1.35-1.26 (m, 38H, CH_3-$C_{14}H_{28}$-CH_2-CH_2-COO and Ph-CH_2-CH_2-$C_5\underline{H}_{10}$-CH_2-CH_2-CH=CH), 0.88 (t, *J* = 6.7 Hz, 3H, C$\underline{H}_3$-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.8, 166.1, 162.6, 142.9, 128.4 (2C), 128.2 (2C), 125.5, 114.6, 71.4, 62.1, 61.7, 41.1, 36.0, 34.1, 31.9, 31.5, 29.7 (7C), 29.6, 29.5 (2C), 29.4 (3C), 29.3 (2C), 29.2, 29.1, 27.4, 24.9, 22.7, 19.0, 14.1. IR (neat): 3524 (br.), 2922, 2852, 1741, 1719, 1645, 1464, 1455, 1218, 1142, 1051 cm^{-1}. HRMS (ESI +) $C_{40}H_{68}O_5$, m/z [M+H^+] 629.5145, found 629.5133.

1-Stearoyl-2-((*Z*)-3-methyl-12-phenyldodec-2-enoyl)-*sn*-glycerol (38)

Following the same procedure as described for the synthesis of compound **33**, starting from **28** (0.33 mmol, 249 mg) in CH_2Cl_2 (4.5 mL) and phosphate buffer (pH = 7, 256 µL) and DDQ (2 × 0.29 mmol, 2 × 67 mg), to give **38** as white amorphous solid in 62 % yield (130 mg). R_f = 0.25 (heptane/EtOAc 3:1). $[\propto]_D^{20}$= -6.46 ° (c = 1.78, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.14 (m, 5H, $C_6\underline{H}_5$), 5.68 (m, 1H, $C(CH_3)$=C<u>H</u>-COO), 5.12-5.06 (m, 1H, CH_2-C<u>H</u>-CH_2), 4.31 (dd, *J* = 11.9, 4.9 Hz, 1H, $C_{17}H_{35}COO$-C$\underline{H}_2$), 4.26 (dd, *J* = 11.9, 5.4 Hz, 1H, $C_{17}H_{35}COO$-C$\underline{H}_2$), 3.74 (m, 2H, CH_2-CH-C$\underline{H}_2$-OH), 2.63-2.57 (m, 4H, C$\underline{H}_2$-Ph and C$\underline{H}_2$-C(CH_3)=CH-COO), 2.32 (t, *J* = 7.5 Hz, 2H, $C_{16}H_{33}$-C$\underline{H}_2$-COO), 2.8 (t, *J* = 6.5, 1H, OH), 1.90 (d, *J* = 1.3 Hz, 3H, C(C$\underline{H}_3$)=CH-COO), 1.65-1.56 (m, 4H, $C_{15}H_{31}$-C$\underline{H}_2$-CH_2-COO and C$\underline{H}_2$-CH_2-Ph), 1.48-1.40 (m, 2H, C$\underline{H}_2$-CH_2-C(CH_3)=CH), 1.35-1.26 (m, 38H, CH_3-$C_{14}H_{28}$-CH_2-CH_2-COO and Ph-CH_2-CH_2-$C_5\underline{H}_{10}$-CH_2-CH_2-CH=CH), 0.88 (t, *J* = 6.7 Hz, 3H, C$\underline{H}_3$-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.8, 165.6, 163.0, 142.9, 128.4 (2C), 128.2 (2C), 125.5, 115.1, 71.3, 62.1, 61.7, 36.0, 34.1, 33.6, 31.9, 31.5, 29.8, 29.7 (8C), 29.6(2C), 29.5 (2C), 29.4, 29.3 (2C), 29.1, 28.2, 25.3, 24.9, 22.7, 14.1. IR (neat): 3492 (br.), 2922, 2852, 1741, 1720, 1645, 1456, 1377, 1226, 1142, 1051 cm^{-1}. HRMS (ESI +) $C_{40}H_{68}O_5$, m/z [M+H^+] 629.5145, found 629.5130.

1-Stearoyl-2-((*E*)-12-phenyldodec-2-enoyl)-*sn*-glycero-3-phospho-(*S*)-glycerol (39)

To a solution of **33** (0.49 mmol, 302 mg) and 2,3-di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycero-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (**32**) (0.66 mmol, 345 mg) in anhydrous CH_2Cl_2 (4 mL) was added 0.45 M tetrazole in MeCN (0.66 mmol, 1.47 mL), and the mixture was stirred at 20 °C. After 45 min, a solution of ^{t}BuOOH 5.5 M in nonane (0.68 mmol, 0.123 mL) was added, and the mixture was stirred at 20 °C for 1 h before concentration *in vacuo*. The residue was purified by flash chromatography (hexane/EtOAc 5:1) to give 390 mg of a clear oil that was dissolved in CH_2Cl_2 (3 mL). DBU (0.39 mmol, 58 µL) was added and the mixture was stirred at 20 °C for 1 h. After that time, the mixture was concentrated *in vacuo* and the crude mixture was purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to give 330 mg of the intermediate compound. This was then dissolved in CH_2Cl_2 (12 mL) and MeCN (32 mL) and the solution was cooled to 0 °C, treated with 40 % w/w aqueous HF (1.3 mL), and the mixture was stirred vigorously at 0 °C for 3 h. The excess reagent was quenched by addition of $MeOSiMe_3$ (12 mL) at 0 °C, the mixture was stirred for 30 min at 20 °C, and $NaHCO_3$ (3.26 mmol, 274 mg) was added to the reaction mixture. The desired phospholipid was isolated in 46% yield (177 mg) after flash chromatography (CH_2Cl_2/MeOH/H_2O 9:1:0 to 65:25:4) as white amorphous solid. R_f = 0.15 (CH_2Cl_2/MeOH 9:1). ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD 4:1): δ 7.28-7.25 (m, 2H, Ph -*meta*), 7.18-7.15 (m, 3H, Ph –*ortho* and -*para*), 7.01 (dt, *J* = 15.5, 7.0 Hz, 1H, C<u>H</u>=CH-COO), 5.81 (d, *J* = 15.5 Hz, 1H, CH=C<u>H</u>-COO), 5.29-5.26 (m, 1H, $C_{17}H_{35}$-COO-CH_2-C<u>H</u>-CH_2), 4.38 (dd, *J* = 11.9, 2.8 Hz, 1H, $C_{17}H_{35}$-COO-C<u>H_2</u>), 4.28-4.20 (1H, $C_{17}H_{35}$-COO-C<u>H_2</u>, under HDO signal), 4.02 (t, *J* = 5.6 Hz, 2H, $C_{17}H_{35}$-COO-CH_2-CH-C<u>H_2</u>), 3.97-3.89 (m, 2H, P-O-C<u>H_2</u>-CH-CH_2-OH), 3.84-3.80 (m, 1H, P-O-CH_2-C<u>H</u>-CH_2-OH), 3.65 (dd, *J* = 11.4, 4.8 Hz, 1H, P-O-CH_2-CH-C<u>H_2</u>-OH), 3.61 (dd, *J* = 11.4, 5.0 Hz, 1H, P-O-CH_2-CH-C<u>H_2</u>-OH), 2.61-2.58 (m, 2H, C<u>H_2</u>-Ph), 2.30 (t, *J* = 7.5 Hz, 2H, $C_{16}H_{33}$-C<u>H_2</u>-COO), 2.20 (q, *J* = 7.0 Hz, 2H, C<u>H_2</u>-CH=CH-COO), 1.62-1.57 (m, 4H, C<u>H_2</u>-CH_2-Ph and C<u>H_2</u>-CH_2-COO), 1.46-1.40 (m, 2H, C<u>H_2</u>-CH_2-CH=CH), 1.34-1.22 (m, 38H, CH_3-C_{14}<u>H_{28}</u>-CH_2-CH_2-COO and Ph-CH_2-CH_2-C_5<u>H_{10}</u>-CH_2-CH_2-CH=CH), 0.88 (t, *J* = 6.8 Hz, 3H, C<u>H_3</u>-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD 4:1): δ 174.1, 166.3, 151.1, 142.9, 128.4 (2C), 128.3 (2C), 125.6, 120.4, 70.9 (d, *J* = 3.8 Hz), 70.5 (d, *J* = 9.1 Hz), 66.5 (d, *J* = 5.1 Hz), 63.8 (d, *J* = 4.2 Hz), 62.7, 62.2, 36.1, 34.1, 32.5, 32.0, 31.6, 29.8 (13C), 29.6, 29.5, 29.4 (2C), 28.1, 25.0, 22.8, 14.1. ^{31}P NMR (202 MHz, $CDCl_3$/CD_3OD 4:1): δ -1.362. IR (neat): 3341, 2916, 2819, 1737, 1717, 1456, 1255, 1236, 1129, 1094, 1056, 1012, 874, 843, 814, 695 cm^{-1}. HRMS (ESI -) $C_{42}H_{72}O_{10}PNa$ $[M - Na]^-$ 767.4863, found 767.4877.

1-Stearoyl-2-(*(Z)*-12-phenyldodec-2-enoyl)-*sn*-glycero-3-phospho-(*S*)-glycerol (40)

To a solution of **34** (0.40 mmol, 245 mg) and 2,3-di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycero-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (**32**) (0.54 mmol, 281 mg) in anhydrous CH_2Cl_2 (3 mL) was added 0.45 M tetrazole in MeCN (0.54 mmol,1.2 mL), and the mixture was stirred at 20 °C. After 45 min, a solution of ^{t}BuOOH 5.5 M in nonane (0.55 mmol, 0.1 mL) was added, and the mixture was stirred at 20 °C for 1 h before concentration *in vacuo*. The residue was purified by flash chromatography (hexane/EtOAc 5:1) to give 258 mg of a clear oil that was dissolved in CH_2Cl_2 (2 mL). DBU (0.36 mmol, 63 µL) was added and the mixture was stirred at 20 °C for 1 h. After that time, the mixture was concentrated *in vacuo* and the crude mixture was purified by flash

chromatography (CH_2Cl_2/MeOH 9:1) to give 226 mg of the intermediate compound. This was then dissolved in CH_2Cl_2 (9 mL) and MeCN (26 mL) and the solution was cooled to 0 °C, treated with 40 % w/w aqueous HF (880 µL), and the mixture was stirred vigorously and allowed to reach slowly 20 °C over 3 h. The excess reagent was quenched by addition of $MeOSiMe_3$ (9 mL) at 0 °C, the mixture was stirred for 30 min at 20 °C, and $NaHCO_3$ (2.21 mmol, 186 mg) was added to the reaction mixture. The desired phospholipid was isolated in 17% yield (55 mg) after flash chromatography (CH_2Cl_2/MeOH/H_2O 9:1:=0 to 65:25:4) as white amorphous solid. R_f = 0.15 (CH_2Cl_2/MeOH 4:1). ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD 4:1): δ 7.30-7.13 (m, 5H, $C_6\underline{H}_5$), 6.31 (dt, *J* = 11.5, 7.5 Hz, 1H, C<u>H</u>=CH-COO), 5.76 (dt, *J* = 11.5, 1.6 Hz, 1H, CH=C<u>H</u>-COO), 5.30-5.24 (m, 1H, $C_{17}H_{35}$-COO-CH_2-C<u>H</u>-CH_2), 4.35-4.42 (1H, $C_{17}H_{35}$-COO-C<u>H_2</u>, under HDO signal), 4.22 (dd, *J* = 12.0, 6.9 Hz, 1H, $C_{17}H_{35}$-COO-C<u>H_2</u>), 4.02 (m, 2H, $C_{17}H_{35}$-COO-CH_2-CH-C<u>H_2</u>), 3.97-3.90 (m, 2H, P-O-C<u>H_2</u>-CH-CH_2-OH), 3.85-3.78 (m, 1H, P-O-CH_2-C<u>H</u>-CH_2-OH), 3.67-3.58 (m, 2H, P-O-CH_2-CH-C<u>H_2</u>-OH), 2.75-2.51 (m, 4H, C<u>H_2</u>-Ph and C<u>H_2</u>-CH=CH-COO), 2.31 (t, *J* = 7.6 Hz, 2H, $C_{16}H_{33}$-C<u>H_2</u>-COO), 1.66-1.54 (m, 4H, C<u>H_2</u>-CH_2-Ph and C<u>H_2</u>-CH_2-COO), 1.48-1.40 (m, 2H, C<u>H_2</u>-CH_2-CH=CH), 1.36-1.22 (m, 38H, CH_3-$C_{14}\underline{H}_{28}$-CH_2-CH_2-COO and Ph-CH_2-CH_2-$C_5\underline{H}_{10}$-CH_2-CH_2-CH=CH), 0.88 (t, *J* = 6.8 Hz, 3H, C<u>H_3</u>-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD 4:1): δ 174.2, 165.8, 152.7, 143.0, 128.5 (2C), 128.3 (2C), 125.7, 118.8, 71.0 (d, *J* = 5.4 Hz), 70.0 (d, *J* = 8.6 Hz), 66.5 (d, *J* = 5.6 Hz), 63.9 (d, *J* = 4.2 Hz), 62.8, 62.3, 36.1, 34.2, 32.1, 31.7, 29.8 (11C), 29.7 (2C), 29.5 (3C), 29.3 (2C), 29.1, 25.0, 22.8, 14.1. ^{31}P NMR (202 MHz, $CDCl_3$/CD_3OD 4:1): δ -1.659. IR (neat): 3500 (br.), 2919, 2850, 1722, 1237, 1183, 1106, 1070 cm^{-1}. HRMS (ESI -) $C_{42}H_{72}NaO_{10}P$, m/z $[M\text{-}Na]^-$ 767.4863, found 767.4880.

