Synthesis of structured ether lipids and n-3 polyunsaturated fatty acids

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90 ECTS thesis submitted in partial fulfillment of a Magister Scientiarum degree in chemistry

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Reykjavik, June 2013
Synthesis of structured ether lipids and n-3 polyunsaturated fatty acids
Synthesis of structured DAGEs and n-3 PUFAs
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Abstract

Ether lipids of the 1-Ω-alkyl-2,3-diacyl-sn-glycerol type, made of pure saturated or monounsaturated 1-Ω-alkyl-sn-glycerols and long chain n-3 polyunsaturated fatty acids (PUFAs), are interesting compounds since their parts are thought of as biologically active. Twelve different types 1-Ω-alkyl-2,3-diacyl-sn-glycerols can be made by using enantiopure chimyl (16:0), batyl (18:0) and selachyl (18:1) alcohols and two different PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in the sn-2 and sn-3 positions. In the first part of the synthesis an immobilized Candida antarctica lipase (CAL) was used for the acylation of the primary hydroxyl group at the end position, using an acetoxyime ester of the corresponding PUFA. Then in the second part, the mid position was esterified, with the same or the other PUFA, with chemical acylation using EDAC as a coupling agent.

The n-3 polyunsaturated fatty acids α-linolenic acid (ALA) and docosapentaenoic acid (DPA) are likewise interesting compounds. They have various biological effects and partake in many different biological pathways. In their total synthesis, the PUFAs were constructed in two parts that were then coupled together. Part one consisted of the synthesis of the polyyne tails, which were made through a series of copper mediated coupling reactions. Part two involved the protection of the carboxyl acid group, followed by an alkylation which formed a terminal acetylene. The two parts were then combined with a coupling reaction, followed by partial hydrogenation used to gain the cis-configuration of the double bonds, and then the protection group was removed to afford the ALA and DPA, respectively.
Útldráttur


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Abbreviations

TAG – Triacylglycerol
DAG – Diacylglycerol
MAG – Monoacylglycerol
DAGE – Diacylglyceryl ether
MAGE – Monoacylglyceryl ether
CAL – Candida antarctica lipase
FA – Fatty acid
FFA – Free fatty acid
EFA – Essential fatty acid
PUFA – Polyunsaturated fatty acid
AO – Acetoxime ester
ALA – α-linolenic acid
EPA – Eicosapentaenoic acid
DPA – Docosapentaenoic acid
DHA – Docosahexaenoic acid
LA – Linoleic acid
DGLA – Dihomo-γ-linolenic acid
AA – Arachidonic acid
EDAC – 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
DMAP – 4-dimethylaminopyridine
TBAB – Tetra-n-butylammonium bromide
TBAF – Tetra-n-butyl ammonium fluoride
HMPA – Hexamethylphosphoramide
THF – Tetrahydrofuran
DMF – N,N-dimethylformamide
NMR – Nuclear magnetic resonance
HRMS – High resonance mass spectrometry
TLC – Thin layer chromatography
HPLC – High-performance liquid chromatography
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1 Introduction

This thesis is comprised of two projects both of which employ polyunsaturated fatty acids (PUFAs). In the first project, twelve different types of 1-O-alkyl-2,3-diacyl-sn-glycerols were synthesised using enantiopure chimyl, batyl and selachyl alcohols and two different PUFAs, eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA), in the mid- and end-positions. The second project involved the total synthesis of two different PUFAs, α-linolenic acid (ALA) and docosapentaenoic acid (EPA).

1.1 TAGs of natural fats and oils

Fats and oils are mostly made out of triacylglycerols (TAGs). TAGs are made out of a glycerol backbone and three fatty acids (FA) that have a determined positional distribution. The glycerol backbone has three different binding sites to which each fatty acid is attached. These attachment sites are specified by the sn-terminology where the pro-S end-position of the prochiral glycerol becomes the sn-1 site, the mid-position the sn-2 site and the remaining end position the sn-3 site. The sn-terminology is demonstrated in Figure 1, which shows the Fischer projection of the prochiral glycerol and of a TAG. [1, 2]

![Fischer projection of TAGs](image)

*Figure 1 Steriospecific numbering (sn-1, 2 and 3). Fischer projections of (A) the prochiral glycerol and (B) a TAG, with various R-groups representing different acyl chains.*

Both the fatty acids and their position on the TAG determine the characteristic properties of fats, i.e. the physical, the chemical and the metabolic ones. [2] In the human body TAGs are often stored in the adipose tissue as energy reserves. This tissue is the body's means of storing the metabolic energy until it is needed. Then the fatty acids can undergo β-oxidation to produce energy for the body. [2, 3]

TAGs can be either symmetrical, comprising the same fatty acid in the sn-1 and the sn-3 positions, or asymmetrical, comprising different fatty acids in these positions. In fact there are four possibilities of an assembly, AAA and ABA, which are symmetrical, and ABB and ABC, which are asymmetrical. The most common fatty acids in oil are palmitic (C16:0), stearic (C18:0) and oleic (C18:1) acids. [3]

The distribution of fatty acids may vary but most of them are predominantly found at a set location. [4, 5] In the adipose tissue of mammals, palmitic acid is predominantly found in the sn-1 position, while oleic acid is the major fatty acid found in the sn-2 position. In
regards to polyunsaturated fatty acids (PUFAs), linoleic acid (LA) is largely situated in the sn-2 position, while α-linolenic acid (ALA) can be found in both sn-1 and sn-3 in plants. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are prevalent in fish oil where DHA is to a large extent in the sn-2 position (about 80%), but EPA is more evenly distributed between the sn-2 (about 50%) and the combined sn-1,3 positions (about 50%). When it comes to dietary intake of the TAGs, the fatty acids in the 1,3-positions are often immediately cleaved from the glycerol backbone in the digestive system and used straight away for energy, while the 2-monoacylglycerol (2-MAG), containing the glycerol backbone, is incorporated or transferred into the body where it is cleaved from the backbone to produce the corresponding fatty acid. [6]

1.2 1-O-alkyl-sn-glycerols – alkylglycerols – glyceryl ethers

The 1-O-alkyl-sn-glycerols are made from a glycerol backbone and an alkyl chain connected to it through an ether bond. The O-alkyl glycerols found in Nature usually have even number of carbons, with saturated or monounsaturated chains ranging from 14-22 carbon atoms, with the 16 and 18 being the most abundant. [5, 7] The most abundant 1-O-alkyl-sn-glycerols are batyl, chimyl and selachyl alcohols which are composed of stearyl (C18:0), palmityl (C16:0) and oleyl (C18:1) fatty alkyl chains, respectively. The trivial names derive from the species they were first isolated from, i.e. Batoides (rays), Chimaeras (ratfish) and Selachii (sharks). [8] Figure 2 shows the structures of the three aforementioned 1-O-alkyl-sn-glycerols.

![Figure 2 The three most abundant 1-O-alkyl-sn-glycerols in Nature: (1) Chimyl alcohol (C16:0), (2) Batyl alcohol (C18:0), and (3) Selachyl alcohol (C18:1).](image)

The 1-O-alkyl-sn-glycerols are chiral compounds which, according to the sn-terminology, means that the ether moiety is located at the sn-1 position implying the S absolute configuration of the chiral carbon atom being in the mid-position of the glycerol moiety. That means that the O-alkyl group is attached to the pro-S (sn-1) position of the prochiral glycerol molecule. The central carbon of the glycerol backbone is assigned as the sn-2 position and the end-position remaining as the sn-3 position. [2] In Nature these positions are usually acylated and the resulting 1-O-alkyl-2,3-diacyl-sn-glycerols are referred to as diacylglycerol ethers (DAGEs).
The 1-\(O\)-alkyl-sn-glycerols and the 1-\(O\)-(alk-1’-enyl)-sn-glycerols are the most common glycerol ether backbones, with the second one being essential in animal lipid membranes. [7] DAGEs belong to the 1-\(O\)-alkyl-sn-glycerols family, and, as noted earlier, the chains of C16 and C18, both saturated and monounsaturated, are the most abundant. They in fact account for 80% of the 1-\(O\)-alkylglycerols found in DAGEs, with saturated chains accounting for about 60% in mammals. The most prevalent polyunsaturated alkyl chain being LA (C18:2). Odd numbered or even branched chains can be found in Nature but only in a minimal amount. [5, 9] The fact that 1-\(O\)-alkyl chains are found in a narrow range suggests that they are synthesised by a common biosynthetic pathway, which selectively chooses the fatty alcohol precursors based on their chain length and unsaturation. [10]

Glycerol ether backbones include DAGEs and neutral-plasmalogens, which have a double bond next to the ether bond, and plasmanyl-phospholipids and plasmalogen which are their phospholipid analogues and can be found widely in animal cell membranes. [5, 11, 12] Figure 3 shows 1-\(O\)-alkyl-sn-glycerols and 1-\(O\)-(alk-1’-enyl)-sn-glycerols neutral ether lipids and phosphoether lipids.

![Figure 3](image)

**Figure 3** Glycerol ether backbones include (A) DAGEs, (B) plasmanyl-phospholipids, (C) neutral plasmalogen, and (D) plasmalogen. R groups represent saturated and monounsaturated hydrocarbon chains, R’ represent saturated, monounsaturated and polyunsaturated hydrocarbon chains and X represents complicated groups found in natural phosphoether lipids.

DAGEs are widely spread among marine and terrestrial animals but appear to be almost absent in plant cells. [6] They are ubiquitously found in mammals, including humans, but most often as minor lipid components. [11] The phospholipids, found in mammals, seem to have a higher ratio of alkylglycerols than the neutral lipids. [5] 1-\(O\)-alkyl-sn-glycerols can be found in particular high amounts in the liver oils of various chondrichthians (sharks, rays and chimaeras). The amount of alkylglycerols varies both between the species and members within the same species. The alkylglycerols are believed to play an important role in the buoyancy control of some marine animals, due to lower density, which partly explains this difference. [4, 5]

The 1-\(O\)-alkyl-sn-glycerols are believed to be highly beneficial to health or to have high therapeutic potential. They possess immune stimulating as well as antineoplastic properties, and have been used before, during and after cancer treatments to prevent further complication and help the patients regain their former strength. [12] Alkylglycerols also seem to enhance antibody production and stimulate macrophages, and are found in relatively large amounts in human colostrum and milk, presumably to help mature the underdeveloped immune system of new-borns. They also seem to increase spermatozoa motility and fertility in artificial inseminations. [6] Throughout the years Greenland shark liver oil has been used to battle gastric ulcers, colon inflammation and arthritis. They also seem to have some antibacterial and antifungal properties. [5] The 1-\(O\)-alkyl-sn-glycerols
might also be used to facilitate the transfer of drugs through the blood brain barrier, but a racemic mixture of 1-O-alkyl-glycerols has already been used to deliver antibiotics and antineoplastic drugs to the brain by increasing its permeability. [13]

Methoxylated alkylglycerols or (2’R)-1-O-(2’-methoxyalkyl)-sn-glycerols are an interesting class of glycerol ethers. They are a minor component of the glycerol ether moiety but might have a high therapeutic value. What distinguishes them from other 1-O-alkyl-sn-glycerols is that they possess a methoxyl group positioned at the second carbon atom of the 1-O-alkyl chain. [9, 14] Figure 4 shows an example of a methoxylated alkylglycerol.