1-Stearoyl-2-((*E*)-2-methyl-12-phenyldodec-2-enoyl)-*sn*-glycero-3-phospho-(*S*)-glycerol (41)

To a solution of **35** (0.16 mmol, 100 mg) and 2,3-di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycero-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (**32**) (0.22 mmol, 112 mg) in anhydrous CH_2Cl_2 (1.5 mL) was added 0.45 M tetrazole in MeCN (0.22 mmol, 478 µL), and the mixture was stirred at 20 °C. After 45 min, a solution of ^{t}BuOOH 5.5 M in nonane (0.22 mmol, 40 µL) was added, and the mixture was stirred at 20 °C for 1 h before concentration *in vacuo*. The residue was purified by flash chromatography (hexane/EtOAc 5:1) to give 103 mg of a clear oil that was dissolved in CH_2Cl_2 (0.7 mL). DBU (0.10 mmol, 15 µL) was added and the mixture was stirred at 20 °C for 1 h. After that time, the mixture was concentrated *in vacuo* and the crude mixture was purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to give 70 mg of the intermediate compound. This was then dissolved in CH_2Cl_2 (3 mL) and MeCN (8.2 mL) and the solution was cooled to 0 °C, treated with 40 % w/w aqueous HF (276 µL), and the mixture was stirred vigorously and allowed to reach slowly 20 °C over 3 h. The excess reagent was quenched by addition of $MeOSiMe_3$ (3 mL) at 0 °C, the mixture was stirred for 30 min at 20 °C, and $NaHCO_3$ (0.70 mmol, 58 mg) was added to the reaction mixture. The desired phospholipid was isolated in 17 % yield (22 mg) after flash chromatography (CH_2Cl_2/MeOH/H_2O 9:1:=0 to 65:25:4) as white amorphous solid. R_f = 0.15 (CH_2Cl_2/MeOH 4:1). ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD 4:1): δ 7.30-7.13 (m, 5H, $C_6\underline{H}_5$), 6.79 (tq, *J* = 7.5, 1.1 Hz, 1H, C<u>H</u>=C(CH_3)-COO), 5.30-5.23 (m, 1H, $C_{17}H_{35}$-COO-CH_2-C<u>H</u>-CH_2), 4.40 (dd, *J*

= 12.0, 3.2 Hz, 1H, $C_{17}H_{35}$-COO-C$\underline{H}_2$), 4.30-4.23 (1H, $C_{17}H_{35}$-COO-C$\underline{H}_2$, under HDO signal), 4.06-3.98 (m, 2H, $C_{17}H_{35}$-COO-CH$_2$-CH-C$\underline{H}_2$), 3.96-3.90 (m, 2H, P-O-C$\underline{H}_2$-CH-CH$_2$-OH), 3.84-3.78 (m, 1H, P-O-CH$_2$-C$\underline{H}$-CH$_2$-OH), 3.65 (dd, *J* = 13.1, 4.8 Hz, 1H, P-O-CH$_2$-CH-C$\underline{H}_2$-OH), 3.61 (dd, *J* = 13.1, 5.1 Hz, 1H, P-O-CH$_2$-CH-C$\underline{H}_2$-OH), 2.62 (m, 2H, C$\underline{H}_2$-Ph), 2.31 (t, *J* = 7.6 Hz, 2H, $C_{16}H_{33}$-C$\underline{H}_2$-COO), 2.17 (q, *J* = 7.5 Hz, C$\underline{H}_2$-CH=C(CH$_3$)-COO), 1.81 (d, *J* = 1.1 Hz, 3H, CH=C(C$\underline{H}_3$)-COO), 1.66-1.52 (m, 4H, C$\underline{H}_2$-CH$_2$-Ph and C$\underline{H}_2$-CH$_2$-COO), 1.46-1.22 (m, 40H, CH$_3$-$C_{14}\underline{H}_{28}$-CH$_2$-CH$_2$-COO and Ph-CH$_2$-CH$_2$-$C_6\underline{H}_{12}$-CH$_2$-CH=C(CH$_3$)), 0.88 (t, *J* = 6.7 Hz, 3H, C$\underline{H}_3$-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$ 4:1): δ 174.1, 167.9, 144.5, 143.0, 128.5 (2C), 128.3 (2C), 127.0, 125.7, 71.0 (d, *J* = 4.9 Hz), 70.8 (d, *J* = 9.2 Hz), 66.5 (d, *J* = 5.1 Hz), 63.8 (d, *J* = 4.3 Hz), 62.7, 62.3, 36.1, 34.2, 32.1, 31.7, 29.8 (10C), 29.6 (3C), 29.5 (2C), 29.4, 29.3, 29.0, 28.7, 25.0, 22.8, 14.1, 12.2. ^{31}P NMR (202 MHz, $CDCl_3/CD_3OD$ 4:1): δ -1.636. IR (neat): 3500 (br.), 2918, 2850, 1736, 1713, 1466, 1275, 1255, 1228, 1174, 1095, 1076, 1030 cm^{-1}. HRMS (ESI -) $C_{43}H_{74}O_{10}PNa$ [M – Na]$^-$ 781.5020, found 781.5037.

1-Stearoyl-2-((*Z*)-2-methyl-12-phenyldodec-2-enoyl)-*sn*-glycero-3-phospho-(*S*)-glycerol (42)

To a solution of **36** (0.42 mmol, 266 mg) and 2,3-di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycero-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (**32**) (0.57 mmol, 298 mg) in anhydrous CH_2Cl_2 (3.3 mL) was added 0.45 M tetrazole in MeCN (0.57 mmol, 130 µL), and the mixture was stirred at 20 °C. After 45 min, a solution of ^{t}BuOOH 5.5 M in nonane (0.58 mmol, 100 µL) was added, and the mixture was stirred at 20 °C for 1 h before concentration *in vacuo*. The residue was purified by flash chromatography (hexane/EtOAc 5:1) to give 274 mg of a clear oil that was dissolved in CH_2Cl_2 (2 mL). DBU (0.270 mmol, 41µL) was added and the mixture was stirred at 20 °C for 1 h. After that time, the mixture was concentrated *in vacuo* and the crude mixture was purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to give 245 mg of the intermediate compound. This was then dissolved in CH_2Cl_2 (9.5 mL) and MeCN (27 mL) and the solution was cooled to 0 °C, treated with 40 % w/w aqueous HF (940 µL), and the mixture was stirred vigorously and allowed to reach slowly 20 °C over 3 h. The excess reagent was quenched by addition of $MeOSiMe_3$ (9.5 mL) at 0 °C, the mixture was stirred for 30 min at 20 °C, and $NaHCO_3$ (2.37 mmol, 100 mg) was added to the reaction mixture. The desired phospholipid was isolated in 17% yield (57 mg) after flash chromatography (CH_2Cl_2/MeOH/H_2O 9:1:0 to 65:25:4) as white amorphous solid. R_f = 0.19 (CH_2Cl_2/MeOH 4:1). ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 4:1): δ 7.30-7.13 (m, 5H, $C_6\underline{H}_5$), 6.01 (tq, *J* = 7.3, 1.3 Hz, 1H, C$\underline{H}$=C(CH$_3$)-COO), 5.34-5.26 (m, 1H, $C_{17}H_{35}$-COO-CH$_2$-C$\underline{H}$-CH$_2$), 4.42 (dd, *J* = 12.0, 2.9 Hz, 1H, $C_{17}H_{35}$-COO-C$\underline{H}_2$), 4.30-4.20 (1H, $C_{17}H_{35}$-COO-C$\underline{H}_2$, under HDO signal), 4.10-3.98 (m, 2H, $C_{17}H_{35}$-COO-CH$_2$-CH-C$\underline{H}_2$), 3.98-3.88 (m, 2H, P-O-C$\underline{H}_2$-CH-CH$_2$-OH), 3.86-3.78 (m, 1H, P-O-CH$_2$-C$\underline{H}$-CH$_2$-OH), 3.668-3.58 (m, 2H, P-O-CH$_2$-CH-C$\underline{H}_2$-OH), 2.62-2.57 (m, 2H, C$\underline{H}_2$-Ph), 2.43 (q, *J* = 7.3 Hz, C$\underline{H}_2$-CH=C(CH$_3$)-COO), 2.30 (t, *J* = 7.6 Hz, 2H, $C_{16}H_{33}$-C$\underline{H}_2$-COO), 1.88 (d, *J* = 1.3 Hz, 3H, CH=C(C$\underline{H}_3$)-COO), 1.68-1.54 (m, 4H, C$\underline{H}_2$-CH$_2$-Ph and C$\underline{H}_2$-CH$_2$-COO), 1.42-1.22 (m, 40H, CH$_3$-$C_{14}\underline{H}_{28}$-CH$_2$-CH$_2$-COO and Ph-CH$_2$-CH$_2$-$C_6\underline{H}_{12}$-CH$_2$-CH=C(CH$_3$)), 0.88 (t, *J* = 6.6 Hz, 3H, C$\underline{H}_3$-$C_{16}H_{32}$-COO). ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$ 4:1): δ 174.1, 167.5, 145.4, 143.0, 128.5 (2C), 128.3 (2C), 126.3, 125.6, 71.0 (d, *J* = 5.7 Hz), 70.4 (d, *J* = 7.4 Hz), 66.5 (d, *J* =

3.7 Hz), 63.8 (d, J = 4.1 Hz), 62.8, 62.3, 36.1, 34.2, 32.0, 31.7, 29.9, 29.8 (10C), 29.7 (3C), 29.6 (2C), 29.5 (2C), 29.3, 25.0, 22.8, 20.5, 14.1. ^{31}P NMR (202 MHz, $CDCl_3/CD_3OD$ 4:1): δ -1.659. IR (neat): 3500 (br.), 2918, 2849, 1734, 1709, 1466, 1455, 1387, 1221, 1135, 1098, 1072 cm^{-1}. HRMS (ESI -) $C_{43}H_{74}O_{10}PNa$ $[M - Na]^-$ 781.5020, found 781.5024.

1-Stearoyl-2-(*(E)*-3-methyl-12-phenyldodec-2-enoyl)-*sn*-glycero-3-phospho-(*S*)-glycerol (43)

To a solution of **37** (0.165 mmol, 104 mg) and 2,3-di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycero-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (**32**) (0.223 mmol, 116 mg) in anhydrous CH_2Cl_2 (1.3 mL) was added 0.45 M tetrazole in MeCN (0.223 mmol, 500 µL), and the mixture was stirred at 20 °C. After 45 min, a solution of ^{t}BuOOH 5.5 M in nonane (0.228 mmol, 41 µL) was added, and the mixture was stirred at 20 °C for 1 h before concentration *in vacuo*. The residue was purified by flash chromatography (hexane/EtOAc 5:1) to give 108 mg of a clear oil that was dissolved in CH_2Cl_2 (780 µL), DBU (0.106 mmol, 16 µL) was added and the mixture was stirred at 20 °C for 1 h. After that time, the mixture was concentrated *in vacuo* and the crude mixture was purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to give 95 mg of the intermediate compound. This was then dissolved in CH_2Cl_2 (3.7 mL) and MeCN (10 mL) and the solution was cooled to 0 °C, treated with 40 % w/w aqueous HF (365 µL), and the mixture was stirred vigorously and kept at 0 °C for 3 h. The excess reagent was quenched by addition of $MeOSiMe_3$ (3.7 mL) at 0 °C, the mixture was stirred for 30 min at 20 °C, and $NaHCO_3$ (0.919 mmol, 77 mg) was added to the reaction mixture. The desired phospholipid was isolated in 45 % yield (59 mg) after flash chromatography (CH_2Cl_2/MeOH/H_2O 9:1:0 to 65:25:4) as white amorphous solid. R_f = 0.13 (CH_2Cl_2/MeOH 9:1). ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 4:1): δ 7.31-7.11 (m, 5H, $C_6\underline{H}_5$), 5.66 (q, J = 1.1 Hz, 1H, $C(CH_3)$=C$\underline{H}$-COO), 5.31-5.19 (m, 1H, $C_{17}H_{35}$-COO-CH_2-C$\underline{H}$-CH_2), 4.41-4.39 (m, 1H, $C_{17}H_{35}$-COO-C$\underline{H}_2$, under HDO signal), 4.23 (dd, J = 11.9, 6.5 Hz, 1H, $C_{17}H_{35}$-COO-C$\underline{H}_2$,), 4.10-3.89 (m, 4H, $C_{17}H_{35}$-COO-CH_2-CH-C$\underline{H}_2$, and P-O-C$\underline{H}_2$-CH-CH_2-OH), 3.86-3.78 (m, 1H, P-O-CH_2-C$\underline{H}$-CH_2-OH), 3.69-3.56 (m, 2H, P-O-CH_2-CH-C$\underline{H}_2$-OH), 2.60 (m, 2H, C$\underline{H}_2$-Ph), 2.32 (t, J = 7.5 Hz, 2H, $C_{16}H_{33}$-C$\underline{H}_2$-COO), 2.18-2.11 (m, 5H, C$\underline{H}_2$-C(C$\underline{H}_3$)=CH-COO), 1.66-1.54 (m, 4H, C$\underline{H}_2$-CH_2-Ph and C$\underline{H}_2$-CH_2-COO), 1.50-1.20 (m, 40H, CH_3-$C_{14}\underline{H}_{28}$-CH_2-CH_2-COO and Ph-CH_2-CH_2-$C_6\underline{H}_{12}$-CH_2-C(CH_3)=CH), 0.88 (t, J = 6.8 Hz, 3H, C$\underline{H}_3$-$C_{16}H_{32}$-COO). ^{13}C NMR (50 MHz, $CDCl_3/CD_3OD$ 4:1): δ 174.2, 166.3, 162.9, 143.0, 128.5 (2C), 128.3 (2C), 125.7, 114.7, 71.1, 69.7 (d, J = 7.4 Hz), 66.5, 63.9, 62.9, 62.3, 41.3, 36.1, 34.2, 32.0, 31.6, 29.8 (10C), 29.6 (4C), 29.5 (2C), 29.3, 27.6, 25.0, 22.8, 19.0, 14.1. ^{31}P NMR (202 MHz, $CDCl_3/CD_3OD$ 4:1): δ -3.009 IR (neat): 3500 (br.), 2919, 2850, 1739, 1719, 1651, 1466, 1454, 1216, 1138, 1093, 1074, 1052 cm^{-1}. HRMS (ESI -) $C_{43}H_{74}O_{10}PNa$ $[M - Na]^-$ 781.5020, found 781.5031.