![Figure 4 Methoxylated alkylglycerol, a 1-O-alkyl-sn-glycerol with a methoxyl group positioned at the second carbon atom of the 1-O-alkyl chain.](image)

Among the properties they seem to possess are antibacterial, antiviral and antifungal properties. They have also been shown to have antibiotic activity and antitumor quality, since they inhibit some cancer cell lines and metastasis formation in mice. [15]

### 1.3 Polyunsaturated fatty acids (PUFAs)

Linoleic acid (LA) (C18:2n-6) and α-linolenic acid (ALA) (C18:3n-3) are so-called essential fatty acids (EFA). They are polyunsaturated fatty acids (PUFA) which belong to the n-6 and n-3 families, respectively. PUFAs are fatty acids possessing more than one double bond, situated on the sixth carbon from the methyl end of the fatty acyl chain in the case of n-6 and on the third for the n-3. [16] The structure of the two essential polyunsaturated fatty acids can be seen in Figure 5.

![Figure 5 The essential fatty acids (A) linoleic acid (LA) (C18:2n-6), and (B) α-linolenic acid (ALA) (C18:3n-3).](image)

The EFAs are indispensable for the development and maintenance of the human body but they must be obtained from diet. [16, 17] They are called essential fatty acids because humans cannot synthesise them, the reason being that we cannot incorporate a double bond further away than 9 carbons from the carbonyl group of the fatty acid. [16, 18] Furthermore dietary LA and ALA can be metabolised, by series of desaturation and elongation reactions, to the five other PUFAs that are needed for the development and regulation of the body. The other PUFAs are dihomo-γ-linolenic acid (DGLA) (C20:3n-6)
and arachidonic acid (AA) (C20:4n-6), the n-6 fatty acids derived from LA, and the n-3 fatty acids EPA (C20:5n-3), DPA (C22:5n-3) and DHA (C22:6n-3) derived from the parent ALA. [16, 19] The two n-6 PUFAs derived from LA are shown in Figure 6 and the three n-3 PUFAs derived from ALA are shown in Figure 7.

**Figure 6** The n-6 derivatives of LA (A) dihomo-γ-linolenic acid (DGLA) (C20:3n-6), and (B) arachidonic acid (AA) (C20:4n-6).

PUFAs have been shown to have many beneficial biological functions and seem to play important roles in several different biological processes. A deficiency of PUFAs in the diet may have serious side effects to health, which can be reflected in several diseases. This is because PUFAs play a role in membrane functions, eicosanoid production and regulation of gene expressions. The genes PUFAs regulate are for example involved in lipid oxidation and they regulate cellular inflammation. [19] Dietary intake of n-3 PUFAs is recommended as a secondary prevention against heart disease as well as prevention and treatment of disorders with inflammatory components. Deficiency in a diet can have bad consequences, for example heart related problems or psychiatric disorders. [20]

**Figure 7** The n-3 derivatives of ALA (A) eicosapentaenoic acid (EPA) (C20:5n-3), (B) docosapentaenoic acid (DPA) (C22:5n-3), and (C) docosahexaenoic acid (DHA) (C22:6n-3).

Both n-3 and n-6 polyunsaturated fatty acids, of 20-22 carbons, in membranes are precursors for eicosanoids and related compounds. [20] Eicosanoids are signalling molecules in the body and control many physiological systems. They include prostaglandins, thromboxanes, leukotrienes, lipoxins, resolvins, protectins and others. [4, 20] Eicosanoids are important for biological function and are involved in controlling, handling, and the duration and intensity of inflammation and immune response. [19] Arachidonic acid (AA, C20:4n-6) is derived from LA and has an effect on pro-inflammatory responses and can act as a vasodilator. Its biological activity and availability is inversely related to the n-3 fatty acids. [20] LA and ALA partly compete against each other due to shared metabolic pathway [17], so higher composition of EPA, DPA and DHA in membranes competitively lowers the availability of AA for the production of the
eicosanoids. Greater intake of EPA and DHA influences the tissue composition directly, since conversion from ALA to EPA/DHA is poor. [20]

As noted before the essential fatty acids LA and ALA can be metabolized into longer PUFAs, that are needed to maintain bodily function. The processes are very similar but herein the focus will be on the n-3 analogues. ALA can be metabolized, by desaturation and elongation enzymes, to form a series of highly unsaturated n-3 PUFAs, as seen in Scheme 1. [17, 21]

![Scheme 1](image)

Scheme 1 A general pathway for the conversion of ALA to longer-chain n-3 PUFAs. The conversion is as follows: (a) ALA (18:3n3) is desaturated using ∆6-desaturase, (b) (20:3n3) is elongated with elongase, (c) (20:4n3) is desaturated using ∆5-desaturase, (d) EPA (20:5n3) is elongated with elongase, (e) DPA (22:5n3) is elongated with elongase, (f) (24:5n3) is desaturated using ∆6-desaturase, (g) (24:6n3) is shortened using β-oxidation, producing (h). DHA (22:6n3).

The major products of this conversion of ALA are EPA, DPA and DHA. [19] Studies show that conversion of ALA to EPA occurs, as well as conversion to n-3 DPA, but that the conversion is limited. Further transformation into DHA is even lower. [17, 19] The
conversion of ALA to n-3 PUFA is greater in women than in men, which may be due to a regulatory effect of oestrogen on elongation pathways. [19] The capacity to convert ALA to EPA and DHA is only about 8% and 0-4%, respectively, for men, but considerably higher for women of reproductive age, or about 21% and 9%, respectively. [4] The explanation of this difference is most likely the demand for PUFA in foetuses and newborns for their development. Deficiency of ALA in the mother’s diet has a negative effect on their neurological function. The n-3 PUFAs are critical in the function and development of the central nervous system which further supports ALA’s role as a precursor for EPA and DHA. [17] It further underlines the necessity of obtaining both EPA and DHA by other means through diet. [21]

When consumption of ALA is chronically increased the conversion to EPA increases, resulting in higher concentration in plasma and cell pools. It does not however increase the level of DHA. [17] A factor that may limit the synthesis of DHA from ALA is the competition between 24:5n-3 and ALA for the D6-desaturase enzyme. The enzyme takes part in two steps of the metabolic pathway, first in the desaturation of ALA, from 18:3n-3 to 18:4n-3, and secondly in the desaturation of 24:5n-3 to 24:6n-3 which is a precursor of DHA. This means that when ALA levels (or LA levels) are high, they might inhibit the second time the enzyme is used in the metabolism, thus limiting the availability of the precursor to form DHA. [19, 21] In fact, demands for PUFAs, especially EPA and DHA, has been growing from both the food industry and pharmaceutical industry, where the goal is to use them as food supplements and to improve drugs, respectively. [6, 22, 23]

LA and ALA are found in oils from plants where they occur as triacylglycerols (TAGs). LA is found in large amounts in several oils, for example sunflower oil and soybean oil. ALA is the principal n-3 PUFA the western world obtains, but is less common in Nature than LA. It can, however, be found in large amounts in linseed oil and canola oil. [16, 24] ALA is easily absorbed in the intestine and efficiently secreted into the bloodstream. The body often seems to prefer it as an energy source and sends it to β-oxidation, rather than storing it in the adipose tissue. In fact only small amounts of it are found in phospholipids in plasma, cells and tissue. It is possible that the β-oxidation might be used in the body to regulate conversion of ALA to other PUFAs. As noted before, women have a higher conversion rate of ALA to other PUFAs than men. Women might also have slightly more ALA stored in their bodies at a given time due to a higher percentage of adipose tissue, 23% for women versus 15% for men. [17]

EPA and DHA are biosynthesised by the photosynthetic phytoplankton at the first trophic level and passed to fish through zooplankton. They are found in fish oil in TAG where they count for about 20% of the fatty acid content. [4, 23] The n-3 PUFAs are characteristic for marine fat and EPA and DHA are the most prevalent ones. [23, 25] The beneficial dietary effect of EPA and DHA on human health is considerable, as reviewed by Magnusson, they are good for cardiovascular and heart diseases, autoimmune diseases such as rheumatoid arthritis, inflammatory bowel diseases and psoriasis, and other inflammatory disorders such as asthma, and together EPA and DHA have overlapping actions in lowering blood lipid levels. [5, 19] EPA is especially good for various inflammatory disorders and DHA is important in pre- and post-natal nutrition and for brain and nervous system development. DHA has unique effect on cognitive health and in promoting normal brain function. [19, 22] They might even help various mental disorders such as schizophrenia, Alzheimer’s dementia and depression. [20, 25]
The n-3 DPA fatty acid has not been as intensively studied as EPA or DHA, but studies suggest it has beneficial health effects as well. DPA can be retro-converted back to EPA in a number of tissues, but is, as stated before, not easily metabolised to DHA. [17, 19] One of the processes that n-3 DPA seems to have effect on, or control over, is endothelial cell migration and proliferation, which are important processes in the control of wound-healing responses of blood vessels. In fact, it seems to have a greater ability to influence the cell migration than EPA, so during these processes EPA is elongated to DPA. [19] The order of effectiveness of these PUFAs, when dealing with an inflammatory response, was found to be DHA > n-3 DPA >> EPA. [19]

1.4 Lipases

In general it is desirable to speed up reactions by using catalysts, especially when dealing with unstable materials. Enzymes are natural catalysts. They have intrinsic chirality, which makes them useful when it comes to synthesis of complex compounds. They have chemo-, regio- and stereoselective properties, which makes them highly suitable when it comes to compounds with multiple chiral centres and/or functional chemical groups. They are effective at room temperature and work under mild conditions, which makes them convenient when dealing with unstable or delicate compounds. Immobilization of enzymes has made them even more competitive against traditional catalysts, it elongates their lifetime, makes them more stable and even reusable. Enzymes are now used widely in the chemical industry, both in the manufacturing processes and in the synthesis of complex compounds. [4, 16, 26, 27]