1-Stearoyl-2-((*Z*)-3-methyl-12-phenyldodec-2-enoyl)-*sn*-glycero-3-phospho-(*S*)-glycerol (44)

To a solution of **38** (0.165 mmol, 104 mg) and 2,3-di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycero-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (**32**) (0.223 mmol, 116 mg) in anhydrous CH_2Cl_2 (1.3 mL) was added 0.45 M tetrazole in MeCN (0.223 mmol, 500 µL), and the mixture was stirred at 20 °C. After 45 min, a solution of ^{t}BuOOH 5.5 M in nonane (0.228 mmol, 41 µL) was added, and the mixture was stirred at 20 °C for 1 h before concentration *in vacuo*. The residue was purified by flash chromatography (hexane/EtOAc 5:1) to give 113 mg of a clear oil that was dissolved in CH_2Cl_2 (1 mL). DBU (0.112 mmol, 17 µL) was added and the mixture was stirred at 20 °C for 1.5 h. After that time, the mixture was concentrated *in vacuo* and the crude mixture was purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to give 75 mg of the intermediate compound. This was then dissolved in CH_2Cl_2 (2.8 mL) and MeCN (8 mL) and the solution was cooled to 0 °C, treated with 40 % w/w aqueous HF (277 µL), and the mixture was stirred vigorously and kept at 0 °C for 3 h. The excess reagent was quenched by addition of $MeOSiMe_3$ (2.7 mL) at 0 °C, the mixture was stirred for 30 min at 20 °C, and $NaHCO_3$ (0.697 mmol, 58 mg) was added to the reaction mixture. The desired phospholipid was isolated in 35 % yield (43 mg) after flash chromatography (CH_2Cl_2/MeOH/H_2O 9:1:=0 to 65:25:4) as white amorphous solid. R_f = 0.13 (CH_2Cl_2/MeOH 9:1). ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 4:1): δ 7.30-7.13 (m, 5H, $C_6\underline{H}_5$), 5.65-5.63 (m, 1H, $C(CH_3)$=C$\underline{H}$-COO), 5.26-5.19 (m, 1H, $C_{17}H_{35}$-COO-CH_2-C$\underline{H}$-CH_2), 4.36 (dd, *J* = 12.0, 3.3 Hz, 1H, $C_{17}H_{35}$-COO-C$\underline{H}_2$), 4.24-4.15 (1H, $C_{17}H_{35}$-COO-C$\underline{H}_2$, under HDO signal), 4.04-3.89 (m, 4H, $C_{17}H_{35}$-COO-CH_2-CH-C$\underline{H}_2$ and P-O-C$\underline{H}_2$-CH-CH_2-OH), 3.84-3.76 (m, 1H, P-O-CH_2-C$\underline{H}$-CH_2-OH), 3.68-3.59 (m, 2H, P-O-CH_2-CH-C$\underline{H}_2$-OH), 2.74-2.46 (m, 4H, C$\underline{H}_2$-Ph and C$\underline{H}_2$-$C(CH_3)$=CH-COO), 2.30 (t, *J* = 7.6 Hz, 2H, $C_{16}H_{33}$-C$\underline{H}_2$-COO), 1.90 (d, *J* = 1.1 Hz, 3H, C(C$\underline{H}_3$)=CH-COO), 1.66-1.54 (m, 4H, C$\underline{H}_2$-CH_2-Ph and C$\underline{H}_2$-CH_2-COO), 1.50-1.22 (m, 40H, CH_3-$C_{14}\underline{H}_{28}$-CH_2-CH_2-COO and Ph-CH_2-CH_2-$C_6\underline{H}_{12}$-CH_2-$C(CH_3)$=CH), 0.88 (t, *J* = 6.7 Hz, 3H, C$\underline{H}_3$-$C_{16}H_{32}$-COO). ^{13}C NMR (50 MHz, $CDCl_3/CD_3OD$ 4:1): δ 174.2, 165.6, 163.4, 143.0, 128.5 (2C), 128.3 (2C), 125.7, 115.2, 71.0, 69.5 (d, *J* = 7.8 Hz), 66.5, 64.0, 62.9, 62.3, 36.1, 34.2, 33.7, 32.0, 31.7, 29.8 (10C), 29.7 (3C), 29.5 (2C), 29.3, 28.4, 25.3, 25.0, 22.8, 18.9, 14.1. ^{31}P NMR (202 MHz, $CDCl_3/CD_3OD$ 4:1): δ -2.986. IR (neat): 3500 (br.), 2921, 2852, 1719, 1645, 1455, 1227, 1141,1103, 1065 cm^{-1}. HRMS (ESI -) $C_{43}H_{74}O_{10}PNa$ $[M - Na]^-$ 781.5020, found 781.5032.

1-Stearoyl-2-(12-phenyldodecanoyl)-*sn*-glycero-3-phospho-(*S*)-glycerol (45)

To a solution of **33** (0.16 mmol, 99 mg) and 2,3-di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycero-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (**32**) (0.22 mmol, 113 mg) in anhydrous CH_2Cl_2 (1.5 mL) was added 0.45 M tetrazole in MeCN (0.22 mmol, 483 µL), and the mixture was stirred at 20 °C. After 45 min, a solution of ^{t}BuOOH 5.5 M in nonane (0.22 mmol, 40 µL) was added, and the mixture was stirred at 20 °C for 1 h before concentration *in vacuo*. The residue was purified by flash chromatography (hexane/EtOAc 5:1) to give 122 mg of a clear oil that was dissolved in CH_2Cl_2 (1 mL). DBU (0.13 mmol, 19 µL) was added and the mixture was stirred at 20 °C for 1 h. After that time, the mixture was concentrated *in vacuo* and the crude mixture was purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to give 105 mg of the intermediate compound. This was then dissolved in EtOAc (1 mL) under Ar. 10 % (w/w) Pd/C (0.005 mmol, 5mg) was added carefully,

and the atmosphere was exchanged with H_2. The suspension was stirred at 20 °C for 15 h, and the mixture was filtered through celite. The Pd residue was rinsed with EtOAc, and the combined organic phases were concentrated *in vacuo*, to give 82 mg of white compound, that was dissolved in CH_2Cl_2 (3.2 mL) and MeCN (8 mL) and the solution was cooled to 0 °C, treated with 40 % w/w aqueous HF (312 µL), and the mixture was stirred vigorously at 0 °C for 3 h. The excess reagent was quenched by addition of $MeOSiMe_3$ (3 mL) at 0 °C, the mixture was stirred for 30 min at 20 °C, and $NaHCO_3$ (0.8 mmol, 64 mg) was added to the reaction mixture. The desired phospholipid was isolated in 47% yield (60 mg) after flash chromatography (CH_2Cl_2/MeOH/H_2O 9:1:0 to 65:25:4) as white amorphous solid. R_f = 0.13 (CH_2Cl_2/MeOH 9:1). ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD 4:1): δ 7.30-7.213 (m, 5H, $C_6\underline{H}_5$), 5.27-5.19 (m, 1H, $C_{17}H_{35}$-COO-CH_2-C$\underline{H}$-CH_2), 4.38 (dd, *J* = 12.1, 3.0 Hz, 1H, $C_{17}H_{35}$-COO-C$\underline{H}_2$), 4.21-4.10 (1H, $C_{17}H_{35}$-COO-C$\underline{H}_2$, under HDO signal), 4.03-3.90 (m, 4H, $C_{17}H_{35}$-COO-CH_2-CH-C$\underline{H}_2$ and -O-C$\underline{H}_2$-CH-CH_2-OH), 3.86-3.78 (m, 1H, P-O-CH_2-C$\underline{H}$-CH_2-OH), 3.68-3.60 (m, 2H, P-O-CH_2-CH-C$\underline{H}_2$-OH), 2.62-2.57 (m, 2H, C$\underline{H}_2$-Ph), 2.32 (t, *J* = 7.5 Hz, 2H, Ph-$C_{10}H_{20}$-C$\underline{H}_2$-COO), 2.30 (t, *J* = 7.5 Hz, 2H, $C_{16}H_{33}$-C$\underline{H}_2$-COO), 1.67-1.55 (m, 6H, C$\underline{H}_2$-CH_2-Ph, C$\underline{H}_2$-CH_2-COO and Ph-C_9H_{18}-C$\underline{H}_2$-CH_2-COO), 1.39-1.21 (m, 46H, CH_3-$C_{14}\underline{H}_{28}$-CH_2-CH_2-COO and Ph-CH_2-CH_2-$C_9\underline{H}_{18}$-CH_2-CH_2-COO), 0.88 (t, *J* = 6.7 Hz, 3H, C$\underline{H}_3$-$C_{16}H_{32}$-COO). ^{13}C NMR (50 MHz, $CDCl_3$/CD_3OD 4:1): δ 174.0, 173.6, 143.1, 128.5 (2C), 128.3 (2C), 70.9, 70.0, 67.7, 64.7, 62.3 (d, *J* = 9.5 Hz), 36.1, 34.3, 32.1, 31.7, 29.8 (13C), 29.6 (6C), 29.4, 29.3 (2C), 25.0 (2C), 22.8, 14.1. ^{31}P NMR (202 MHz, $CDCl_3$/CD_3OD 4:1): δ -1.659. IR (neat): 3500 (br.), 2916, 2849, 1732, 1467, 1106, 1056 cm^{-1}

1-Stearoyl-2-*O*-(*tert*-butyl-dimethylsilyl)-3-*O*-(*para*-methoxybenzyl)-*sn*-glycerol (46)

To a solution of **19** (1.25 mmol, 600 mg) in DMF (12 mL), were added TBSCl (1.38 mmol, 208 mg) and imidazole (2.5 mmol, 170 mg), and the resulting mixture was stirred at 20 °C for 3.5 h. The reaction was then quenched with 1% HCl (20 mL). The organic layer was separated, and the aqueous phase was washed with Et_2O. The combined organic layers were washed with brine, dried over $MgSO_4$, concentrated *in vacuo* and the crude oil thereby obtained was purified by flash chromatography (heptane/EtOAc 20:1) to afford the desired product as clear oil in 70 % yield (510 mg). R_f = 0.63 (heptane/EtOAc 4:1). $[\propto]_D^{20}$= 9.35 ° (c = 1.39, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29-7.26 and 6.92-6.88 (AA'XX' pattern, *J* = 8.7 Hz, 4H, aromatic), 4.49 (s, 2H, C$\underline{H}_2$-Ph), 4.22 (dd, *J* = 13.6, 6.6 Hz, 1H, $C_{18}H_{35}$-COO-C$\underline{H}_2$-CH-CH_2), 4.08-4.00 (m, 2H, $C_{18}H_{35}$-COO-C$\underline{H}_2$-C$\underline{H}$-CH_2), 3.84 (s, 3H, O-C$\underline{H}_3$), 3.45 (d, *J* = 5.1 Hz, 2H, $C_{18}H_{35}$-COO-CH_2-CH-C$\underline{H}_2$), 2.31 (t, *J* = 7.6 Hz, 2H, C$\underline{H}_2$COO), 1.71-1.59 (m, 2H, C$\underline{H}_2$-CH_2-COO), 1.41-1.20 (m, 28H, CH_3-$C_{14}\underline{H}_{28}$-CH_2-CH_2-COO), 0.96-0.87 (m, 12H, $C_{17}H_{32}$-C$\underline{H}_3$ and Si-C-(C$\underline{H}_3$)$_3$), 0.10 (s, 6H, Si-(C$\underline{H}_3$)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.7, 159.1, 130.2, 129.2 (2C), 113.7 (2C), 73.1, 71.5, 69.7, 66.2, 55.2, 34.3, 31.9, 29.7 (7C), 29.6, 29.5, 29.4, 29.3, 29.2, 25.7 (3C), 24.9, 22.7, 18.1, 14.1, -4.7, -4.8. IR (neat): 2923, 2853, 1739, 1613, 1513, 1463, 1247, 1172, 1143, 1099, 1036, 1005, 830, 777 cm^{-1}.HRMS (ESI +) $C_{35}H_{64}O_5Si$, m/z [M+Na$^+$] 615.4421, found 615.4421.

1-Stearoyl-2-*O*-(*tert*-butyl-dimethylsilyl)-*sn*-glycerol (47)

Compound **46** (0.80 mmol, 472 mg) was dissolved in a mixture of CH_2Cl_2 (4 mL) and phosphate buffer (pH=7) (0.6 mL), and DDQ (1.4 mmol, 320 mg) was added in two portions over 45 min. The two-phase mixture was stirred vigorously at 20 °C for 1.5 h, and ice sat. aq. $NaHCO_3$ (5 mL) and CH_2Cl_2 (5 mL) were added at 0 °C. The organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL) and the combined organic layers were washed with cold brine (15 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The product was purified by flash chromatography (heptane/EtOAc 7:1) and obtained as colourless oil in 87% yield (330 mg). R_f = 0.30 (heptane/EtOAc 3:1). $[\propto]_D^{20}$= -4.31 ° (c = 1.95, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 4.10 (dd, *J* = 11.0, 5.7 Hz, 1H, $C_{18}H_{35}$-COO-C$\underline{H_2}$-CH-CH_2), 4.07 (dd, *J* = 11.0, 5.5 Hz, 1H, $C_{18}H_{35}$-COO-C$\underline{H_2}$-CH-CH_2), 3.95-3.91(m, 1H, $C_{18}H_{35}$-COO-CH_2-C$\underline{H}$-CH_2), 3.63-3.53 (m, 2H, $C_{18}H_{35}$-COO-CH_2-CH-C$\underline{H_2}$), 2.31 (t, *J* = 7.6 Hz, 2H, C$\underline{H_2}$COO), 1.97 (t, *J* = 5.9 Hz, 1H, OH), 1.64-1.59 (m, 2H, C$\underline{H_2}$-CH_2-COO), 1.35-1.22 (m, 28H, CH_3-$C_{14}\underline{H_{28}}$-CH_2-CH_2-COO), 0.90 (s, 9H, Si-C-(C$\underline{H_3}$)$_3$), 0.88 (t, *J* = 6.9 Hz, 3H, $C_{17}H_{32}$-C$\underline{H_3}$), 0.11 (s, 3H, Si-(C$\underline{H_3}$)$_2$, 0.10 (s, 3H, Si-(C$\underline{H_3}$)$_2$. ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.8, 70.5, 64.7, 63.8, 34.2, 31.9, 29.7 (6C), 29.6 (2C), 29.4 (2C), 29.2, 29.1, 25.7 (3C), 24.9, 22.7, 18.0, 14.1, -4.7, -4.8. IR (neat): 3500 (br.), 2923, 2853, 1741, 1463, 1251, 1172, 1115, 1057, 992, 835, 777 cm^{-1}.HRMS (ESI +) $C_{27}H_{56}O_4Si$, m/z [M+Na$^+$] 495.3846, found 495.3831.