Lipases (triacylglycerol hydrolases, EC 3.1.1.3) are a group of hydrolytic enzymes whose natural function is to hydrolyse the ester bonds of triacylglycerols. They are without a doubt the most widely applied biocatalysts in synthetic chemistry. Lipases are both used for their hydrolysis properties and to catalyse the reverse ester formation. They are activated by means of an organic solvent or an oily phase, because their activation depends on the substrate forming a non-water-miscible micelles on the lipid water interface. Lipases exhibit a high substrate tolerance which means that they can accept structures that differ from their natural substrates. [4, 26, 27]

Lipases have been vastly applied to induce chirality and have been exploited to prepare many enantiomerically pure bioactive compounds. The Candida antarctica lipase (CAL B or CAL) is highly regioselective towards the 1,3-end of the glycerol backbone which results in good yields of entiomerically pure compounds. [28] This quality is highly useful when it comes to making or modifying TAG or DAGE, since CAL will be regioselective towards the less hindered primary alcohol sites. [6, 21] The 1-O-alkyl-sn-glycerols have also proven to be ideal substrates for lipases and, with the help of CAL, several types of structurally modified enantiomerically pure 1-O-alkyl-2,3-diacyl-sn-glycerols (DAGE) have been synthesised using the lipase to link an acyl chain to the sn-3-site followed by a chemical coupling of an acyl chain to the sn-2-site. [6, 22]

1.5 Structured lipids

TAGs that have a predetermined composition and distribution of the fatty acids at the glycerol backbone are often referred to as structured lipids. As noted before, there are four basic forms of the TAGs, AAA and ABA, that are achiral, and AAB and ABC, that are
chiral and can be found as racemates or as separated enantiomers. [29] Structured lipids are quite useful and can be used to enhance the nutritional value of a certain food or to help incorporate pharmaceuticals into the body. [4]

Lipases have been utilised to synthesise structured lipids. Their high regioselectivity towards the primary position of the glycerol moiety combined with the mild and neutral reaction condition they operate at has made them very useful. Those qualities suppress acyl-migration and enhance the regio-purity giving the desired compounds that are hard to make by conventional chemical synthesis. [26, 27]

In the body the sn-1,3-positions of TAGs are often rapidly absorbed and hydrolysed by a lipase in the digestion track rendering the free fatty acids that can be used immediately. The 2-MAG is absorbed as a whole and then used either as a free fatty acid, after being cleaved of the glycerol backbone, or by being incorporated into another molecule from which it can be released upon demand for its desired biological functions. [25, 30] This preference can be used as an advantage when it comes to developing pharmaceuticals and applying them. [4]

1.6 Acyl migration

Acyl migration is a spontaneous intramolecular rearrangement. [31] It is the transfer of an acyl group to an adjacent hydroxyl group within the same molecule, which causes a loss of regioselectivity and regiocontrol. Acyl-migration takes place with a transfer through a cyclic five membered hemiorthoester type intermediate, which results in a rearrangement of the acyl group. [25, 28, 32] Acyl migration may happen between the two forms of MAG, i.e. 1-MAG and 2-MAG, or between the two forms of DAG, i.e. 1,3-DAG and 1,2-DAG. These rearrangements are demonstrated in Scheme 2.

\[ \text{1-MAG} \rightleftharpoons \text{2-MAG} \]
\[ \text{1,3-DAG} \rightleftharpoons \text{1,2-DAG} \]

Scheme 2 Acyl migration (A) between 1-MAG and 2-MAG, and (B) between 1,3-DAG and 1,2-DAG.

Many factors influence the speed of acyl migration, for example temperature and acidity. When using lipases, the solvent type, water activity, reaction type and lipase support can also influence the speed or magnitude of the acyl migration. [31, 33, 34] The acyl-chain length can also play a role, but longer chains are less prone to acyl migration. [6] The equilibrium between the differently sited monoacylglycerols and diacylglycerols, i.e. between 1-MAG and 2-MAG and between 1,2-DAG and 1,3-DAG, also plays a role in the rate of acyl-migration. But the equilibrium ratio is roughly 9:1 for 1(3)-MAG against 2-MAG and 7:3 for the corresponding 1,3-DAG against 1(3),2-DAG. [33, 34]
2 Results and discussion

The project goals were twofold. The goal of the first part was to construct enantiopure diacyl glyceryl ethers comprising polyunsaturated fatty acids. The synthesis consisted of activation of starting materials, construction of intermediate compounds and full synthesis of the DAGEs made of three different alkylglycerols and two different polyunsaturated fatty acids. The goal of the second part was the total synthesis of two polyunsaturated fatty acids, α-linolenic acid and docosapentaenoic acid. The complete fatty acid synthesis involved a series of small reactions, owing to complications during their synthesis. Following are detailed descriptions of the two projects, their synthetic routes and of the compounds.

2.1 Part one: synthesis of enantiopure diacyl glyceryl ethers comprising polyunsaturated fatty acids

As noted in the introduction, ether lipids of the 1-O-alkyl-2,3-diacyl-sn-glycerol type (DAGE), made of pure saturated or monounsaturated 1-O-alkyl-sn-glycerols and long chain n-3 polyunsaturated fatty acids (PUFAs) are compounds of interest since the parts they are comprised of are considered biologically active.

Twelve different types of 1-O-alkyl-2,3-diacyl-sn-glycerols can be made from enantiopure chimyl (16:0), batyl (18:0) and selachyl (18:1) alcohols and two different PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Thus, there are in total six adducts of the ALL type (alkyl-long-long), comprising the same PUFA in both positions, and six adducts of the ALL’ type, comprising different PUFAs in the mid and end positions. A synthetic route for the twelve different compounds is shown in Scheme 3.

In the first part of the synthesis an immobilized Candida antarctica lipase (CAL) was used for the acylation of the primary hydroxyl group at the end position, of an alkyl glycerol backbone, using an acetoxime ester of the corresponding PUFA. Then, in the second part, the mid position was esterified, using either the same or the other PUFA, with chemical acylation using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) as a coupling agent and 4-dimethylaminopyridine (DMAP) serving as a catalyst and a base. [22, 28]
For further clarity for the compound numering, see Table 1 and 2.

Following is a detailed description of the synthesis of the twelve adducts defined above, starting with the synthesis of the starting materials leading up to the more complicated synthesis of the six 1-O-alkylacyl-sn-glycerols and finally ending with the twelve 1-O-alkyl-2,3-diacyl-sn-glycerols.

2.1.1 The alkyl glycerol backbones

The three ether lipids employed in the synthesis can be formed by reacting enantiomerically pure (S)-solketal ((S)-2,3-O-Isopropylidene-sn-glycerol) with stearyl, palmityl and oleyl bromides. This affords the corresponding chimyl (16:0), batyl (18:0) and selachyl (18:1) alcohols, which are both enantiopure and of the natural sn-1 configuration. Their general synthesis of the alkyl glycerols is shown in Scheme 4, and subsequently a description of the synthesis of selachyl alcohol.

Scheme 4 Synthesis of the three 1-O-alkylglycerols, (S)-chimyl, (S)-batyl and (S)-selachyl alcohols. The R-group represents palmityl (C16:0), stearyl (C18:0) and oleyl (C18:1) alkyl chains. Reaction conditions were as follows: (a) KOH, TBAB at 35-40°C, and (b) p-TsOH in H₂O-THF and reflux.

The synthesis for the three 1-O-alkylglycerols was conducted in the same manner, with the only difference being the alkylbromide starting materials, i.e. the stearyl, palmityl and oleyl bromides. [6, 22] The oleyl bromide (cis-1-bromooctadec-9-ene) was prepared, prior to the synthesis of selachyl alcohol, by brominating an oleyl alcohol (cis-octadec-9-en-1-ol). The bromination was accomplished by dissolving and cooling triphenylphosphine and carbon-tetrabromide in dichloromethane to 0°C under inert conditions, and then adding the
oleyl bromide and stirring the resulting mixture for 2 hours. The solution was then petrified using hexane and passed through a silica gel affording the product, after removal of solvents, as a yellow oil in excellent yields (97%).

The oleyl bromide could then be reacted with the enantiomerically pure (S)-solketal in the presence of freshly ground potassium hydroxide and catalytic amount of tetra-n-butylammonium bromide (TBAB) without any solvent. The mixture was stirred at 35°C overnight under inert conditions, quenched with water and the intermediate product extracted. TBAB is essential for the reaction. It enables the reaction by working as a phase transfer catalyst by replacing the bromide ion for the hydroxide ion, the reaction does not occur without it. Maintaining the temperature at 35°C is also essential, as it both speeds up the substitution and ensures that the alkyl bromide stays in a liquid form. [4] The intermediate product was not isolated before undergoing the hydrolytic deprotection, where it was refluxed in slightly acidic conditions, using catalytic amounts of p-toluenesulfonic acid dissolved in water and tetrahydrofuran (THF), at inert conditions, overnight. After aqueous workup and purification on a silica gel column, the product was afforded as a clear oil (67% yield).

2.1.2 Activation of EPA and DHA

The synthetic route starts off with an introduction of the PUFAs to the alkyl-glycerol backbone using a lipase reaction. CAL has shown superb regioselectivity towards the end positions of both glycerol and the glycerol backbone of alkylglycerols and is therefore an ideal choice for preserving the stereo-chemistry of the compounds. CAL also accommodates the intrinsic n-3 PUFA structures very well into its catalytic site. In order to speed up the reaction whilst, at the same time, minimising the chance of acyl migration, EPA and DHA were activated by turning them into acetoxime esters (AO). [22]

Thus, the first step of the overall synthesis was the activation of free fatty acids (FFA) of EPA and DHA into acetoxime esters, making them desirable and effective substrates for CAL. In order to obtain the required free fatty acids, eicosapentaenoic and docosahexaenoic ethyl esters were cleaved into their respective free fatty acids using basic hydrolysis. [35] See Scheme 5.

![Scheme 5](image)

Scheme 5 Hydrolysis of ethyl esters of EPA and DHA into their respective to FFA, and their activation to acetoxime esters. PUFA stands for EPA and DHA. Reaction conditions were as follows: (a) NaOH, Na$_2$EDTA in H$_2$O-EtOH and reflux, (b) AO, EDAC, DMAP in CH$_2$Cl$_2$.

The FFA were obtained by refluxing the ethyl esters and hydrolysing their ester bonds. This was done using a mixture of freshly ground sodium hydroxide pellets dissolved in water/ethanol blend (1:1), with minute quantities of the sodium EDTA ligand. The mixture was refluxed for 2 hours under nitrogenous atmosphere, followed by aqueous-workup after neutralisation of the basic reaction mixture, which afforded the FFA as pure compounds, both yellowish oils in excellent yields, 95% and 93% for EPA and DHA, respectively.
The free fatty acids were then converted into their corresponding acetoxime esters using a coupling reaction. One of the gains of activating the FFA with acetoximes is that the coupling reaction occurs under mild reaction conditions which preserves the polyunsaturated chains. Vinyl esters of saturated fatty acids have previously been used very successfully with CAL in esterification of 1-0-alkylglycerols. They are however not a feasible option when it comes to activating PUFAs. This is because their formation is conducted at high temperature and makes use of metals, which could cause coordination of the π-orbitals of the double bonds which in turn could increase the likelihood of cis/trans isomerisation and possibly double bond migration. This gives the acetoxime esters an edge over the vinyl esters, and is the reason why they are chosen to activate the PUFAs. [22]

In the reaction, the respective free fatty acids were dissolved in dichloromethane along with the coupling agent EDAC and in the presence of DMAP, which acted both as a base and a catalyst. The acetoxime was then added slowly to the mixture and the resulting solution stirred for 4 hours under nitrogenous atmosphere. Upon completion of the reaction the crude mixture was passed through a silica gel column, as a method of purification, and resulted in pure eicosapentaenoic acid and docosahexaenoic acid acetoxime esters, both in 84% yield. [6, 22] The proportions between EDAC, DMAP and the starting materials were altered slightly in the favour of the coupling agent and the base due to the age of the compounds.

2.1.3 The lipase reaction

The next step was to acylate the 1-O-alkyl-sn-glycerols, (S)-1-3, with EPA and DHA using the acetoxime esters of the PUFAs and CAL, via an enzymatic reaction. As noted before, six different types of 1-O-alkyl-3-acyl-sn-glycerols or monoacyl glyceryl ethers (MAGEs) can be made from the enantiopure chimyl (16:0), batyl (18:0) and selachyl (18:1) alcohols and the two different PUFAs occupying the end position of the glycerol backbones.

Two different but similar approaches, which were used to make the six MAGE adducts, are described below, one approach for the fully saturated alkylglycerol backbones (1 and 2) and another for the monounsaturated one (3). In both cases molecular sieves were used to improve the reactions, because whilst the reaction between the alkylglycerol backbone and the acetoxime esters provides an almost irreversible system, the molecular sieves push them towards completion by removing some of the released acetoxime. This is demonstrated in Scheme 6.

![Scheme 6 The lipase reaction. Acylation of 1-O-alkyl-sn-glycerols to 1-O-alkyl-3-acyl-sn-glycerols via lipase reaction using EPA and DHA acetoxime esters. The order of the glycerol backbones are as follows: Chimyl 1, 4 and 7; batyl 5, 7, and 8; and selachyl 3, 6 and 9. Compounds 4-6 were acylated with EPA and compounds 7-9 with DHA.](image-url)
Compounds 4, 5, 7 and 8 were made by adding an excess of the respective PUFAs to a mixture of the saturated alkylglycerols, the immobilised lipase and the molecular sieves in dichloromethane. The mixtures were stirred for 3-4 hours under nitrogen at room temperature. Upon completion the lipases were removed and the compounds crystallized, affording the pure products in very high to excellent yields (Table 1). Compounds 6 and 9 were made without any solvent, under vacuum (10⁻² mmHg), using the monounsaturated alkylglycerol, 3, the immobilised lipase and the respective acetoxime esters in low excess. The mixture was heated to 30°C and stirred for 2 hours or until the reaction was completed. The heating pushed the reaction towards completion by causing the acetoxime leaving group to evaporate, its boiling point is 135°C at 1 atm which makes it volatile at 10⁻² Torr. The crude mixture was then purified on a basic silica gel column resulting in pure products afforded in very high yields (Table 1). [6, 22]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Alkyl</th>
<th>PUFA</th>
<th>Yields (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-4</td>
<td>-C₁₆H₃₃</td>
<td>EPA</td>
<td>89</td>
</tr>
<tr>
<td>(R)-5</td>
<td>-C₁₈H₃₇</td>
<td>EPA</td>
<td>85</td>
</tr>
<tr>
<td>(R)-6</td>
<td>-C₁₈H₃₅</td>
<td>EPA</td>
<td>86</td>
</tr>
<tr>
<td>(R)-7</td>
<td>-C₁₆H₃₃</td>
<td>DHA</td>
<td>89</td>
</tr>
<tr>
<td>(R)-8</td>
<td>-C₁₈H₃₇</td>
<td>DHA</td>
<td>86</td>
</tr>
<tr>
<td>(R)-9</td>
<td>-C₁₈H₃₅</td>
<td>DHA</td>
<td>91</td>
</tr>
</tbody>
</table>

The incorporation of EPA and DHA to the end positions of the alkylglycerol backbones was successful because of good control of the lipase reactions. This was done by making the reaction faster by activating the PUFAs, which kept the mid-positions of the alkylglycerols clean of any acyl migration impurities. The yields reported in Table 1 were not maximised since the compounds had previously been synthesised and characterised, making them slightly lower than those previously reported.[6]

As a further demonstration for CAL preference to the end position of glycerol and alkylglycerol backbones, CAL was employed to incorporate two equivalents of the same PUFA to a glycerol backbone. This was done using glycerol, acetoxime ester of the corresponding PUFA and CAL in dichloromethane, under inert conditions at 0-4°C. The resulting mixture was stirred for 4 hours before the lipase was filtered away and the product purified on a 4% boric acid impregnated silica gel column, affording the product as a clear oil (82% and 79% yield for EPA and DHA, respectively). The conditions were kept at this low temperature to avoid acyl migration and prevent impurities in the mid position of the products. This shows the selectivity of CAL towards the end positions as well as demonstrating a good control of the lipase reaction using mild reaction conditions. [22]

### 2.1.4 The coupling reaction

The esterification of the mid-position was carried out after the successful incorporation of EPA and DHA to the end positions of the alkylglycerol backbones. As noted before, twelve different types of DAGEs can be made by adding a second PUFA to the six 1-O-alkyl-3-acyl-sn-glycerols, 4-9, i.e. six compounds with the same PUFA occupying both
positions and six compounds with different PUFAs in the sn-2 and sn-3 positions. See Scheme 7.

\[
\begin{align*}
\text{O-R} & = \text{b - a} \\
\text{O-O} & = \text{P} - \text{U} - \text{A} \\
\text{O-O} & = \text{P} - \text{U} - \text{A} \\
\text{O-O} & = \text{P} - \text{U} - \text{A} \\
\text{O-O} & = \text{P} - \text{U} - \text{A} \\
\text{O-O} & = \text{P} - \text{U} - \text{A} \\
\text{O-O} & = \text{P} - \text{U} - \text{A} \\
\text{O-O} & = \text{P} - \text{U} - \text{A} \\
\text{O-O} & = \text{P} - \text{U} - \text{A} \\
\text{O-O} & = \text{P} - \text{U} - \text{A} \\
\end{align*}
\]

\[\text{PUFA} + \text{PUFA'} \rightarrow \text{PUFA} \]

\(\text{PUFA'} = \text{EPA (a)}\)
\(\text{PUFA'} = \text{DHA (b)}\)

\(\text{(R)-4; 7}\)
\(\text{(R)-5; 8}\)
\(\text{(R)-6; 9}\)

Scheme 7 Introduction of PUFA to the mid position of the glycerol backbone. Compounds 4-6 are comprised of the alkylglycerols and EPA but 7-9 of DHA. Reaction circumstances: (a) EDAC, DMAP in CHCl₃.

The esterification of the MAGEs (1-O-acyl-3-acyl-sn-glycerols) was accomplished by introducing EPA and DHA, in the form of FFA, to the mid-position with chemical coupling using the coupling agent EDAC and in the presence of DMAP. In some of the cases the proportions between the materials were altered slightly. This was done to push the reactions towards completion, but EDAC and DMAP were not fresh so in some cases a higher ratio was needed. The coupling reaction was conducted in dichloromethane under nitrogen atmosphere. The reaction took a considerably longer time than the previous coupling reactions (approximately 16 hours). This is due to the rate of the coupling reaction being slower when the reaction site is situated next to the ether bond of the alkylglycerols rather than next to an ester bond. The compounds were purified with silica gel column chromatography, affording pure compounds 4a-9a and 4b-9b as yellowish oils in very high to excellent yields. [6, 22, 25] This is shown in Table 2 along with the specific optical activity for each of the products.

Table 2 The DAGEs (compounds 4a-9a and 4b-9b), their respective yields and optical activity measurements. The order of DAGEs is as follows: chimyl-derivatives with EPA at sn-3 (4a and 4b) and DHA at sn-3 (7a and 7b), batyl-derivatives with EPA at sn-3 (5a and 5b) and DHA at sn-3 (8a and 8b), and selachyl-derivatives with EPA at sn-3 (6a and 6b) and DHA at sn-3 (9a and 9b).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Alkyl, R</th>
<th>PUFA at sn-2</th>
<th>PUFA at sn-3</th>
<th>Yields (%)</th>
<th>([\alpha]_D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>-C₁₆H₃₃</td>
<td>EPA</td>
<td>EPA</td>
<td>93</td>
<td>-6.5</td>
</tr>
<tr>
<td>4b</td>
<td>-C₁₆H₃₃</td>
<td>DHA</td>
<td>EPA</td>
<td>86</td>
<td>-6.4</td>
</tr>
<tr>
<td>7b</td>
<td>-C₁₆H₃₃</td>
<td>DHA</td>
<td>DHA</td>
<td>87</td>
<td>-6.7</td>
</tr>
<tr>
<td>7a</td>
<td>-C₁₆H₃₃</td>
<td>EPA</td>
<td>DHA</td>
<td>87</td>
<td>-6.1</td>
</tr>
<tr>
<td>5a</td>
<td>-C₁₈H₃₇</td>
<td>EPA</td>
<td>EPA</td>
<td>92</td>
<td>-6.6</td>
</tr>
<tr>
<td>5b</td>
<td>-C₁₈H₃₇</td>
<td>DHA</td>
<td>EPA</td>
<td>87</td>
<td>-6.1</td>
</tr>
<tr>
<td>8b</td>
<td>-C₁₈H₃₇</td>
<td>DHA</td>
<td>DHA</td>
<td>89</td>
<td>-6.8</td>
</tr>
<tr>
<td>8a</td>
<td>-C₁₈H₃₇</td>
<td>EPA</td>
<td>DHA</td>
<td>88</td>
<td>-6.3</td>
</tr>
<tr>
<td>6a</td>
<td>-C₁₈H₃₅</td>
<td>EPA</td>
<td>EPA</td>
<td>87</td>
<td>-6.4</td>
</tr>
<tr>
<td>6b</td>
<td>-C₁₈H₃₅</td>
<td>DHA</td>
<td>EPA</td>
<td>92</td>
<td>-6.5</td>
</tr>
<tr>
<td>9b</td>
<td>-C₁₈H₃₅</td>
<td>DHA</td>
<td>DHA</td>
<td>87</td>
<td>-6.4</td>
</tr>
<tr>
<td>9a</td>
<td>-C₁₈H₃₅</td>
<td>EPA</td>
<td>DHA</td>
<td>86</td>
<td>-6.3</td>
</tr>
</tbody>
</table>
The incorporation of EPA and DHA to the mid positions of the alkylglycerol backbones was very successful and resulted in very good to excellent yields of the products. The optical activity measurements for the aforementioned compounds fell within a small range, between -6.1 and -6.8, which suggest similarities between the compounds. It is possible that a correlation exists between the position of the PUFAs, that is whether they are positioned in the mid or the end position, and the optical activity values, but without a bigger sample size it is hard to draw such conclusions. For example, the values for two DHAs seem to be higher than those for two EPAs but it is hard to see if it is associated with the mid or the end positions of the compounds, when compared to those comprised of both PUFAs, or perhaps a mixture of the two. The values are, however, in a narrower range and slightly higher than those obtained from compounds comprised of the three alkylglycerol backbones, a PUFA in the mid position and a short chain saturated fatty acid in the end position.[6]