1-Stearoyl-*sn*-glycero-3-phospho-(*S*)-glycerol (48)

To a solution of **47** (0.70 mmol, 330 mg) and 2,3-di-*O*-(*tert*-butyldimethylsilyl)-*sn*-glycero-2-cyanoethyl-*N,N*-diisopropylphosphoramidite (**32**) (0.94 mmol, 492 mg) in anhydrous CH_2Cl_2 (5 mL) was added 0.45 M tetrazole in MeCN (0.94 mmol,2 mL), and the mixture was stirred at 20 °C. After 45 min, a solution of ^{t}BuOOH 5.5 M in nonane (0.96 mmol, 175 µL) was added, and the mixture was stirred at 20 °C for 1 h before concentration *in vacuo*. The residue was purified by flash chromatography (hexane/EtOAc 5:1) to give 470 mg of a clear oil that was dissolved in CH_2Cl_2 (3.9 mL), DBU (0.54 mmol, 81 µL) was added and the mixture was stirred at 20 °C for 1 h. After that time, the mixture was concentrated *in vacuo* and the crude mixture was purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to give 396 mg of the intermediate compound. This was then dissolved in CH_2Cl_2 (28 mL) and MeCN (67 mL) and the solution was cooled to 0 °C, treated with 40 % w/w aqueous HF (2.7 mL), and the mixture was stirred vigorously at 0 °C over 3 h. The excess reagent was quenched by addition of $MeOSiMe_3$ (27 mL) at 0 °C, the mixture was stirred for 30 min at 20 °C, and $NaHCO_3$ (4.3 mmol, 364 mg) was added to the reaction mixture. The desired phospholipid was isolated in 52% yield (194 mg) after flash chromatography (CH_2Cl_2/MeOH/H_2O 9:1:0 to 65:25:4) as white amorphous solid. R_f = 0.14 (CH_2Cl_2/MeOH 4:1). ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD 4:1): δ 4.16-4.10 (m, 2H,), 4.05-3.77 (m, 6H,), 3.66-3.56 (m, 2H), 3.54-3.52 (br. s, 1H, OH), 3.36-3.33 (m, 1H, C$\underline{H}$-CH_2-OH), 3.26-3.24 (br. s, 1H, OH) 2.35 (t, *J* = 7.6 Hz, 2H, $C_{16}H_{33}$-C$\underline{H_2}$-COO), 1.66-1.57 (m, 3H, C$\underline{H_2}$-CH_2-COO), 1.33-1.22 (m, 28H, CH_3-$C_{14}\underline{H_{28}}$-CH_2-CH_2-COO), 0.88 (t, *J* = 6.8 Hz, 3H, C$\underline{H_3}$-$C_{16}H_{32}$-COO). ^{13}C NMR (50 MHz, $CDCl_3$/CD_3OD 4:1): δ

174.7, 71.2, 70.0, 67.0, 65.0, 62.6, 34.2, 32.1, 29.8 (8C), 29.7 (2C), 29.5 (2C), 29.3, 25.0, 22.8, 14.1.

13-Phenyl-3,6,9,12-tetraoxatridecan-1-ol (49)

To a solution of tetraethyleneglycol (10.30 mmol, 2g) in anhydrous DMF (10 mL) cooled to 0 °C under Ar, was added NaH (60 % in mineral oil 10.29 mmol, 412 mg) in four portions over 10 min and the resulting suspension was stirred at 0 °C for 10 min, then at 20 °C for additional 10 min. BnBr (2.06 mmol, 245 µL) was then added dropwise and the reaction mixture was stirred at 20 °C for 20 h. The reaction mixture was poured into ice water (50 mL) and extracted with Et_2O (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and purified by flash chromatography (heptane /EtOAc 1:1 to 0:1) to afford **49** as a colourless oil in 92 % yield (540 mg), and unreacted tetraethyleneglycol was recovered (7.10 mmol, 1.380 g). R_f = 0.2 (EtOAc/heptane 2:1). ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.35-7.27 (m, 5H, $C_6\underline{H}_5$), 4.57 (s, 2H, $C\underline{H}_2$-Ph), 3.70-3.58 (m, 16H, O-$C\underline{H}_2$-$C\underline{H}_2$-O), 2.54 (t, *J* = 5.5 Hz, 1H, O$\underline{H}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.2 ($\underline{C}_6H_5$), 128.3 (2C, $\underline{C}_6H_5$), 127.7 (2C, $\underline{C}_6H_5$), 127.5 ($\underline{C}_6H_5$), 73.2 ($\underline{C}H_2$-Ph), 72.5 (O-$\underline{C}H_2$-$\underline{C}H_2$-O-), 70.6 (4C, O-$\underline{C}H_2$-$\underline{C}H_2$-O-), 70.3 (O-$\underline{C}H_2$-$\underline{C}H_2$-O-), 69.4 (O-$\underline{C}H_2$-$\underline{C}H_2$-O-), 61.7 ($\underline{C}H_2$-OH).

13-Phenyl-3,6,9,12-tetraoxatridecanyl methanesulfonate (50)

To a solution of **49** (7.52 mmol, 2.14 g) in CH_2Cl_2 (15 mL) was added Et_3N (13.53 mmol, 1.9 mL). The reaction mixture was cooled to -30 °C and MsCl (12.03 mmol, 930 µL) was added dropwise and the mixture was stirred for 10 min at that temperature then allowed to reach 20 °C slowly over 1 h and stirred for additional 14 h. Sat. aq. NH_4Cl (20 mL) was added, the organic layer was separated, and the aqueous layer was washed with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with H_2O (20 mL), sat. aq. $NaHCO_3$ (20 mL), and brine (20 mL) and dried over $MgSO_4$ to afford 2.64 g of crude oil (crude yield 97%) used without further purification. R_f = 0.42 (EtOAc/heptane 9:1). ^{1}H NMR (300 MHZ; $CDCl_3$): δ 7.34-7.26 (m, 5H, $C_6\underline{H}_5$), 4.56 (s, 2H, $C\underline{H}_2$-C_6H_5), 4.37-4.34 (m, 2H, $C\underline{H}_2$-OMs), 3.76-3.73 (m, 2H, $C\underline{H}_2$-CH_2-OMs), 3.65-3.64 (m, 12H, (O-$C\underline{H}_2$-$C\underline{H}_2$)$_3$-OBn), 3.05 (s, 3H, SO_2-$C\underline{H}_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.2, 128.3 (2C), 127.7 (2C), 127.5, 73.2, 70.6 (3C), 70.5 (2C), 69.4, 69.3, 69.0, 37.7; HRMS (ESI +) $C_{16}H_{26}O_7S$, m/z [M+Na^+] 385.1297, found 385.1294.

1,2-*O*-Isopropylidene-3-*O*-(13-phenyl-3,6,9,12-tetraoxatridecyl)-*sn*-glycerol (51)

To a flame dried round bottomed flask was added powdered KOH (17.95 mmol, 1 g) and anhydrous DMSO (20 mL) under Ar, and the resulting suspension was stirred at 20 °C for 1 h. A solution of (*S*)-(+)-1,2-isopropylideneglycerol (10.77 mmol, 1.42 g), crude **50** (7.18 mmol, 2.6 g) in anhydrous DMSO (20 mL) was added, and the reaction mixture was stirred at 40 °C for 20 h. Aq. NH_4Cl (50

% w/w, 40 mL) was added, the aqueous layer was extracted with EtOAc (3 × 50 mL), the combined organic layers were washed with H_2O (100 mL), dried over $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography (heptane/EtOAc 1:1) to afford 2.1 g of **51** as colourless oil (76 %). R_f = 0.18 (heptane/EtOAc 1:1). $[\propto]_D^{20}$= +3.51 ° (c = 1.51, $CHCl_3$). ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.34-7.24 (m, 5H, C_6H_5), 4.56 (s, 2H, $C\underline{H_2}$-C_6H_5), 4.32-4.22 (m, 1H, CH_2-$C\underline{H}$-CH_2), 4.04 (dd, *J* = 8.3, 6.4 Hz, 1H, $C\underline{H_a}H_b$-CH-CH_2-OTEG), 3.72 (dd, *J* = 8.3, 6.4 Hz, 1H, $CH_a\underline{H_b}$-CH-CH_2-OTEG), 3.69-3.60 (m, 16H, (O-$\underline{CH_2}$-$\underline{CH_2}$)$_4$), 3.57 (dd, *J* = 10.0, 5.7 Hz, 1H, CH_2-CH-$C\underline{H_a}H_b$-OTEG), 3.48 (dd, *J* = 10.0, 5.5 Hz, 1H, CH_2-CH-$CH_a\underline{H_b}$-OTEG), 1.41 (s, 3H, $C\underline{H_3}$), 1.35 (s, 3H, $C\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.5 (aromatic), 128.6 (2C, aromatic), 128.0 (2C, aromatic), 127.8 (aromatic), 109.6 (CH_3-$\underline{C}$-CH_3), 74.9 (CH_2-$\underline{C}H$-CH_2), 73.5 ($\underline{C}H_2$-C_6H_6), 72.6 ((O-CH_2-CH_2)$_4$), 71.2 ((O-CH_2-CH_2)$_4$), 70.9 (2C, (O-CH_2-CH_2)$_4$), 70.8 (3C, (O-CH_2-CH_2)$_4$), 70.7 ((O-CH_2-CH_2)4), 69.6 ($\underline{C}H_2$-CH-CH_2-OTEG), 67.0 (CH_2-CH-$\underline{C}H_2$-OTEG), 27.0 (CH_3), 25.6 (CH_3); IR (neat): 2985, 2866, 1454, 1370, 1252, 1212, 1095, 1049, 843, 738, 698 cm^{-1}. HRMS (ESI +) $C_{21}H_{34}O_7$, m/z [M+Na^+] 421.2202, found 421.2193.

3-*O*-(13-Phenyl-3,6,9,12-tetraoxatridecyl)-*sn*-glycerol (52)

To a solution of **51** (11.4 mmol, 4.5 g) in MeOH (100 mL), was added HCl 1M in MeOH (100 mL), and the reaction mixture was stirred at 20 °C for 18 h, after what the solvent was removed *in vacuo*, and the desired product was isolated by flash chromatography (EtOAc/MeOH 90:10 to 75:25) as a clear oil in 93 % yield (3.8 g). R_f = 0.2 (EtOAc). $[\propto]_D^{20}$= -1.27 ° (c =2.68, $CHCl_3$). ^{1}H NMR (300 MHz; CD_3OD): δ 7.33-7.21 (m, 5H, $C_6\underline{H_5}$), 4.52 (s, 2H, $C\underline{H_2}$-C_6H_5), 3.75-3.40 (m, 21H, $C\underline{H_2}$-$C\underline{H}$-$C\underline{H_2}$- and O-$C\underline{H_2}$-$C\underline{H_2}$-O), 3.28 (s (br), 2H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.2 ($\underline{C}_6H_5$), 128.3 ($\underline{C}_6H_5$, 2C), 127.7 ($\underline{C}_6H_5$, 2C), 127.6 ($\underline{C}_6H_5$), 73.2 ($\underline{C}H_2$-Ph), 72.9 (TEG + glycerol), 70.7 (TEG + glycerol), 70.6 (TEG + glycerol, 2C), 70.5 (TEG + glycerol, 4C), 70.4 (TEG + glycerol), 69.3 (TEG + glycerol), 63.9 ($\underline{C}H_2$-OH). IR (neat): 3419 (br.), 2867, 1453, 1350, 1296, 1249, 1090, 1040, 924, 753 cm^{-1}. HRMS (ESI +) $C_{18}H_{30}O_7$, m/z [M+H^+] 359.2070, found 359.2076.

1,2-Di-*O*-octadecyl-3-*O*-(13-phenyl -3,6,9,12-tetraoxatridecyl)-*sn*-glycerol (53)

In a flame-dried 25 mL round bottom flask, were added **52** (0.84 mmol, 300 mg) and DMSO (5 mL), followed by KOH (4.19 mmol, 235 mg), and the resulting mixture was stirred at 20 °C for 3 h in order to solubilise KOH fully. When all the materials were into solution, 1-bromooctadecane was added slowly and the reaction mixture was stirred for 22 h. NH_4Cl (10 mL) was then added and the aqueous phase was washed with EtOAc (3 × 20 mL), the combined organic layers were dried ($MgSO_4$), concentrated *in vacuo*, and the desired product was obtained after purification by flash chromatography (heptane/EtOAc 1:0 to 1:3) in 68 % yield (490 mg) as a white amorphous solid. Mp.: 36.0-36.5 °C. R_f = 0.69 (EtOAc). $[\propto]_D^{20}$= +0.16 ° (c = 1.26, $CHCl_3$). ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.34-7.23 (m, 5H, $C_6\underline{H_5}$), 4.56 (s, 2H, $C\underline{H_2}$-C_6H_5), 3.69-3.36 (m, 21H, $C_{18}H_{37}$-O-$C\underline{H_2}$-$C\underline{H}$(O-$C_{18}H_{37}$)-$C\underline{H_2}$-O-($C\underline{H_2}$-$C\underline{H_2}$-O)$_4$-Bn), 1.57-1.50 (m, 4H, $C_{17}H_{35}$-$C\underline{H_2}$-O), 1.32-1.19 (m, 64H,

CH_3-$C_{16}\underline{H_{32}}$-CH_2-O), 0.88 (t, *J* = 6.7 Hz, 6H, $C\underline{H_3}$-$C_{17}H_{34}$-O). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.2 (C_5H_5-$\underline{C}$-CH_2), 128.3 ($\underline{C_6}H_6$, 2C), 127.7 ($\underline{C_6}H_6$, 2C), 127.6 ($\underline{C_6}H_6$), 77.8 (CH_2-$\underline{C}$H-CH_2), 73.2 (C_6H_6-$\underline{C}H_2$), 71.6 ($C_{18}H_{37}$-O-$\underline{C}H_2$-CH-CH_2), 71.4 (C_{17}-H_{35}-$\underline{C}H_2$-O- and TEG), 70.8 (C_{17}-H_{35}-$\underline{C}H_2$-O- and TEG, 2C), 70.6 (C_{17}-H_{35}-$\underline{C}H_2$-O- and TEG, 5C), 70.5 (C_{17}-H_{35}-$\underline{C}H_2$-O- and TEG, 2C), 69.4 (CH_2-CH-$\underline{C}H_2$-OTEG), 31.9 (CH_3-CH_2-$\underline{C}H_2$-CH_2, 2C), 30.1 (CH_3-CH_2-CH_2-CH_2-$\underline{C_{14}}H_{28}$, 2C), 29.7 (CH_3-CH_2-CH_2-CH_2-$\underline{C_{14}}H_{28}$, 20C), 29.5 (CH_3-CH_2-CH_2-CH_2-$\underline{C_{14}}H_{28}$, 2C), 29.4 (CH_3-CH_2-CH_2-CH_2-$\underline{C_{14}}H_{28}$, 2C), 26.1 (CH_3-CH_2-CH_2-CH_2-$\underline{C_{14}}H_{28}$, 2C), 22.7 (CH_3-CH_2-$\underline{C}H_2$-CH_2-$C_{14}H_{28}$, 2C), 14.1 ($\underline{C}H_3$-CH_2-CH_2-CH_2-$C_{14}H_{28}$, 2C). IR (neat): 2916, 2849, 1466, 1099 cm^{-1}. HRMS (ESI +) $C_{54}H_{102}O_7$, m/z [M+Na^+] 885.7523, found 885.7545.

Octadecyl-*para*-toluenesulfonate (54)[106]

p-Toluenesulfonyl chloride (2.04 mmol, 387 mg) was added to a stirred solution of pyridine (1 mL) and octadecanol (1.85 mmol, 500 mg) maintained at 0 °C. The reaction mixture was stirred for 3 h and then quenched with H_2O (5 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with 3 M HCl (3 × 10 mL) followed by 10% $NaHCO_3$ (10 mL), dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by flash chromatography (heptane/EtOAc 9:1), to afford the desired compound as white powder in 70 % yield (555 mg). R_f = 0.4 (heptane EtOAc 9:1). Mp.: 55.8-56.2 °C. ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.79 (d, *J* = 8.3 Hz, 2H, 2×SO_2-C-C$\underline{H}$-CH-C-CH_3), 7.34 (d, *J* = 8.3 Hz, 2H, 2×SO_2-C-CH-C$\underline{H}$-C-CH_3), 4.01 (t, *J* = 6.5 Hz, 2H, $C\underline{H_2}$-O-SO_2), 2.46 (s, 3H, O-$C\underline{H_3}$), 1.67-1.58 (m, 2H, $C\underline{H_2}$-CH_2-SO_2), 1.33-1.17 (m, 30H, CH_3-$(C\underline{H_2})_{15}$), 0.88 (t, *J* = 6.7 Hz,3H, $C\underline{H_3}$-$(CH_2)_{15}$). ^{13}C NMR (75 MHz; $CDCl_3$) δ 144.6, 133, 2, 129.8 (2C), 127.9 (2C), 70.7, 31.9, 29.7 (9C), 29.6 (3C), 29.5, 29.4(2C), 28.9, 28.8.

1,2-Di-*O*-octadecyl-3-*O*-(12-hydroxy-3,6,9-trioxadodecyl)-*sn*-glycerol (55)

Compound **53** (4.64 mmol, 4 g) was dissolved in EtOAc (45 mL) under N_2 atmosphere, and w/w 10 % Pd/C (0.23 mmol, 247 mg) was added. The atmosphere was then exchanged with H_2, and the reaction mixture was stirred at 20 °C under H_2. After 20 h, some starting material still remained, and the atmosphere was exchanged with N_2, w/w 10 % Pd/C (0.23 mmol, 247 mg) was added, and the atmosphere was exchanged to H_2 again. After 24 h, TLC showed full conversion of **53**, the reaction mixture was filtered through celite, the residue was rinced with EtOAc and the solvent was removed in vacuo. The crude solid was purified by flash chromatography (heptane/EtOAc 1:1) to afford **55** as white crystals in 83 % yield (2.97 g). Mp.: 46-47 °C. R_f = 0.16 (heptane/EtOAc). $[\propto]_D^{20}$= -0.7 ° (c =1.0 , $CHCl_3$). ^{1}H NMR (300 MHz; $CDCl_3$): δ 3.74-3.36 (m, 25H, glycerol + TEG+ $C_{17}H_{35}$-$C\underline{H_2}$-O), 2.0 (s, 1H, OH), 1.60-1.51 (m, 4H, CH_3-$C_{15}H_{30}$-$C\underline{H_2}$-CH_2-O), 1.36-1.24 (m, 60H, CH_3-$C_{15}\underline{H_{30}}$-CH_2-CH_2), 0.88 (t, *J* = 6.7 Hz, 6H, $C\underline{H_3}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 77.8 (CH_2-$\underline{C}$H-CH_2), 72.5 ($C_{18}H_{35}$-O-$\underline{C}H_2$-CH-CH_2), 71.6 ($C_{17}H_{33}$-$\underline{C}H_2$-O-CH_2-CH-CH_2), 71.4 (TEG), 70.8 (TEG), 70.7 (TEG), 70.6 (3C, TEG), 70.5 (2C, $C_{17}H_{33}$-$\underline{C}H_2$-O-CH-CH_2 and TEG), 70.3 ($\underline{C}H_2$-OTEG), 61.7 ($\underline{C}H_2$-OH), 31.9 (2C, $\underline{C_{15}}H_{30}$), 30.1 ($\underline{C_{15}}H_{30}$), 29.7 (14C, $\underline{C_{15}}H_{30}$), 29.6 (7C, $\underline{C_{15}}H_{30}$),

29.5 (2C, $\underline{C}_{15}H_{30}$), 29.3 (2C, $\underline{C}_{15}H_{30}$), 26.1 (2C, $\underline{C}_{15}H_{30}$), 22.7 (2C, $\underline{C}H_2$-CH_3), 14.1 (2C, $\underline{C}H_3$). IR (neat): 3461 (br.), 2916, 2849, 1467, 1109 cm^{-1}. HRMS (ESI +) $C_{47}H_{96}O_7$, m/z [M+Na^+] 795.7054, found 795.7055.

1,2-Di-*O*-octadecyl-3-*O*-(13-oxo-3,6,9,12-tetraoxaheptadec-16-ynyl)-*sn*-glycerol (56)

To a solution of **55** (0.129 mmol, 100 mg) and 4-pentynoic acid (0.129 mmol, 13 mg) in anhydrous CH_2Cl_2 (1.5 mL) was added DMAP (0.220 mmol, 27 mg) followed by EDC•HCl (0.194 mmol, 37 mg) and the resulting mixture was stirred at 20 °C for 17 h, after what 0.3 more equivalents of 4-pentynoic acid (0.04 mmol, 6 mg) were added and the reaction mixture was stirred for 6 h. The crude mixture was then concentrated on silica and purified by flash chromatography (heptane/EtOAc 5:1 to 4:1), to afford 102 mg (93 % yield) of **56** as white amorphous solid. R_f = 0.26 (heptane/EtOAc 3:1). $[\propto]_D^{20}$= -1.00 ° (c = 1.00, $CHCl_3$). ^{1}H NMR (300 MHz; $CDCl_3$): δ 4.27-4.24 (m, 2H, C$\underline{H}_2$-O-C=O), 3.71-3.46 (m, 21H, glycerol + TEG+ $C_{17}H_{35}$-C$\underline{H}_2$), 3.42 (t, *J* = 6.9 Hz, 2H, glycerol + TEG+ $C_{17}H_{30}$-C$\underline{H}_2$), 2.61-2.56 (m, 2H, C$\underline{H}_2$-C$\underline{H}_2$-CΞCH), 2.53-2.47 (m, 2H, C$\underline{H}_2$-C$\underline{H}_2$-CΞCH), 1.97 (t, *J* = 2.4 Hz, 1H, CΞC$\underline{H}$), 1.56-1.52 (m, 4H, $C_{16}H_{33}$-C$\underline{H}_2$-CH_2-O-), 1.35-1.22 (m, 60H, CH_3-$C_{15}\underline{H}_{30}$-CH_2-CH_2), 0.87 (t, *J* = 6.7 Hz, 6H, C$\underline{H}_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.7 ($\underline{C}$(O)O), 82.40 (CH_2-$\underline{C}$ΞCH), 77.8 (CH_2-$\underline{C}$H-CH_2), 71.6 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 71.4 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.8 (2C, TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.6 (5C, TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.5 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 69.0 (2C, $\underline{C}H_2$-O-C(O) + CΞ$\underline{C}$H), 63.8 ($\underline{C}H_2$-O-TEG), 33.2 ($\underline{C}H_2$-C(O)-O), 31.9 (2C, $\underline{C}_{15}H_{30}$), 30.1 ($\underline{C}_{15}H_{30}$), 29.7 (21C, $\underline{C}_{15}H_{30}$), 29.5 (2C, $\underline{C}_{15}H_{30}$), 29.4 (2C, $\underline{C}_{15}H_{30}$), 26.1 (2C, $\underline{C}_{15}H_{30}$), 22.7 (2C, $\underline{C}H_2$-CH_3), 14.3 ($\underline{C}H_2$-CΞCH), 14.1 (2C, $\underline{C}H_3$). IR (neat): 3272, 2956, 2916, 2849, 1736, 1467, 1108 cm^{-1}. HRMS (ESI +) $C_{52}H_{100}O_8$, m/z [M+Na^+] 875.7316, found 875.7339.

1,2-Di-*O*-octadecyl-3-*O*-(17-azido-13-oxo-3,6,9,12-tetraoxaheptadecyl)-*sn*-glycerol (57)

To a solution of **55** (0.129 mmol, 100 mg) and 5-azidopentanoic acid (0.182 mmol, 26 mg) in anhydrous CH_2Cl_2 (1.5 mL) was added DMAP (0.220 mmol, 27 mg) followed by EDC.HCl (0.194 mmol, 37 mg) and the resulting mixture was stirred at 20 °C for 17 h, after what 0.3 more equivalents of 5-azidopentanoic acid (0.04 mmol, 6 mg) were added and the reaction mixture was stirred for 6 h. The crude mixture was then concentrated onto silica and purified by flash chromatography (heptane/EtOAc 5:1 to 4:1), to afford 100 mg (86 % yied) of **57** as white amorphous solid. R_f = 0.2 (heptane/EtOAc 3:1). $[\propto]_D^{20}$= -0.26 ° (c = 1.15, $CHCl_3$). ^{1}H NMR (300 MHz; $CDCl_3$): δ 4.25-4.21 (m, 2H, C$\underline{H}_2$-O-C=O), 3.70-3.40 (m, 23H, glycerol + TEG+ $C_{17}H_{35}$-C$\underline{H}_2$-O), 3.29 (t, *J* = 6.5 Hz, 2H, C$\underline{H}_2$-C=O-O); 2.37 (t, *J* = 7.1 Hz, 2H, C$\underline{H}_2$-N_3), 1.78-1.60 (m, 4H, C$\underline{H}_2$-C$\underline{H}_2$-CH_2-N_3), 1.58-1.50 (m, 4H, CH_3-$C_{15}H_{30}$-C$\underline{H}_2$-CH_2-O), 1.36-1.22 (m, 60H, CH_3-$C_{15}\underline{H}_{30}$-CH_2-CH_2), 0.87 (t, *J* = 6.6 Hz, 6H, C$\underline{H}_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.7 ($\underline{C}$(O)O), 77.8 (CH_2-$\underline{C}$H-CH_2), 71.6 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 71.4 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.8 (TEG + glycerol + $\underline{C}_{17}H_{33}$-CH_2), 70.7 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.6 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$,

4C), 70.5 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$, 2C), 69.1 ($\underline{C}H_2$-O-C(O)), 63.5 ($\underline{C}H_2$-O-TEG), 51.0 ($\underline{C}H_2$-N_3), 33.5 ($\underline{C}H_2$-C(O)-O), 31.9 ($C_{15}H_{30}$, 2C), 30.1 ($C_{15}H_{30}$), 29.7 ($C_{15}H_{30}$, 14C), 29.6 ($C_{15}H_{30}$, 7C), 29.5 ($C_{15}H_{30}$, 2C), 29.3 ($C_{15}H_{30}$, 2C), 28.2 (CH_2-CH_2-N_3), 26.1 ($C_{15}H_{30}$, 2C), 22.7 (CH_2-CH_3, 2C), 22.0 (CH_2-CH_2-CH_2-N_3), 14.1 (CH_3, 2C). IR (neat): 2916, 2849, 2096, 1735, 1467, 1109 cm^{-1}. HRMS (ESI +) $C_{52}H_{103}N_3O_8$, m/z [M+Na^+] 920.7643, found 920.7669.

1,2-Di-*O*-octadecyl-3-*O*-(12-(methanesulfonyloxy)-3,6,9-trioxadodecyl)-*sn*-glycerol (58)

To a solution of **55** (1.29 mmol, 1 g) and Et_3N (2.33 mmol, 325 µL) in anhydrous dichloromethane (4 mL) cooled to 0 °C, was added MsCl (6.47 mmol, 500 µL), and the reaction mixture was allowed to reach slowly 20 °C. After stirring for 16 h, **55** was not fully converted, and MsCl (6.47 mmol, 500 µL) was added at 0 °C, and the reaction mixture was stirred at 20 °C for additional 20 h. Sat. aq. NH_4Cl (20 mL) was added, the organic phase was separated, and the aqueous phase was washed with dichloromethane (3 × 20 mL), the combined organic layers were dried over $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography (heptane/EtOAc 3:1 to 1:1) to afford **58** as white solid in 80 % yield (883 mg). Mp.: 51.0-51.5 °C R_f = 0.26 (heptane/EtOAc 1:1). $[\propto]_D^{20}$= +0.6 ° (c =1.04, $CHCl_3$). ^{1}H NMR (300 MHz; $CDCl_3$): δ 4.40-4.37 (m, 2H, $C\underline{H}_2$-OMs), 3.78-3.75 (m, 2H, $C\underline{H}_2$-CH_2-OMs), 3.68-3.40 (m, 21H, $C_{17}H_{33}$-$C\underline{H}_2$ + O-$C\underline{H}_2$-$C\underline{H}_2$- + $C\underline{H}_2$-$C\underline{H}$-$C\underline{H}_2$), 3.08 (s, 3H, S-$C\underline{H}_3$), 1.55 (tt, *J* = 6.5, 6.5 Hz, 4H, CH_3-$C_{15}H_{30}$-$C\underline{H}_2$-CH_2-O), 1.35-1.23 (m, 60H, CH_3-$C_{15}\underline{H}_{30}$-CH_2-CH_2), 0.87 (t, *J* = 6.7 Hz, 6H, $C\underline{H}_3$-$C_{17}H_{34}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 77.8 (CH_2-$\underline{C}H$-CH_2), 71.6 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 71.4 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.8 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.7 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.6 (4C, TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.5 (2C, TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 69.2 ($\underline{C}H_2$-CH_2-OMs), 69.0 (CH_2-$\underline{C}H_2$-OMs), 37.7 (SO_2-$\underline{C}H_3$), 31.9 (2C, $\underline{C}_{15}H_{30}$), 30.1 ($\underline{C}_{15}H_{30}$), 29.7 (14C, $\underline{C}_{15}H_{30}$), 29.6 (7C, $\underline{C}_{15}H_{30}$), 29.5 (2C, $\underline{C}_{15}H_{30}$), 29.3 (2C, $\underline{C}_{15}H_{30}$), 26.1 (2C, $\underline{C}_{15}H_{30}$), 22.7 (2C, $\underline{C}H_2$-CH_3), 14.1 (2C, $\underline{C}H_3$). IR (neat): 2916, 2849, 1467, 1350, 1173, 1107 cm^{-1}. HRMS (ESI +) $C_{48}H_{98}O_9S$, m/z [M+Na^+] 873.6829, found 873.6834.