The coupling reaction employed above is very effective when it comes to the merger of the PUFAs and the alkylglycerol backbones. In fact, it can be used to produce DAGEs, comprised of the same PUFA in both positions, without employing the intermediate products (MAGEs). When these DAGEs are synthesised, that is when a reaction is used to combine the alkylglycerols with two equivalents of one PUFA, there is no need to ensure that no acyl-migration takes place. This makes the coupling reaction highly beneficial as it eliminates the intermediate products, shortening the synthetic route and in the end resulting in better overall yields. This method was applied to several of the compounds that consisted of the same PUFA in both the mid and end positions of the alkylglycerol backbones, i.e. compounds 4a, 5a and 6a composed of two EPAs, and 9b composed of two DHAs.

2.1.5 Summary of part one

The structures of the starting materials and the intermediate compounds in part one were all confirmed by $^1$H-NMR analysis. The three ether lipids, the EPA and DHA free fatty acids and their corresponding acetoxime esters and the monoacyl glycerols had all previously been made, so only proton NMR was used to identify them and establish their purity. These compounds only served as intermediates in the synthesis of the DAGEs so the yields were not optimised to their full extent. In the case of the DAGEs the yields were fully optimised. The structures and purity of the DAGEs were confirmed by $^1$H-NMR and $^{13}$C-NMR analysis, their exact mass measured and authenticated using high resonance mass spectrometry (HRMS) and known vibrational signals of the structure confirmed by infrared spectroscopy (IR). Finally, their specific optical activity, which they have due to the chirality of carbon in the mid position of the compounds, was measured with polarisation measurements [$\alpha$]D.

2.2 Part two: Synthesis of the polyunsaturated fatty acids $\alpha$-linolenic acid (ALA) and docosapentaenoic acid (DPA)

The goal of the second part of the project was to synthesise the polyunsaturated fatty acids ALA (C18:3n-3) and DPA (22:5n-3). The two PUFAs are compounds of interest, as mentioned before, and have various biological effects and partake in many different biological pathways. The structures of ALA and DPA are quite similar, with both of them
having a relatively long alkyl chain separating the carboxyl acid end from the polyunsaturated omega-3 tail. See Figure 8. This similarity means they can be synthesised using almost identical protocols, which simplifies their retrosynthetic analysis and, of course, their total synthesis.

![Figure 8 Comparison of the structures of ALA and DPA. (A) ALA is C18:3n-3 and (B) DPA is C22:5n-3.](image)

As their classification as PUFAs suggests, both ALA and DPA have multiple double bonds, which have a fixed cis-configuration. This orientation needs to be taken into account during their total synthesis, where the incorporation of the double bonds, with the right stereochemistry, could become troublesome. One way of tackling the problem would be to synthesise the long unsaturated n-3 tail separately, and bypass complications related to the cis-trans configuration of the double bonds by using triple bonds in the synthesis. The compounds could then be partially hydrogenated to achieve the desired structure and orientation of the double bonds.

### 2.2.1 Retrosynthetic analysis

The ideal approach for synthesis of the two PUFAs would be to create the head parts and the polyyne tail parts in two separate syntheses and to combine them at a later point. This idea is demonstrated in the complete retrosynthetic analysis of the head part of ALA in Scheme 8 and of DPA in Scheme 9, and in Scheme 10 and Scheme 11 which shows retrosynthetic analysis of the polyunsaturated tails of the PUFAs.

Scheme 8 shows the ideal retrosynthetic analysis of the head part of the fatty acid ALA. ALA has three cis-configurated double bonds, and in order to keep or regain the stereochemistry, the first step of the retrosynthesis requires their preservation, the easiest way being simply to turn them into triple bonds. Another requirement is to ensure the carboxyl acid end does not interfere with the chain synthesis by deactivating it with protection which prevents interference. The unsaturated chain could then be incorporated into the synthesis via coupling as triple bonds. Thus, for the second step, the ideal approach would be to cleave off the long triple bond acyl chain and synthesise it separately. Further retro-synthetic analysis of the diyne chain can be found below and seen in Scheme 10. The third step would be to incorporate the acetylene triple bond, which again would need to be protected in order to prevent polymerisation.
Scheme 8 Retrosynthetic analysis of ALA. Analysis of the tail part can be found in Scheme 10.

The last step of the retro-synthesis of the head part would be the addition of the carboxyl acid protecting group. The retrosynthetic analysis for DPA is identical to the one for ALA apart from changing the starting material and the polyunsaturated tail. See Scheme 9.
Scheme 9 Retrosynthetic analysis of DPA. Analysis of the tail part can be found in Scheme 11.

The retrosynthetic analysis for DPA is an analogue to the one given for ALA. In the first step the double bonds are converted into triple bonds to preserve the right stereochemistry and the carboxyl acid end is protected. The second step handles the incorporation of the polyyne chain via coupling, so the retro synthetic analysis cleaves the tetrayne from the head of the fatty acid. For further retro-synthesis of the tetrayne chain see Scheme 11. The third step would be the incorporation of the protected acetylene triple bond and the last step the addition of the carboxyl acid protecting group.

This summarises the total retrosynthetic analysis for the head parts of ALA and DPA, aside from the polyyne tails. As noted before, the problem concerning the all-cis configuration of the polyunsaturated chains may be avoided by using triple bonds. This prevents the undesired trans-configuration of the double bonds, since the triple bonds can undergo
partial hydrogenation in the final stages of the total synthesis, providing the all-cis configuration.

The two fatty acids have a different degree of unsaturation, with ALA having three double bonds while DPA has five. This makes the synthesis of ALAs tail relatively simple when compared to the synthesis of the DPA tail. The two polyyne tails can be made using a series of copper mediated coupling reactions, so the following retro-synthetic analysis shows how their tails can be cleaved into convenient pieces for the coupling reactions for each PUFA. The diyne used in the synthesis of ALA, retrosynthesised in Scheme 10, is a component of the more complicated synthesis of the tetryne used for DPA, retrosynthesised in Scheme 11.

\[
\text{Br} \quad \text{OH} \quad \text{Br} \quad \text{OH} \quad + \\
\text{11} \quad \text{10} \quad \text{Br}^+ \quad \text{OH}
\]

Scheme 10 Retrosynthetic analysis of diyne, 11.

The synthesis of ALA makes use of 11 (1-bromo-octa-2,5-diyne). The diyne needs to be brominated to undergo a coupling reaction with the propargyl end of the OBO protected acyl chain. The untreated/simple diyne could furthermore be disconnected to form commercially available monoyne parts.

The retrosynthesis of the polyunsaturated tail of DPA is considerably longer, as can be seen in scheme 11, and starts with 15 (1-bromotetradeca-2,5,8,11-tetryne). The tetryne, needed for the DPA synthesis, can be disconnected into two diynes, 13 (hexa-2,5-diyn-1-ol) and 11 (1-bromo-octa-2,5-diyne), with the latter being the tail needed for ALA. The same applies as before, the diyne can be further disconnected into two monoyns. The terminal alkyne of 13 (hexa-2,5-diyn-1-ol) would need protection in order to avoid polymerisation, for example using a tetramethyilsilane (TMS) group, before it could be disconnected into the two simple monoyne parts.
Following is a description of the synthetic routes that correspond to the two fatty acids retrosynthesised above, first for the ALA tail then for the DPA tail, and followed by a description of the complete synthesis of ALA and DPA.

As noted before, ALA is the simpler of the two with only three double bonds while DPA has five double bonds. The synthesis of the latter builds on the synthesis of the first, with the polyunsaturated tail needed for ALA, being used in the longer and more complicated tail of DPA and the synthesis of the two head parts being almost identical.

2.2.2 Synthesis of the polyunsaturated acyl tail

The approach used to synthesise the fatty acids' polyunsaturated n-3 end entailed condensation of terminal acetylenes with propargyl halides. The cross-coupling used for this purpose is generally conducted at room temperature under inert gas and requires the presence of copper (I) iodide, sodium iodide and potassium carbonate or, in the case of the longer, more delicate chains, caesium carbonate. [36-38] Other reactions required for the
tail synthesis are activation of the compounds for the coupling reaction, i.e., removal of the trimethylsilyl propargylic (TMS) protection group to create the terminal acetylene, and halogenation (bromination) of the alcohol groups to create the propargyl halides needed for the reaction. [39, 40].

The compound, 11 (1-bromoocta-2,5-diyne), was used in the synthesis of ALA and DPA. Firstly as the main polyyne chain in the case of ALA, where it was coupled to the head part of the ALA fatty acid. Secondly as a component of the longer polyyne chain in the case of DPA, where it was first coupled to another diyne to form tetrayne, before being made suitable for another coupling to the head part of DPA fatty acid. The synthesis of 11 is shown in Scheme 12.

**Scheme 12** Synthesis of the diyne used for ALA. Reaction circumstances: (a) CuI, NaI, K$_2$CO$_3$ in DMF, (b) PPh$_3$, CBr$_4$ in CH$_2$Cl$_2$ at $-15^\circ$C to rt.

The synthesis of 11, began with the merger of a terminal acetylene and a propargyl halide via coupling with copper (I) iodide, sodium iodide and potassium carbonate, using dimethylformamide as a solvent at room temperature under inert conditions. The overnight reaction resulted in a reddish oil, after aqueous workup, which was further purified using chromatography. The pure product 10 was afforded as an orange oil in good yield (83%). [36-38] The initial reaction was then followed by halogenation to form a suitable compound for another coupling reaction, either to the head part of ALA or to another diyne 13, to form tetrayne 15 in the case of DPA. In the halogenation, triphenyl phosphine and carbon tetrabromide were dissolved in dichloromethane under inert conditions and stirred vigorously at 0°C for 15 minutes before the dialkyne was added to the solution. This was done to ensure that the right reaction would take place. The mixture was then stirred for an hour at the same temperature before it was slowly allowed to reach room temperature. The product 11 was afforded as a yellowish oil, after chromatographic purification, in good yield (80%). [40]

The synthesis of the polyyne chain used for DPA, was slightly more complicated, due to it having more double bonds and thus many more steps to the synthesis. In the synthesis of the tetrayne, two diyne were coupled to together to form the desired compound. The first one is shown above, in scheme 12, and the second one is shown below, in Scheme 13.
The synthesis of the second diyne 13 (hexa-2,5-diyne-1-ol), began with the same type of copper mediated coupling reaction as was used for 11, where a trimethylsilyl-protected propargyl bromide was combined with a terminal acetylene. A TMS protected alkyl halide was chosen for the reaction to prevent unwanted polymerisation. [36] The overnight reaction resulted in a pure reddish oil, 12, in excellent yields (96%), which needed no further purification. [36-38] The product’s terminal acetylene end was then deprotected to form appropriate material for further coupling reactions. The deprotection was executed in an overnight reaction, using silver nitrate in a mixture of solvents (methanol, water and dichloromethane) at room temperature. The product, 13 (hexa-2,5-diyne-1-ol), formed as a dark orange oil, after aqueous-work up and chromatographic purification, in moderate yield (47%). [39]

Finally the two diynes, 11 (1-bromoocta-2,5-diyne) and 13 (hexa-2,5-diyne-1-ol), were combined to form the tetrayne 15 needed for the synthesis of DPA, using a similar type of coupling reaction as before, followed by bromination. See Scheme 14.
replacing potassium carbonate with caesium carbonate. The latter is a highly soluble base which offers even better reaction conditions in the sensitive synthesis, and ensures that the multiple triple bonds are left undamaged through the coupling process. The product 14 was afforded as an orange to brown oil in moderate yield (44%). The yields were lower than those previously obtained for the synthesis of the tetrayne (76%), because they were affected by a loss of starting material during the set up and loss of product in last step of the synthesis due to its sensitivity towards direct light and oxidation. [36-38] The compound was then brominated using the same method as before, i.e. using PPh₃ and CBr₄, and resulted in the product 15 as a yellow oil after purification in adequate yield (64%). [40]

2.2.3 Synthesis of the PUFA head groups

The overall synthesis of the head parts of the two PUFAs is almost identical. The first course of action was to disable the carboxyl acid end of the starting material. This was done by using a protection group, thus disabling the function of the carboxyl acid and limiting any problems that might arise during the rest of the synthesis. This was followed by an alkylation with a protected compound, and its deprotection, to prepare the material for a copper mediated coupling reaction of the acyl tail, producing the polyyne compounds with a protected carboxyl acid end. This product would in the end be partially hydrogenated and de-protected to give the desired PUFA.

Synthesis of the ALA head group

The first part of the fatty acid head group synthesis involved the protection of the carboxyl acid. This was done by converting it into an oxybicyclo[2,2,2]octyl (OBO) orthoester through a two-step reaction. The OBO-group is highly useful in the synthesis since it is stable towards strongly basic reagents as well as nucleophiles (such as Grignard) and can easily be removed, allowing for the carboxylic acid moity to be regenerated, under mildly acidic conditions. [41]

The OBO-group was created using a two-step process. The first step involved the creation of an oxetane ester and the second step was its conversion into the OBO-orthoester. The protection process of the starting material for ALA is shown in Scheme 15.

![Scheme 15](image)

Scheme 15 Synthesis of the OBO-protection group for ALA. Reaction conditions: (a) EDAC, DMAP in CH₂Cl₂, (b) BF₃*etherate in CH₂Cl₂ at -15°C.
The first step in making the OBO-group for ALA involved a coupling reaction between a 3-methyl-3-oxetanemethanol and 8-bromooctanoic acid, using EDAC as a coupling agent and DMAP as a catalytic base in dry dichloromethane. The reaction took place under inert conditions and was completed in only 3-4 hours. The afforded oxetane ester was then passed through silica gel to afford the pure compound 16 as colourless oil in excellent yield (98%). [28]

The creation of the OBO-group itself is driven on by the effects of BF$_3$ which, as a Lewis acid, causes the rearrangement of the oxetane ester and thus releases the ring angle strain in the four-membered ring. BF$_3$ provides these circumstances when it coordinates with the oxetane alcohol and adds to the ring strain, which pushes the compound towards heterolysis of the C-O bond, making the carbon more susceptible to a nucleophilic attack from the carbonyl ester. This creates a zwitterion, which collapses to give the isomeric-bridged OBO ester, aided by the gain in stability going from a four-membered to a six-membered ring. The mechanism is shown in Scheme 16. [41]

![Scheme 16 The mechanism of the creation of the OBO-protection group.](image)

In the second step, the OBO-group was created using the oxetane ester with BF$_3$-etherate in dichloromethane under inert conditions at -15°C. The reaction conditions were kept at such a low temperature to circumvent possible side reactions and to ensure that the OBO-group formation would be pushed towards completion. Carrying out the reaction at a higher temperature caused formation of unidentified side products that could not be easily separated from the desired product. Unfortunately, the reaction conditions slow the reaction rate considerably, from a couple of hours up to 10-12 hours. Upon completion the reaction was quenched with triethyl amine (TEA), which formed a complex with the BF$_3$, that was easily separated off by filtration. For purification the product was passed through a basic TEA impregnated (5%) silica gel column to keep the OBO-group intact, since its sensitivity to acidic conditions renders the slightly acidic silica gel useless. This afforded the OBO-protected compound 17 as clear oils in good yields (80%). [41]

With the carboxylic acid protected, preparation for the PUFA chain could be carried out at the other end of the compound. The next step in the synthesis was to introduce a terminal acetylene to the chain that could in turn be coupled to the polyunsaturated tail. This was done by replacing the terminal bromide of 17 with a trimethylsilyl (TMS)-protected acetylene using n-butyl lithium to aid the substitution, followed by a deprotection of the TMS-group. This is illustrated in Scheme 17.
Scheme 17 Acylation and de-protection of the OBO protected chain of ALA. Reaction conditions: (a) n-BuLi in HMPA and THF at -78°C, (b) K₂CO₃ in MeOH.

The organometallic reaction was conducted under inert conditions at -78°C to avoid unwanted side reactions. The reaction started with the removal of the terminal alkyne proton of the ethynyltrimethylsilane by n-BuLi in tetrahydrofuran (THF). [42-45] The reaction was aided by use of hexamethylphosphoramide (HMPA). HMPA coordinates extremely powerfully to the lithium atom, and subsequently solvates the cation, leaving the anion more reactive and making n-BuLi an even stronger base. [18] The mixture was stirred for 3 hours before adding the OBO-protected material, and stirred for a further 1 ½ hours at -78°C before being allowed to slowly reach room temperature. This was done, as noted before, in order to hinder undesirable side reactions that occur at a higher temperature, for example the butyl chain itself substituting the bromide instead of the ethynyltrimethylsilane. The reaction ended with a slightly basic water workup, affording the compound 18 as colourless to yellow oil in excellent yields (95%).

The TMS-protection group of 18 was then removed using basic conditions to leave the OBO-group unharmed, making the terminal acetylene susceptible for the final coupling reaction with the diyne to afford the desired triyne ALA is comprised of. This was done by mixing the 18 with freshly ground potassium carbonate dissolved in dry methanol and stirring the mixture under inert conditions for 4 hours. A slightly basic aqueous workup afforded the product 19 in excellent yields (89%). [46, 47]

Ideally the next step in the process would be to couple the OBO-protected head part to the corresponding polyyne chains, which in the case of ALA is the diyne, 11. This would be done via a copper mediated coupling similar to those described above for the polyyne chain. This would then be followed by the partial hydrogenation of the polyyne chains and finally removal of the OBO-protection group resulting in ALA as a free fatty acid. This is demonstrated in Scheme 18.

Attempts to use a coupling reaction to connect 19 and 11 failed to produce the desired product 20. The attempts were carried out using CuI, NaI and Cs₂CO₃ in DMF under inert conditions at room temperature or at a slightly higher temperature, i.e. 40°C. Neither condition gave the desired product in acceptable yields nor did they react to a completion, judging by analysis of the crude compounds. The experiments were performed on a small scale and only resulted in scant amounts of a crude compound. Purification proved to be
more difficult than expected, since amounts of the material seemed to get lost or damaged during the purification process, either in the mass amount of emulsion formed during aqueous workup or lost during chromatographic purification.

\[
\begin{align*}
\text{19} & \quad \text{+} \quad \text{Br} \quad \text{11} \\
\text{20} & \quad \text{b} \\
\text{21} & \quad \text{c} \\
\text{ALA} & 
\end{align*}
\]

Scheme 18 The final stages in the synthesis of ALA, starting up with a coupling reaction, followed by partial hydrogenation and removal of the OBO-protection group. Reaction circumstances: (a) CuI, NaI, CsCO\textsubscript{3} in DMF, (b) H\textsubscript{2}/Lindlar’s catalyst, quinoline in benzene, (c) deprotection using slightly acidic conditions.

To summarise: the compounds were either not pure enough or not in high enough yields for full diagnostics. The reaction could perhaps benefit from a different reaction temperature, a different method of purification or more freshly made starting materials alternatively another approach is needed to synthesise the desired product.

It is plausible that the problem regarding the coupling reaction lies with the coupling of a single triple bond to multiple triple bonds. If this is the case the problem could be circumvented by changing the starting materials, for example by changing the ratios of triple bonds between the two compounds. This could be achieved by elongating the alkyl chain of the head part with a triple bond, whilst simultaneously shortening its counterpart.

This alternative route to the originally proposed synthesis could be made by coupling a short protected propargyl bromide to the previously synthesised head part and working on elongation of the chain from there, by deprotecting and repeating as many times as needed. See Scheme 19.
Scheme 19 Alternative approach to the synthesis of ALA. Elongation of the chain by a triple bond. Reaction conditions: (a) CuI, NaI, CsCO₃ in DMF, (b) TBAF in THF at -78°C to -50°C.