1,2-Di-*O*-octadecyl-3-*O*-(3,6,9-trioxa-12-azapentadec-14-ynyl)-*sn*-glycerol (59)

To a solution of **58** (0.47 mmol, 400 mg) in anhydrous THF (1 mL) under argon, was added Et_3N (1.175 mmol, 164 µL) followed by Bu_4NI (0.235 mmol, 87 mg) and propargyl amine (0.940 mmol, 80 mg). The reaction mixture was stirred at 20 °C for 18 h, then at 70 °C for 3.5 h, after what the crude mixture was concentrated *in vacuo* on silica and **59** was obtained as yellow amorphous solid in 79 % yield (300 mg) after purification by flash chromatography (EtOAc/heptane/Et_3N 66:33:1). R_f = 0.16 (EtOAc/heptane 3:1). $[\propto]_D^{20}$= -0.45 ° (c = 1.32, $CHCl_3$). ^{1}H NMR (300 MHZ; $CDCl_3$): δ 3.89 (t, *J* = 4.74 Hz, 2H, $C\underline{H}_2$-CH_2-NH), 3.81 (d, *J* = 2.0 Hz, 2H, NH-$C\underline{H}_2$-CΞCH), 3.71-3.40 (m, 21H, glycerol + TEG+ $C_{17}H_{35}$-$C\underline{H}_2$-O), 3.19-3.22 (m, 2H, $C\underline{H}_2$-NH-CH_2-CΞCH), 2.48 (t, *J* = 2.0 Hz, 1H, CΞC$\underline{H}$), 1.58-1.51 (m, 4H, 2×CH_3-$C_{15}H_{30}$-CH_2-CH_2-O), 1.35-1.20 (m, 60H, 2×CH_3-$C_{15}\underline{H}_{30}$-CH_2-CH_2), 0.87 (t, *J* = 6.64 Hz, 6H, 2×$C\underline{H}_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 82.0 ($\underline{C}$ΞCH), 77.8 (CH2-$\underline{C}$H-CH2), 71.6 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}$H2), 71.4 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 71.3 (CΞ$\underline{C}$H), 70.8 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.7 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.6

(3C, TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.5 (2C, TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.4 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.3 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 48.0 ($\underline{C}H_2$-NH-CH_2-CΞCH), 38.2 (CH_2-NH-$\underline{C}H_2$-CΞCH), 31.9 (2C, $C_{15}H_{30}$), 30.1 ($C_{15}H_{30}$), 29.7 (14C, $C_{15}H_{30}$), 29.6 (7C, $C_{15}H_{30}$), 29.5 (2C, $C_{15}H_{30}$), 29.3 (2C, $C_{15}H_{30}$), 26.1 (2C, $C_{15}H_{30}$), 22.6 (2C, 2×$\underline{C}H_2$-CH_3), 14.1 (2C, 2×$\underline{C}H_3$). IR (neat): 3250 (br.), 2915, 2849, 1467, 1106 cm^{-1}. HRMS (ESI +) $C_{50}H_{99}NO_6$, m/z [M+Na^+] 832.7370, found 832.7371.

Methyl 10-oxodecanoate (60)

In a flame dried 3-neck flask, a solution of DMSO (163.13 mmol, 11.6 mL) in CH_2Cl_2 (300 mL) was cooled to -78 °C. A solution of $(ClCO)_2$ (81.57 mmol, 6.9 mL) in CH_2Cl_2 (15 mL) was added dropwise over 1 h at -78 °C, and the resulting mixture was stirred at that temperature for 30 min. A mixture of methyl 10-hydroxydecanoate (74.15 mmol, 15 g) in CH_2Cl_2 (75 mL) was added dropwise over 2.5 h at -78 °C, and the reaction mixture was stirred for 1h at that temperature before Et_3N (370 mmol, 51 mL) was added slowly at -78 °C. The reaction mixture was stirred at that temperature for 15 min, and allowed to warm to 20 °C over 1 h. H_2O (400 mL) was then added, the organic layer was separated, and the aqueous layer extracted with CH_2Cl_2, the combined organic layers were washed with aq. HCl 1%, H_2O, Na_2CO_3 and brine, dried over $MgSO_4$ and concentrated *in vacuo* to afford the desired product as colourless oil (12.3 g, 83%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.74 (t, *J* = 1.8 Hz, 1H, C$\underline{H}$=O), 3.65 (s, 3H, $CO_2C\underline{H}_3$), 2.40 (td, *J* = 7.4, 1.8 Hz, 2H, $C\underline{H}_2$-CH=O), 2.28 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2$-CO_2CH_3), 1.55-1.65 (m, 4H, $C\underline{H}_2$-CH_2-CO_2CH_3 and $C\underline{H}_2$-CH_2-CH=O), 1.32-1.23 (m, 8H, ($C\underline{H}_2$)$_4$-$COOCH_3$).[217]

6-*tert*-Butyldimethylsilyloxy-hex-1-yne (62)

To a solution of 5-hexyn-1-ol (101.90 mmol, 10.0 g) in anhydrous DMF (20 mL) cooled to 0 °C were added imidazole (152.84 mmol, 10.4 g) and *t*-butyldimethylsilyl chloride (122.3 mmol, 18.4 g). The resulting mixture was stirred at 0 °C for 10 min then allowed to warm to 20 °C and stirred at that temperature for additional 2.5 h. After that time, a saturated aqueous solution of NH_4Cl (50 mL) was added, the two phases were separated, and the aqueous phase was extracted with Et_2O (3 × 50 mL). The organic layers were combined, washed with water and brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure and purified through a short plug of silica (heptane/EtOAc 9:1) to yield the protected alcohol **62** as a colourless oil (21.08 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.63 (t, *J* = 6.0 Hz, 2H, $C\underline{H}_2$-OTBS), 2.21 (td, *J* = 6.7, 2.7 Hz, 2H, $C\underline{H}_2$-CΞCH), 1.94 (t, *J* = 2.7 Hz, 1H, CH_2-CΞC$\underline{H}$), 1.68-1.53 (m, 4H, CHΞC-CH_2-($C\underline{H}_2$)$_2$), 0.89 (s, 9H, Si-C-($C\underline{H}_3$)$_3$), 0.05 (s, 6H, Si-($C\underline{H}_3$)$_2$).[218]

6-Benzyloxyhex-1-yne (64).

To a suspension of NaH (60 % w/w in moneral oil, 10.19 mmol, 407 mg) in DMF (4 mL) cooled to 0 °C was added hex-5-yn-1-ol (10.19 mmol, 1g) dropwise. The mixture was stirred at 20 °C for 15 min. To the mixture was added dropwise benzyl bromide (15.28 mmol, 1.8 mL) at 0 °C and the resulting mixture was stirred at 20 °C for 12 h. The mixture was poured in ice water (15 mL) and extracted with Et_2O (3 × 10 mL), the combined organic layers were dried overNa_2SO_4, concentrated in vacuo and purified by flash chromatography (hexane/EtOAc 20:1) to give 64 (1.38 g, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35-7.29 (m, 5H, Ph), 4.51 (s, 2H, O-C$\underline{H_2}$-Ph), 3.50 (t, *J* = 6.2 Hz, 2H, O-C$\underline{H_2}$-CH_2), 2.22 (td, *J* = 6.8, 2.6 Hz, 2H, C$\underline{H_2}$-CΞCH), 1.95 (t, *J* = 2.6 Hz, 1H, CΞC$\underline{H}$), 1.80-1.59 (m, 4H, CHΞC-CH_2-(C$\underline{H_2}$)$_2$).[219] ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.75, 128.3 (2C), 127.6 (2C), 127.5, 84.3, 72.8, 69.7, 68.4, 28.7, 25.2, 18.2.[219]

10-(Benzyloxy)decan-1-ol (66)

Decan-1,10-diol (10 g, 57.38 mmol) was added to a suspension of NaH (60% in mineral oil, 57.38 mmol, 2.30 g) in dry DMF (45 mL) at 0 °C under an argon atmosphere, and the mixture was stirred for 20 min at that temperature. BnBr (5.74 mmol, 680 µL) was then added dropwise at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min then at room temperature for 4 h. The reaction mixture was poured into ice-water (150 mL) and the resulting phase was extracted with EtOAc (3 × 200 mL). The combined organic layers were dried over $MgSO_4$, and concentrated *in vacuo*. The obtained white solid was recrystallized from EtOAc/heptane, to offer the starting material as white crystals (7.9 g) and the filtrate was concentrated *in vacuo* and purified by flash chromatography (heptane/EtOAc 2:1) to afford **66** in 90% yield (1.37 g). R_f = 0.27 (heptane/EtOAc 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29-7.18 (m, 5H, Ph), 4.43 (s, 2H, C$\underline{H_2}$-Ph), 3.55 (t, *J* = 6.6 Hz, 2H, C$\underline{H_2}$-OH), 3.39 (t, *J* = 6.6 Hz, 2H, C$\underline{H_2}$-OBn), 1.97 (s, 1H, OH), 1.58-1.46 (m, 4H, C$\underline{H_2}$-CH_2-OBn and C$\underline{H_2}$-CH_2-OH), 1.32-1.18 (m, 12H, OH-CH_2-CH_2-$C_6\underline{H_{12}}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.6, 128.3 (2C), 127.6 (2C), 127.4, 72.8, 70.5, 63.0, 32.7, 29.7, 29.5 (2C), 29.4 (2C), 26.1, 25.7.

16-Benzyloxy-1-((*tert*-butyldimethylsilyl)oxy)hexadec-5-yn-7-ol (67)

To a solution of DMSO (24.96 mmol, 1.95 g, 1.8 mL) in CH_2Cl_2 (45 mL) at -78 °C under an atmosphere of N_2 was added a solution of oxalyl chloride (12.49 mmol, 1.58 g, 1.1 mL) in CH_2Cl_2 (2.3 mL) slowly, maintaining the temperature below -60 °C while stirring. A solution of **66** (11.35 mmol, 3.00 g) in CH_2Cl_2 (11 mL) was added dropwise to the resulting mixture while still keeping the temperature below -60 °C. After stirring for 1.5 h, Et_3N (56.8 mmol, 5.74 g, 8.2 mL) was added and the reaction was allowed to warm slowly to 20 °C over 3 h. The reaction mixture was poured into water (60 mL), the phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 60 mL). The combined organic phases were washed with 1% aq. HCl (90 mL), water (90 mL), sat. aq. $NaHCO_3$ (90 mL) and sat. aq. NaCl (90 mL) and then dried with $MgSO_4$, concentrated and used without further purification.

To a solution of 6-((*tert*butyldimethylsilyl)oxy)hex-1-yne (**62**) (4.70 mmol, 1.00 g) in THF (4.7 mL) at -78 °C was added ^{t}-BuLi (1.7 M in pentane, 4.24 mmol, 2.50 mL) dropwise. The resulting mixture was allowed to warm to 20 °C and then a solution of the crude aldehyde (4.24 mmol, 1.11 g) in THF (4.2 mL) was added dropwise. The reaction mixture was stirred at 20 °C for 1 h, poured into sat. aq. NH_4Cl (20 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried with $MgSO_4$, filtered, concentrated and purified by column chromatography (EtOAc/heptane 1:9) affording **67** as a colourless oil (1.37 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37-7.24 (m, 5H), 4.50 (s, 2H), 4.38-4.29 (m, 1H), 3.62 (t, *J* = 5.9 Hz, 2H), 3.46 (t, *J* = 6.6 Hz, 2H), 2.24 (td, *J* = 6.4, 3.3 Hz, 2H), 1.72-1.54 (m, 6H), 1.46-1.24 (m, 14H), 0.89 (s, 9H), 0.05 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.83, 128.48 (2C), 127.77 (2C), 127.61, 85.43, 81.66, 73.00, 70.65, 62.91, 62.79, 38.35, 32.06, 32.03, 29.92, 29.64, 29.61, 29.42, 26.33, 26.11 (3C), 25.36, 25.27, 18.64, 18.49, -5.12, -5.14. HRMS (ESI+) $C_{29}H_{50}O_3Si$, m/z [M+Na+] 497.3427, found 497.3413.

16-Benzyloxy-1,7-bis((*tert*-butyldimethylsilyl)oxy)hexadec-5-yne (68)

To a solution of **67** (2.30 mmol, 1.09 g) in DMF (4.6 mL) was added imidazole (3.44 mmol, 234 mg) and TBSCl (2.76 mmol, 415 mg). The resulting mixture was stirred for 3 h, poured into sat. aq. NH_4Cl (20 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic phases were dried with $MgSO_4$, filtered, concentrated and purified bycolumn chromatography (EtOAc/heptane 1:19) affording **68** as a colourless oil (1.27 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37-7.24 (m, 5H), 4.50 (s, 2H), 4.36-4.24 (m, 1H), 3.62 (t, *J* = 5.9 Hz, 2H), 3.46 (t, *J* = 6.6 Hz, 2H), 2.21 (td, *J* = 6.7, 1.8 Hz, 2H), 1.75-1.50 (m, 6H), 1.45-1.23 (m, 14H), 0.90 (s, 9H), 0.89 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H), 0.05 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.85, 128.48 (2C), 127.76 (2C), 127.60, 84.27, 82.35, 72.99, 70.66, 63.35, 62.80, 39.17, 32.09, 32.03, 29.92, 29.69, 29.63, 29.42, 29.18, 26.10 (6C), 26.01, 25.50, 25.31, 18.66, 18.48, -4.29, -4.81, -5.16 (2C).

10,16-Bis((*tert*-butyldimethylsilyl)oxy)hexadecan-1-ol (69)

To a solution of **68** (2.05 mmol, 1.21 g) in EtOAc (20.5 mL) under a N_2 atmosphere was added 10% Pd/C (218 mg) and an atmosphere of H_2 was installed by bubbling H2 through the solution for 5 min. The reaction was stirred under H_2 for 20 h, filtered through Celite and concentrated, affording **69** as a colourless oil (1.02 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.66-3.55 (m, 5H), 1.62-1.21 (m, 26H), 0.89 (s, 9H), 0.88 (s, 9H), 0.04 (s, 6H), 0.03 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 72.49, 63.46, 63.19, 37.25, 32.98, 32.93, 29.98, 29.81, 29.72, 29.56, 26.13 (4C), 26.08 (4C), 25.96, 25.88, 25.47 (2C), 18.52, 18.30, -4.27 (2C), -5.11 (2C).

***cis*-2-Phenyl-1,3-dioxan-5-yl 10,16-bis((*tert*-butyldimethylsilyl)oxy)hexadecanoate (70)**

To a solution of **69** (1.97 mmol, 991 mg) in CH_2Cl_2 (8 mL) and water (4 mL) was added PhI(OAc)2 (1.65 g, 5.12 mmol) and TEMPO (62 mg, 0.39 mmol). The resulting mixture is stirred vigorously for 3 h, poured into 10%. aq. $Na_2S_2O_3$ (20 mL) and extractedwith EtOAc (3 × 25 mL). The combined organic phases were dried with $MgSO_4$, filtered, concentrated and taken up in CH_2Cl_2 (20 mL). To this solution was added *cis*-5-hydroxy-2-phenyl-1,3-dioxane (2.56 mmol, 461 mg), DMAP (3.35 mmol, 409 mg) and EDC-HCl (2.96 mmol, 567 mg). The reaction was stirred for 18 h, SiO_2 was added, the mixture was concentrated and purified by column chromatography (EtOAc:heptane 1: 9) affording **70** as a colourless oil (1.19 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56-4.47 (m, 2H), 7.43-7.34 (m, 3H), 5.57 (s, 1H), 4.75-4.71 (m,1H), 4.29 (d, *J* = 12.9 Hz, 2H), 4.18 (d, *J* = 12.9 Hz, 2H), 3.65-3.56 (m, 3H), 2.44 (t, *J* =7.6 Hz, 2H), 1.76-1.59 (m, 2H), 1.56-1.45 (m, 2H), 1.45-1.18 (m, 20H), 0.89 (s, 9H), 0.88 (s, 9H), 0.05 (s, 6H), 0.03 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.03, 138.11, 129.22, 128.43 (2C), 126.15 (2C), 101.37, 72.49, 69.28 (2C), 65.81, 63.46, 37.28, 37.22, 34.54, 33.00, 29.81, 29.63, 29.42, 29.26, 26.13 (3C), 26.09 (4C), 26.03, 25.97, 25.48, 25.08, 18.38, 18.30, -4.27 (2C), -5.11 (2C).

cis-2-Phenyl-1,3-dioxan-5-yl 10,16-dihydroxyhexadecanoate (71)

To a solution of **70** (0.22 mmol, 150 mg) in MeCN (23 mL) at 0 °C was added 20% aq. HF (10 mmol, 1.0 mL) and the resulting mixture was stirred at 0 °C for 4 h. TMSOMe (28 mmol, 3.9 mL) was added, stirring was continued at 0 °C for 20 min, the mixture was poured into sat. aq. NH_4Cl (30 mL) and extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phases were dried over $MgSO_4$, filtered, concentrated and purified by column chromatography (CH_2Cl_2:EtOAc 1:0 to 1:1) affording **71** (84 mg, 84%) as a white solid, m.p.: 70.5-72.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56-7.48 (m, 2H), 7.42-7.34 (m, 3H), 5.56 (s, 1H), 4.72 (s, 1H), 4.29 (d, *J* = 12.9 Hz, 2H), 4.18 (d, *J* = 12.9 Hz, 2H), 3.64 (t, *J* = 6.6 Hz, 2H), 3.62-3.52 (m, 1H), (t, *J* = 7.5 Hz, 2H), 1.73-1.51 (m, 4H), 1.50-1.21 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.03, 137.94, 129.23, 128.45 (2C), 126.16 (2C), 101.38, 72.07, 69.29 (2C), 65.84, 63.16, 37.61, 37.48, 34.52, 32.85, 29.73, 29.59, 29.53, 29.33, 29.20, 25.85, 25.74 (2C), 25.05. HRMS (ESI+) $C_{26}H_{42}O_6$, m/z [M+Na+] 473.2879, found 473.2872.

1,3-Dihydroxypropan-2-yl 10,16-dihydroxyhexadecanoate (72 [2-MHG])

To a solution of **71** (0.26 mmol, 118 mg) in THF (13 mL) under a N_2 atmosphere was added 10% $Pd(OH)_2$/C (60 mg) and an atmosphere of H_2 was installed by bubbling H_2 through the solution for 5 min. The reaction was stirred under H_2 for 10 h, filtered through Celite, concentrated and crystallized from EtOAc and heptane, affording **71** (79 mg, 83%) as a white solid, Mp.: 66.7-68.1 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 4.68-4.59 (m, 1H), 3.68 (dd, *J* = 12.1, 3.7 Hz, 2H), 3.64 (dd, *J* = 12.1, 3.7 Hz, 2H), 3.54 (t, *J* = 6.6 Hz, 2H), 3.53-3.50 (m, 1H), 2.37 (t, *J* = 7.4 Hz, 2H), 1,71-1.25 (m, 24H). ^{13}C NMR (75 MHz, CD_3OD): δ 175.33, 76.51, 72.40, 62.98, 61.68 (2C), 38.42, 38.38,

35.13, 33.62, 30.79, 30.68, 30.59, 30.38, 30.17, 30.12, 26.95, 26.79, 25.97. HRMS (ESI+) $C_{19}H_{38}O_6$, m/z [M+Na+] 385.2566, found 385.2553.

(5-Bromo-1,3-phenylene)dimethanol (73)

To a solution of dimethyl 5-bromoisophthalate (18.00 mmol, 4.91 g) in anhydrous THF (40 mL) cooled to 0 °C under an atmosphere of Ar, was added a solution of $LiAlH_4$ (39.57 mmol, 1.5 g) in THF (40 mL) *via cannula* at 0 ºC, and the resulting mixture was stirred at that temperature for 10 min, then at 20 °C for 2 h. The mixture was cooled to 0 °C again, and a sat. aq. solution of Rochelle´s salt (150 mL) was added carefully. After stirring for 10 min at 0 °C and 30 min at 20 °C, the reaction ixture was extracted with EtOAc (3 × 150 mL), the combined organic layers were washed with NH_4Cl (300 mL) and brine, dried over $MgSO_4$ concentrated *in vacuo* and used as crude without further purification. R_f = 0.2 (hexane/CH_2Cl_2/MeOH, 7:2:1). ^{1}H-NMR (300 MHz, CD_3OD): δ 7.42 (d, *J* = 1.5 Hz, 2H, 2×C<u>H</u>-*ortho*), 7.28 (t, *J* = 1.5 Hz, 1H, C<u>H</u>-*para*), 4.59-4.58 (s, 4H, Ar-C<u>H_2</u>-OH). ^{13}C NMR (75 MHz, CD_3OD): δ 145.5 (2C, C3 and C5), 129.5 (2C, C2 and C6), 124.9 (C4), 123.4 (C1), 64.3 (2C, 2×<u>C</u>H_2-OH).

5-Bromoisophthalaldehyde (74)

To a solution of anhydrous DMSO (197 mmol, 14 mL) in CH_2Cl_2 (200 mL) cooled to -78 °C under Ar, was added a solution of $(ClCO)_2$ (114.6 mmol, 9.7 mL) in CH_2Cl_2 (23 mL) dropwise at -50 °C to -60 °C. The reaction mixture was stirred at -78 °C for 15 min and a solution of **73** (40.9 mmol, 8.9 g) in a mixture of CH_2Cl_2 (40 mL) and DMSO (10 mL) was added dropwise at -50 °C to -60 °C. After stirring at -78 °C for 1 h, Et_3N (409.5 mmol, 57 mL) was added at -78 °C and the mixture was stirred at that temperature for 15 min and then allowed to warm to 20 °C. Water (300 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 300 mL). The combined organic layers were washed with aq. HCl (1% w/w), water, aq. Na_2CO_3 (5% w/w) and water, dried over $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography (heptane/EtOAc 9:1 to 8:2) to afford 6.88 g of the dialdehyde **74** as a white powder (79 %). R_f = 0.46 (heptane/EtOAc 2:1). mp: 127-128 °C (lit. 118-120 ºC)[220]. ^{1}H NMR (300 MHz; $CDCl_3$): δ 10.04 (s, 2H, CHO), 8.29 (t, *J* = 1.4 Hz, 1H, H4), 8.24 (d, *J* = 1.4 Hz, 2H, H2, H6);[220] ^{13}C NMR (75 MHz, $CDCl_3$): δ 189.5 (2C, <u>C</u>HO), 138.4 (2C, C3, C5), 137.1 (2C, C2, C6), 129.2 (C4), 124.3 (C1). IR (neat): 3063, 2868, 1691 cm^{-1}. HRMS (ESI +) $C_8H_5BrO_2$, m/z [M+H^+] 212.9551, found 212.9545.

1-Bromo-3,5-di((*R*)-1-hydroxybut-3-enyl)benzene (75)

The operations were carried out under an atmosphere of Ar. A solution of (+)-Ipc_2BAll (1M in pentane, 26 mmol, 26 mL) was cooled to -78 °C. A solution of **74** (10.3 mmol, 2.2 g) in anhydrous THF (45 mL) cooled to 0 °C was added to the allylborane solution at -78 °C *via cannula* over 1 h.

The addition was performed on the side of the flask in order to cool the aldehyde mixture before it reaches the allylborane. The resulting reaction mixture was stirred at -78 °C for 4 h, after what MeOH (2 mL) followed by 3N NaOH (4.5 mL) and 35% H_2O_2 (9 mL) were added slowly at -78 °C. The mixture was stirred at -78 °C for 10 min and then at 50 °C for 15 h. sat. aq. NH_4Cl (50 mL) was added and the reaction mixture was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, concentrated on silica *in vacuo* and purified by flash chromatography (heptane/EtOAc 9:1 to 2:1) to afford 2.87 g of **75** as a white solid (94 %). R_f = 0.28 (heptane/EtOAc 2:1). mp = 48-49 °C. $[\propto]_D^{20}$= +0.08 ° (c = 1.48, $CHCl_3$). ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.42 (d, 2H, *J* = 1.5 Hz, H2 and H6), 7.25 (t, *J* = 1.5 Hz, 1H, H4), 5.78 (dddd, *J* = 20.1, 9.7, 7.8, 6.5 Hz, 2H, C<u>H</u>=CH_2), 5.21-5.14 (m, 4H, CH=C<u>H_2</u>), 4.70 (dd, *J* = 4.8, 7.8 Hz, 2H, C<u>H</u>-OH), 2.57-2.38 (m, 4H, C<u>H_2</u>-CH=CH_2), 2.12 (broad s, 2H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 146.4 (2C, C3, C5), 134.0 (2C, <u>C</u>H=CH_2), 128.2 (2C, C2, C6), 122.8 (C1), 122.1 (C4), 119.3 (2C, CH=<u>C</u>H_2), 72.7 (2C, CH-OH), 44.1 (2C, <u>C</u>H-CH=CH_2). IR (neat): 3303, 2932, 2898, 1641, 1601, 1571, 1485, 1429, 1403, 1339, 1230, 1062 918 cm^{-1}. HRMS (ESI+) $C_{14}H_{17}BrO_2$, m/z [M+Na^+] 319.0310, found 319.0302. HPLC (Chiralpak OD-H at 20 °C, λ= 215 nm, hexane with 2-propanol 0-4% over 90 min at 0.5 mL/min flow rate). Retention times: 70.8-71.3 min (*ent*-(-)-**75**); 74.1-74.3 (meso); 78.2-78.5 min ((+)-**75**). To obtain the retention time for *ent*-(-)-**75**, the reaction was performed with (-)-Ipc_2BAll under identical conditions.

1-Bromo-3,5-di((*R*)-1-methoxybut-3-enyl)benzene (76)

To a suspension of NaH (60 % in mineral oil 176.76 mmol, 7.07 g) in anhydrous DMF (400 mL) cooled to 0 °C under Ar, was added a solution of **75** (58.92 mmol, 17.50 g) in anhydrous DMF (120 mL). After stirring at 0 °C for 10 min, the resulting mixture had turned yellow. MeI (235.68 mmol, 14.7 mL) was added slowly at 0 °C, and the resulting reaction mixture was stirred at that temperature for 10 min and then allowed to reach 20 °C slowly. After 14 h, the reaction mixture was poured into 10 % aq. $Na_2S_2O_3$ (400 mL) at 0 °C and extracted with Et_2O (3 × 400 mL). The combined organic layers were washed with H_2O (1 L) and dried over $MgSO_4$, concentrated on silica *in vacuo* and purified by short flash chromatography (heptane:EtOAc 19:1). The desired product was isolated as a colourless viscous oil in 92 % yield (17.71 g). R_f = 0.53 (heptane/EtOAc 9:1). $[\propto]_D^{20}$= +79.63 ° (c = 1.08, $CHCl_3$). ^{1}H NMR (300 MHZ; $CDCl_3$) δ 7.35 (d, *J* = 1.5 Hz, 2H, H2, H6), 7.12 (t, *J* = 1.5 Hz, 1H, H4), 5.80-5.66 (m, 2H, CH_2-C<u>H</u>=CH_2), 5.06-5.03 (m, 2H, CH_2-CH=C<u>H_2</u>), 5.02-4.99 (m, 2H, CH_2-CH=<u>CH_2</u>), 4.14 (dd, *J* = 7.1, 6.0 Hz, 2H, C<u>H</u>-OCH_3), 3.23 (s, 6H, OCH_3), 2.53 (dtt, *J* = 14.2, 7.1, 1.2 Hz, 2H, C<u>H_2</u>-CH=CH_2), 2.38 (dddt, *J* = 14.2, 7.1, 6.0, 1.2 Hz, 2H, C<u>H_2</u>-CH=CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.2 (2C, C3, C5), 134.1 (2C, <u>C</u>H=CH_2), 128.9 (2C, C2, C6), 123.7 (C4), 122.6 (C1), 117.4 (2C, CH=<u>C</u>H_2), 82.9 (2C, <u>C</u>H-OCH_3), 56.9 (2C, O<u>C</u>H_3), 42.3 (2C, <u>C</u>H_2-CH=CH_2). IR (neat): 3076, 2980, 2932, 2904, 2855, 2821, 1430, 1190, 1092, 912 cm^{-1}. HRMS (ESI +) $C_{16}H_{21}BrO_2$, m/z [M+Na^+] 347.0617, found 347.0615.