The coupling reaction is similar to those above and couples the terminal acetylene of OBO-protected tail to a TMS-protected propargyl bromide using copper (I) iodide, sodium iodide and caesium carbonate in DMF. The reaction was carried out overnight and quenched with a basic aqueous solution (0.05 M NaHCO₃), followed by an aqueous workup that resulted in emulsion. The emulsion was dissolved using gravity filtration, but some of the material may have been lost during this process. The product 22 was purified on a basic alumina column and was afforded as light brown oil in moderate yields (52%).

The deprotection of the product was more complicated than the one used after the first triple bond, since the same conditions could not be used due to the risk of allene formation. To circumvent this, the compound, 22, was dissolved in THF under inert condition and cooled to -78°C. Then tetrabutyl ammonium fluoride (TBAF), also cooled to -78°C, was added slowly into the mixture and stirred for 6-7 hours while being cooled to between -50 to -78°C. The crude mixture was then passed through a cold basic alumina column using pet.ether/diethyl ether (1:1) that had been cooled to -15°C as an eluent, affording it as a dark crude product 23 in excellent yields (96%). The product 23 needs to be purified further, so it can be used in subsequent steps in the synthesis.

The ensuing step would be to introduce the third triple bond to the chain to complete the polyunsaturated chain of ALA, followed by its partial hydrogenation, using Lindlar’s catalyst, and removal of the OBO-group, using slightly acidic conditions.

Synthesis of the DPA head group

The synthesis of the DPA head group was almost identical to the one for ALA with the exception being that different starting material was used for the DPA alkyl chain. As before the first reaction involved the creation of the OBO-protection group using a two-step synthesis. See Scheme 20.
Scheme 20 Synthesis of the OBO-protection group for DPA. Reaction conditions: (a) EDAC, DMAP in CH₂Cl₂, (b) BF₃·etherate in CH₂Cl₂ at -15°C.

The first step was the coupling reaction between 3-methyl-3-oxetanemethanol and 6-bromohexanoic, with EDAC and DMAP in dry dichloromethane. As before the reaction was conducted under inert conditions and completed in only 3-4 hours. The afforded oxetane ester was then purified by passing it through a silica gel column, to afford the pure compound 24 as colourless oil in very good yields (86%). [28] The OBO-group was then created using BF₃·etherate in dichloromethane under inert conditions at -15°C for 10-12 hours. Upon completion the reaction was quenched with triethyl amine and the solution filtrated, to separate the complex it formed with BF₃, and then purified on basified silica gel. This afforded the OBO-protected compound 25 as clear oil in good yields (80%). [41]

With the carboxyl acid end OBO-protected, the chain, 25, could be can to a terminal acetylene to elongate it. The product can then be deprotected, to prepare it for further coupling reactions, i.e. to create the polyunsaturated tail. See Scheme 21.

Scheme 21 Acylation and de-protection of the OBO protected chain of DPA. Reaction conditions: (a) n-BuLi in HMPA and THF at -78°C, (b) K₂CO₃ in MeOH.

The coupling reaction required the removal of the terminal alkyne proton of the ethynyltrimethylsilane using n-BuLi in tetrahydrofuran (THF) and HMPA. [42-45] The organometallic reaction was carried out for 3 hours under inert conditions at -78°C, before slowly adding the OBO-protected alkyl chain, 25. That in turn was stirred for 1 ½ hours under the same conditions before being allowed to slowly reach room temperature overnight. The reaction ended with a slightly basic water workup, affording the compound 26 as colourless to yellow oil in excellent yields (95%).

The TMS group of 26 was then removed using basic conditions, leaving it susceptible for other coupling reactions. As before, this was accomplished by dissolving the compound in methanol along with freshly ground potassium carbonate and stirring the mixture under
inert conditions, for 4 hours. A slightly basic aqueous workup afforded the product 27 in excellent yields (94%). [46, 47]

The original idea was to combine this product with the tetrayne 15, described before, in a coupling reaction similar to those above to afford compound 28, the reaction would then be followed by partial hydrogenation and finally removal of the OBO-protection group to afford the DPA FFA. See Scheme 22.

Scheme 22 The final stages in the synthesis of DPA, starting up with a coupling reaction, followed by partial hydrogenation and removal of the OBO-protection group. Reaction circumstances: (a) CuI, NaI, CsCO₃ in DMF, (b) H₂/Lindlar’s catalyst, quinolin in benzene, (c) deprotection using slightly acidic conditions.

As noted before for ALA, attempts to couple a single triple bond with a polyyne chain have proven futile, resulting in only partial coupling of the materials. Similar conditions were attempted for DPA, using a coupling reaction on the terminal acetylene end of the OBO-protected head and its polyyne counterpart. The OBO-protected head and the tetrayne were mixed with CuI, NaI and Cs₂CO₃ in DMF under inert conditions at room temperature. The experiments were performed on a small scale and resulted only in small amounts of the desired compound to be found in the crude mixture, with most of the product lost during work-up or purification.

Given the results, it is likely that this approach will not result in DPA, neither in quality nor quantity, and that the most viable option to complete the synthesis is to use a similar approach as described before for ALA. This could be done by elongating the pre-existing terminal acetylene of the OBO-protected head with a protected propargyl bromide,
deprotecting the product and coupling it with the now shorter, triyne, counterpart. The full length chain could then be partially hydrogenated and finally the OBO-head removed, using slightly acidic conditions, resulting in the fully synthesised DPA free fatty acid.

In conclusion the total synthesis of the aforementioned fatty acids should be possible. In order to form the desired PUFA problems regarding the last coupling reactions need to be solved and the last step of the synthesis, i.e. hydrogenation and de-protection, to be carried out successfully.

### 2.2.4 Summary of part two

The structures and purity of all previously synthesised compounds were confirmed by $^1$H-NMR analysis, but not characterised further, or their yields fully optimised. This includes the parts used for the polyyne synthesis. The yields of the novel compounds created in this project, were on the other hand fully optimised and their structures and purity confirmed by both $^1$H-NMR and $^{13}$C-NMR analysis. This includes all the compounds made for synthesis of the head parts of ALA and DPA, with the exception of the crude compounds in the coupling reactions of the PUFAs terminal acetylene and their polyyne counterparts, and the two compound made in the alternative route in the synthesis of ALA, which were only analysed using $^1$H-NMR. The structure of the novel compounds were furthermore authenticated by measuring their exact mass using high resonance mass spectrometry (HRMS).
3 Materials and methods

The following text details which instruments were used to authenticate the products synthesised, and which compounds were used in the process. It also describes the methods carried out in the experiments and the full analysis of the synthesised products.

3.1 Materials and instruments

$^1$H and $^{13}$C nuclear magnetic resonance spectra were recorded on a Bruker Avance 400 spectrometer in deuterated chloroform as a solvent at 400.12 and 100.61 MHz, respectively. Chemical shifts (δ) are quoted in parts per million (ppm) and the coupling constants (J) in Hertz (Hz). The following abbreviations are used to describe the multiplicity in $^1$H-NMR: s, singlet; s (br), broad singlet; d, doublet; d (br), broad doublet; dd, doublet of doublets; t, triplet; t (br), broad triplet; dt, doublet of triplets; m, multiplet. Other multiplicities are written out in full form: quartet; quartet (br), broad quartet; quartet of triplets; quintet; quintet (br), broad quintet. The number of carbon nuclei behind each $^{13}$C signal is indicated in parentheses after each chemical shift value, when there is more than one carbon responsible for the peak. All Infrared spectra were conducted on a FT-IR (E.S.P.) Spectrophotometer on a ZnSe plate. The optical activities were measured on an Autopol V automatic Polarimeter from Rudolph Research Analytical, using a 40T-2.5-100-0.7 Temp Trol polarimetric cell. The high-resolution mass spectra (HRMS) were acquired on a Bruker microTOF-Q mass spectrometer equipped with E-spray atmospheric pressure ionization chamber (ESI). All chemicals and solvents were used without further purification unless otherwise stated. The immobilized Candida antarctica lipase (Novozym 435; CAL-B) was supplied as a gift from Novozyme A/S (Bagsvaerd, Denmark). EPA (98%) and DHA (≥95%) were obtained as ethyl esters from Pronova Biocare (Sandefjord, Norway) and were hydrolysed to their corresponding free acids. (S)-3-O-hexadecyl-sn-glycerol, (S)-3-O-octadecyl-sn-glycerol obtained from organic laboratory. [6] Glycerol (99%) purchased from Sigma Chemicals (St. Louis, Missouri, USA). (S)-Solketal, ((S)-1,2-O-isopropylidene-sn-glycerol; 98%, 99% ee); oleyl alcohol, technical grade; and commercial grade EDAC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimidemethylhydrochloride); tetrabromomethane (99%); triphenylphosphine (99%); ethylenediaminetetraacetic acid disodium salt dihydrate, reagent grade; along with 6-bromohexanoic acid (97%); 8-bromoocotanoic acid (97%); 3-bromo-1-(trimethylsilyl)-1-propyne (98%); 1-bromo-2-pentyne (97%); 3-methyl-3-oxetanemethanol (98%); n-buthyllithium, 2.0M in cyclohexane; cesium carbonate (99.9%); potassium carbonate, anhydrous (≥99.0%); sodium iodide (≥99.0%); ammonium chloride (≥99.5%); tetrabutyl ammonium fluoride (TBAF), 1.0 M in THF; and N,N-dimethylfromamide (DMF, 99.8%) were obtained from Sigma-Aldrich (Steinheim, Germany) as well as the following solvents in HPLC grade: chloroform; dichloromethane (dried over CaH₂); diethyl ether; ethanol; ethyl acetate; methanol; petroleum ether, boiling range 40-60 °C; and tetrahydrofuran (THF, dried over pressed Na in the presence of benzophenone). 4-dimethylaminopyridine (DMAP, 99%); acetone oxime (98%) and tetra-n-butylammonium bromide (TBAB, 99%); and molecular sieves (4 Å); along with ethynyltrimethylsilane (≥98%); propargylalcohol (99%); copper(I) iodide (98%); silver nitrate and silicagel (0.060-0.020 mm, 60Å) were
purchased from Acros Organics (Geel, Belgium). Triethylamine (TEA, ≥ 99.5%); and sodium bicarbonate purum were purchased Fluka and Sodium hydroxide pellets from Riedel-de Haén. Boron trifluoride etherate (~50%); p-toluenesulfonic acid monohydrate and potassium hydroxide were purchased from Merck (München or Darmstad, Germany) along with pro analysis benzene and hexane. Hexamethylphosphoramide (HMPA), obtained from the Inorganic chemistry laboratory (dried over Li, stored over molecular sieves). Finally silica gel (230-400 mesh, 60 Å); SiliaFlash (F60, 40-63 µm) and preparative TLC plates (250 µm, F-254) were obtained from Silicycle (Quebec, Canada).