Mosher ester analysis of compound 75

(2*S*,2'*S*)-((1*R*,1'*R*)-1,1'-(5-Bromo-1,3-phenylene)bis(but-3-ene-1,1-diyl)) bis(3,3,3-trifluoro-2-methoxy-2-phenylpropanoate) ((*S*)-Mosher ester) 77

To a solution of diol **75** (0.128 mmol, 38 mg) in pyridine (1.3 mL) was added (R)-methoxytrifluoromethylphenylacetyl chloride (0.396 mmol, 100 mg) and the resulting mixture was stirred at 20 °C. After 2.5 h, a saturated aqueous solution of $NaHCO_3$ (3 mL) was added and the organic layer extracted with with CH_2Cl_2 (3 × 3 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude ester which was analysed by NMR spectroscopy. The crude was then purified by flash chromatography (heptane/EtOAc 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44–7.32 (m, 10H, 2×Ph), 7.21 (d, *J* = 1.52 Hz, 2H, 2×H *ortho* to Br), 7.02-7.00 (m, 1H, H *para* to Br), 5.84 (dd, *J* = 8.2, 5.3 Hz, 2H, 2×Ar-C<u>H</u>), 5.74-5.60 (m, 2H, 2×C<u>H</u>=CH_2), 5.14-5.05 (m, 4H, 2×CH-C<u>H_2</u>), 3.55 (s, 3H, -OC<u>H_3</u>), 3.54 (3H, -OC<u>H_3</u>), 2.66-2.44 (m, 4H, 2×C<u>H_2</u>-CH=CH_2). ^{19}F NMR (282 MHz, $CDCl_3$): δ -71.8.

(2*R*,2'*R*)-((1*R*,1'*R*)-1,1'-(5-Bromo-1,3-phenylene)bis(but-3-ene-1,1-diyl)) bis(3,3,3-trifluoro-2-methoxy-2-phenylpropanoate) 78

To a solution of diol **75** (0.128 mmol, 38 mg) in pyridine (1.3 mL) was added (S)-methoxytrifluoromethylphenylacetyl chloride (0.396 mmol, 100 mg) and the resulting mixture was stirred at 20 °C. After 2.5 h, a saturated aqueous solution of $NaHCO_3$ (3 mL) was added and the organic layer extracted with with CH_2Cl_2 (3 × 3 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude ester which was analysed by NMR spectroscopy. The crude was then purified by flash chromatography (heptane/EtOAc 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44–7.32 (m, 12H, 2×Ph and 2×H *ortho* to Br), 7.22-7.20 (m, 1H, H *para* to Br), 5.91 (dd, *J* = 7.9, 5.5 Hz, 2H, 2×Ar-C<u>H</u>), 5.55 (tdd, *J* = 7.0, 10.5, 17.3 Hz, 2H, 2×C<u>H</u>=CH_2), 5.00-4.95 (m, 4H, 2×CH-C<u>H_2</u>), 3.44 (s, 3H, -OC<u>H_3</u>), 3.43 (3H, -OC<u>H_3</u>), 2.66-2.44 (m, 4H, 2×C<u>H_2</u>-CH=CH_2). ^{19}F NMR (282 MHz, $CDCl_3$): δ -71.4.

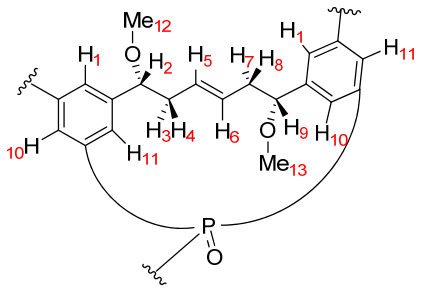
Tris(3,5-bis((*R*)-1-methoxybut-3-enyl)phenyl)-phosphine oxide (79)

Anhydrous THF (3 mL) was added to magnesium turnings (739 mg, 30.77 mmol) under an atmosphere of Ar. Approximately 200 mg of **76** (0.62 mmol) was added onto the magnesium turnings in order to obtain a high concentration of **76** around the turnings, followed by a crystal of iodine. The reaction was started by gentle heating of the unstirred mixture. When the reaction was started, the reaction was stirred at reflux and a solution of **76** (9.8 g, 30.15 mmol) in anhydrous THF (60 mL) was added dropwise over a period of 30 min. The mixture was stirred at reflux for additional 4 h, and turned black over that time. The resulting Grignard mixture was then cooled to 20 °C, and freshly distilled PCl_3 (814 µL, 9.32 mmol) was added dropwise over 15 min. The reaction mixture was stirred at 20 °C for 14 h, after what ^{t}BuOOH (2.5 mL, 13.99 mmol) was

added, and the reaction was stirred for additional 2 h. The crude mixture was poured into sat. aq. NH_4Cl (100 mL). The layers were separated, and the aqueous layer was washed with Et_2O (3 × 100 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography (heptane/EtOAc 1:1 to 1:4) afforded the desired phosphine oxide as viscous oil in 84 % yield (6.10 g). R_f = 0.31 (heptane/EtOAc 1:2). $[\propto]_D^{20}$= 72.3 ° (c =1.09, $CHCl_3$). ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.47 (dd, *J* =12.1, 1.6 Hz, 6H, H2 and H6), 7.43-7.40 (m, 3H, H4), 5.66 (ddt, *J* = 17.5, 10.6, 7.0 Hz, 6H, CH_2-C<u>H</u>=CH_2), 4.99-4.95 (m, 6H, CH_2-CH=C<u>H_2</u>), 4.95-4.89 (m, 6H, CH_2-CH=<u>CH_2</u>), 4.18 (m, 6H, C<u>H</u>-OCH_3), 3.17 (s, 18H, OCH_3), 2.48 (dtt, *J* = 14.1, 7.0, 1.1 Hz, 6H, C<u>H_2</u>-CH=CH_2), 2.35 (dddt, *J* = 14.1, 7.0, 5.9 Hz, 2H, C<u>H_2</u>-CH=CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.6 (d, *J* = 11.7 Hz, 6C, C3, C5), 133.9 (6C, <u>C</u>H=CH_2), 132.6 (d, *J* = 103.2 Hz, 3C, C1), 129.6 (d, *J* = 10.3 Hz, 6C, C2, C4), 128.6 (d, *J* = 2.7 Hz, 3C, C4), 117.4 (6C, CH=<u>C</u>H_2), 83.0 (6C, <u>C</u>H-OCH_3), 56.8 (6C, O<u>C</u>H_3), 42.3 (6C, <u>C</u>H_2-CH=CH_2). IR (neat): 3075, 29.80, 2932, 2903, 2821, 1641, 1428, 1190, 1092, 986, 912 cm^{-1}. HRMS (ESI +) $C_{48}H_{63}O_7P$, m/z [M+Na^+] 805.4209, found 805.4206.

Phosphine oxide 82

To a solution of **79** (0.64 mmol, 500 mg) in anhydrous CH_2Cl_2 (60 mL) were added 2,6-dichloro-1,4-benzoquinone (0.13 mmol, 24 mg) and Grubbs' catalyst 2nd generation (0.06 mmol, 36 mg), and the resulting mixture was stirred at reflux. Five more portions of 2,6-dichloro-1,4-benzoquinone (5 × 0.13 mmol, 5 × 24 mg) and Grubbs' catalyst 2nd generation (5 × 0.06 mmol, 5 × 36 mg) were added at 1 h intervals at 40 °C, and the mixture was stirred for additional 17 h. The solvent was reduced to half volume under reduced pressure, and the curde mixture was purified stepwise by repeated preparative TLC (toluene/MeOH 9:1), then by preparative TLC in a different solvent system (EtOAc/MeOH 95:5), and another time by preparative TLC with the first eluent system (toluene/MeOH 8:2). Thereby, the desired product could be isolated in about 80% purity (judged by HPLC and ^{1}H NMR). Final purification of the compound by preparative HPLC afforded the pure desired compound in 2% yield (9 mg).



^{1}H NMR (300 MHZ; $CDCl_3$): δ 7.60 (s, 1H, H_1), 7.54 (d, *J* = 11.7 Hz, 1H, H_{11}), 6.93 (d, *J* = 12.5 Hz, 1H, H_{10}), 5.40-5.33 (m, 1H, H_5), 5.30-5.23 (m, 1H, H_6), 4.33 (dd, *J* = 10.0 Hz, *J* = 2.3 Hz, 1H, H_2), 4.12 (d, *J* = 8.7 Hz, 1H, H_9), 3.40 (s, 3H, OMe_{13}), 3.30 (s, 3H, OMe_{12}), 2.69 (d, *J* = 11.5 Hz, 1H, H_3), 2.58 (d, *J* = 13.9 Hz, 1H, H_7), 2.36-2.28 (m,2H, H_4 and H_8). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.1 (d, *J* = 12.2 Hz, 3C, <u>C</u>-C-H_2), 142.2 (d, *J* = 11.7 Hz, 3C, <u>C</u>-C-H_9), 130.3 (d, *J* = 9.7 Hz, 3C

C-H_{11}), 129.8 (d, J = 11.7 Hz, 3C, C-H_{10}), 129.2 (3C, C-H_6), 127.5 (3C, C-H_5), 125.1 (m, 3C, C-H_1), 82.2 (3C, C-H_2), 80.9 (3C, C-H_9), 57.1 (3C, OMe_{13}), 56.8 (3C, OMe_{12}), 42.0 (3C, C-H_3H_4), 41.2 (3C, C-H_7H_8) (signal for the aromatic carbons bound to phosphorous is not reported, it could not be observed, neither in hmbc nor in the ^{13}C spectrum, presumably due to a long relaxation time and the coupling to phosphorous).

6.List of abbreviations

Approx. approximately
Aq.: aqueous
Asp: aspartic acid
ATRA: all-*trans* retinoic acid
AUC: area under the curve
Bn: benzyl
br.: broad
^{t}Bu: butyl
cod: cycloocta-1,5-diene
CD. circular dichroism
CuAAC: copper(I)-catalyzed Azide-Alkyne Cycloaddition
Cy: cyclohexyl
d: duplet
DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene
DCC: dicyclohexylcarbodiimide
DDQ: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DDS: drug delivery system
de: diastereomeric excess
DHB: 2,5-dihydroxybenzoic acid
$(DHQD)_2PHAL$: hydroquinidine 1,4-phthalazinediyl diether
DIAD: diisopropyl azodicarboxylate
DIBAL-H: diisopropylaluminium hydride
DIPAMP: ethane-1,2-diylbis[(2-methoxyphenyl)phenylphosphane]
DIPEA: diisopropylethylamine
DIPHOS, bis-diphenylphosphinoethane
DH: dimethylglyoximato
DHB: 2,5-dihydroxybenzoic acid
DLS: dynamic Light Scattering
DLPG: 1,2-dilauroyl-*sn*-glycero-3- phosphatidylglycerol
DMAP: 4-(*N*,*N*-dimethylamino)pyridine
DME: dimethoxyethane
DMF: dimethylformamide
DMSO: dimethylsulfoxide
DMPG: 1,2-dimyristoyl-sn-glycero-3-phosphatidylglycerol
DOPG: 1,2-dioleoyl-*sn*-glycero-3-phosphatidylglycerol
DPPC: 1,2-hexadecanoyl-sn-glycero-3-phosphatidylcholine
DPPG: 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylglycerol
DPPE-PEG2000: 1,2-hexadecanoyl-sn-glycero-3-phosphatidylethanolamine- N-[methoxy(polyethylene glycol)-2000]
DSC: differential scanning calorimetry
DSPC: 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine
DSPG: 1,2-distearoyl-*sn*-glycero-3-phosphatidylglycerol
DSPE: 1,2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine
EDC.HCl: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
EDTA: ethylenediaminetetraacetic acid
ee: enantiomeric excess
EPR: enhanced permeability and retention
equiv.: equivalent
ESI: electron spray ionization

Et: ethyl
FITC: fluorescein isothiocyanate
FRET: fluorescence resonance energy transfer
Gly: glycine
HEPES: 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid
His: histidine
HPLC: high pressure liquid chromatography
HRMS: high resolution mass spectrometry
HWE: Horner–Wadsworth–Emmons
Ipc_2Ball: *B*-allyldiisopinocampheylborane
ITC: isothiocyanate
KHMDS: potassium bis(trimethylsilyl)amide
LC-Ms: liquid chromatography-mass spectrometry
LDA: lithium diisopropylamide
L-DOPA: L-3,4-dihydroxyphenylalanine
LHMDS: lithium bis(trimethylsilyl)amide
MALDI-TOF-MS: matrix-assisted laser desorption/ionization - time of flight - mass spectrometry
MD: molecular dynamics
Me: methyl
Ms: methanesulfonyl
MTPA: α-methoxy-α- trifluoromethylphenylacetate
MW: molecular weight
NMR: nuclear magnetic resonance
PBS: phosphate buffered saine
PC: phosphatidylcholine
Pd/C: palladium on charcoal
PEG: poyethleneglycol
Pg: protecting group
PG: phosphatidylglycerol
PdI: polydispersity index
PMB: *p*-methoxybenzyl
POPC: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine
POPG: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylglycerol
ppm: parts per million
q: quadruplet
qnt: quintet
quant. quantitative
RCM: ring closing metathesis
RES: reticulo-endothelial system
rmsd: root mean square deviation
Rt: retention time
s: singlet
S: substrate
SD: standard deviations
SMEAH: sodium bis(2-methoxyethoxy)aluminium
$sPLA_2$: secretory phospholipase A_2
t: triplet
TBAHS: tetrabutylammonium hydrogen sulfate
TBAF: tetrabutylammonium fluoride
TBAI: tetrabutylammonium iodine

TBS: *tert*-butyldimethylsilyl
TEG: tetraethylelene glycol
TEMPO: 2,2,6,6-tetramethylpiperidine 1-oxyl
THF: tetrahydrofuran
TLC: thin layer chromatography
TMEDA: tetramethylethylenediamine
TMSOK: potassium trimethylsilanolate
TRITC: tetramethylrhodamine isothiocyanate
UV: ultraviolet

7. References

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