3.2 Experimental

The methods employed in the two projects are described below, beginning with the synthesis of enantiopure diacyl glyceryl ethers, and followed by the total synthesis of ALA and DPA. In both cases, the experiments conducted are thoroughly described, along with an analysis of the resulting products.

3.2.1 Synthesis of enantiopure diacyl glyceryl ethers comprising polyunsaturated fatty acids

The synthesis of the twelve adducts of the 1-O-alkyl-2,3-diacyl-sn-glycerol type, consisted of starting materials, intermediate compounds and the twelve final products. The reactions used in the synthesis are described in detail, with 1H-NMR analysis included, confirming the structures and purity of the compounds. Since the starting materials and the intermediate compounds had all previously been synthesised, no further validation was required. The twelve 1-O-alkyl-2,3-diacyl-sn-glycerol intermediates were additionally analysed using 13C-NMR and HRMS, confirming their exact mass, and their polarisation measured [α]D, along with their IR-vibration. The order of the compounds is as follows: the starting materials, which are oleyl bromide, selachyl alcohol, and EPA and DHA both as the free fatty acids and their acetoxime ester analogues; the six 1-O-alkyl-3-acyl-sn-glycerol intermediate compounds; the twelve adducts of the 1-O-alkyl-2,3-diacyl-sn-glycerol type and finally the two diacylglycerols, synthesised to display the preference CAL has for end-positions.

Synthesis of the starting materials

cis-1-bromooc-tadec-9-ene.
A solution of triphenylphosphine (PPh₃) (7.62 g, 29 mmol) and carbontetrabromide (CBr₄) (4.19 g, 14.5 mmol) dissolved in dichloromethane (100 mL) was cooled to 0°C and stirred for 15 minutes under nitrogen atmosphere. Next, Cis-octadec-9-en-1-ol (3.00 g, 11.2 mmol) dissolved in dichloromethane (50 mL) was added, and the resulting mixture stirred at 0°C for 15 minutes, and then at room temperature for 2 hours. Then hexane was used to precipitate the solution and it passed through silica gel, filtrating the salts away. The solvent was then removed resulting in a pale yellow liquid (3.59 g, 97% yield). Notebook reference: EKRII_56A. 1H-NMR (400 MHz,CDCl₃) δ 5.39-5.31 (m, 2H, =CH), 3.40 (t, J = 6.9 Hz, 2H, BrCH₂), 2.02 (quartet (br), J = 6.1 Hz, 4H, =CHCH₂), 1.85 (quintet (br), J = 7.0 Hz, 2H, CH₂CH₃), 1.48-1.39 (m, 2H, BrCH₂CH₂), 1.35-1.27 (m, 20H, CH₂), 0.88 (t, J = 6.8, 3H, CH₃) ppm.
(R)-1-O-cis-octadec-9-enyl-sn-glycerol, 3.

Freshly ground potassium hydroxide (KOH) (257 mg, 4.53 mmol) was added to a solution of cis-1-bromoctadec-9-ene (600 mg, 1.81 mmol), (S)-solketal (248 mg, 1.86 mmol) and tetra-n-butylammonium bromide (TBAB) (123 mg, 0.38 mmol) under nitrogenous atmosphere and stirred overnight at 35°C. The solution was then quenched with water and the intermediate product extracted with ether before being made neutral. It was then solved in water and tetrahydrofuran (2:6) and p-toluenesulfonic acid added and the mixture refluxed overnight under inert conditions. At last the compound was extracted with chloroform and purified on a silica gel column using ethyl acetate/petroleum ether (2:8) affording the product as clear oil (414 mg, 67% yield). Notebook reference: EKRII_60A.

Eicosapentaenoic acid.

To a solution of eicosapentaenoic acid (EPA) (2.0 g, 6.61 mmol), DMAP (804 mg, 6.61 mmol) and EDAC (1.901 g, 9.91 mmol) in dichloromethane (20 mL), acetoxime (483 mg, 7.5 mmol) was added and the resulting solution stirred for 4 hours under nitrogen atmosphere. The reaction solution was passed through a short silica gel column with ethyl acetate/petroleum ether (60:40) as an eluent, affording the product as yellowish oil (414 mg, 67% yield). Notebook reference: EKRII_EPA_FFA.

Docosahexaenoic acid.

A procedure identical to that described above for EPA was followed using NaOH (1.49 g, 37 mmol), 1:1 water/ethanol mixture (70 mL), Na₂-EDTA (16 mg, 0.042 mmol) and docosahexaenoic acid ethyl ester (6.64 g, 19 mmol). The product was afforded as yellowish oil after evaporation of the solvents (4.35 g, 95% yield). Notebook reference: EKRII_21A. ¹H-NMR (400 MHz,CDCl₃) δ 5.45-5.28 (m, 10H, =CH), 2.88-2.74 (m, 8H, =CH₂CH₂N=C(C=)), 2.45-2.37 (m, 4H, CH₂CH₂COO), 2.08 (quintet, J = 7.2 Hz, 2H, =CHCH₂CH₃), 0.97 (t, J = 7.5 Hz, 3H, CH₃) ppm.

Eicosapentaenoic acid acetoxime ester.

To a solution of eicosapentaenoic acid (EPA) (2.0 g, 6.61 mmol), DMAP (804 mg, 6.61 mmol) and acetoxydine (483 mg, 6.61 mmol) was added and the resulting solution stirred for 4 hours under nitrogen atmosphere. The reaction solution was passed through a short silica gel column with ethyl acetate/petroleum ether (60:40) as an eluent, affording the product as yellowish oil after evaporation of the solvents (1.980 g, 84% yield). Notebook reference: EKRII_3A. ¹H-NMR (400 MHz,CDCl₃) δ 5.44-5.28 (m, 10H, =CH), 2.88-2.77 (m, 8H, =CHCH₂CH₂N=C(C=)), 2.42 (t, J = 7.6 Hz, 2H, CH₂COO), 2.18-2.11 (m, 2H, =CHCH₂CH₃), 2.11-2.05 (m, 2H, =CHCH₂CH₂), 2.05 (s, 3 H, N=C(CH₃)₂), 1.99 (s, 3 H, N=C(CH₃)₂), 1.78 (quintet, J = 7.5 Hz, 2H, CH₂CH₂COO), 0.97 (t, J = 7.5 Hz, 3H, CH₃) ppm.
Docosahexaenoic acid acetoxime ester.

A procedure identical to that described above for EPA acetoxime ester was followed using docosahexaenoic acid (DHA) (2.001 g, 6.09 mmol), DMAP (683 mg, 6.09 mmol), EDAC (1518 mg, 6.09 mmol), dichloromethane (20 mL) and acetoxime (445 mg, 6.09 mmol). The product was afforded as yellowish oil after evaporation of the solvents (1.951 g, 84% yield). Notebook reference: EKRII_6A. \(^1\)H-NMR (400 MHz,CDCl\(_3\)) \(\delta\) 5.46-5.27 (m, 12H, =CH), 2.88-2.75 (m, 10H, =CHCH\(_2\)CH=), 2.49-2.44 (m, 4H, CH\(_2\)CH\(_2\)COO), 2.11-2.04 (m, 2H, =CHCH\(_2\)CH\(_3\)), 2.04 (s, 3 H, N=C(CH\(_3\))\(_2\)), 1.99 (s, 3 H, N=C(CH\(_3\))\(_2\)), 0.97 (t, \(J = 7.5\) Hz, 3H, CH\(_3\)) ppm.

**Synthesis of the 1-O-alkyl-3-acyl-sn-glycerols**

(R)-1-O-hexadecyl-3-eicosapentaenoyl-sn-glycerol, 4.

To a mixture of (S)-3-O-hexadecyl-sn-glycerol (S)-1 (100 mg, 0.32 mmol), immobilized *Candida antarctica* lipase (30 mg) and 4 Å molecular sieves (115 mg) in dry dichloromethane (3 mL), EPA acetoxime ester (114 mg, 0.32 mmol) was added and the resulting mixture was then stirred, under nitrogen atmosphere, until the alcohol completely dissolved. Additional EPA acetoxime ester (61 mg, 0.17 mmol) and lipase (15 mg) were then added to the reaction mixture and it stirred for 3h and 30 min at room temperature. The lipase was filtrated away and the solvent evaporated in vacuo from the mixture. Next the compound was crystallized in methanol at -40°C, affording the product as a white solid which melted into a clear oil when allowed to reach room temperature (169 mg, 89% yield). Notebook reference: EKRII_9A. \(^1\)H NMR (400 MHz, CDC\(_3\)) \(\delta\) 5.45-5.27 (m, 10H, =CH), 4.18 (dd, \(J = 11.5, 4.4\) Hz, 1H, CH\(_2\)OCO), 4.12 (dd, \(J = 11.5, 6.2\) Hz, 1H, CH\(_2\)OCO), 4.02-4.96 (m, 1H, CH\(_2\)CH\(_2\)), 3.51-3.40 (m, 4H, CHCH\(_2\)O and OCH\(_2\)CH\(_2\)), 2.88-2.77 (m, 8H, =CHCH\(_2\)CH=), 2.46 (d, \(J = 4.6\) Hz, 1H, CHO\(_2\)H), 2.36 (t, \(J = 7.6\) Hz, 2H, CH\(_2\)COO), 2.16-2.03 (m, 4H, =CHCH\(_2\)CH\(_2\) and =CHCH\(_2\)CH\(_3\) in EPA), 1.72 (quintet, \(J = 7.5\) Hz, 2H, CH\(_2\)CH\(_2\)COO), 1.57 (quintet (br), \(J = 6.9\) Hz, 2H, OCH\(_2\)CH\(_2\)), 1.35-1.18 (m, 26H, CH\(_2\)), 0.98 (t, \(J = 7.5\) Hz, 3H, CH\(_3\) in EPA), 0.88 (t (br), \(J = 6.8\) Hz, 3H, CH\(_3\) in 1-O-alky) ppm.

(R)-1-O-hexadecyl-3-docosahexaenoyl-sn-glycerol, 7.

A procedure identical to that described above for (R)-4 was followed using (S)-3-O-hexadecyl-sn-glycerol (S)-1 (150 mg, 0.47 mmol), immobilized *Candida antarctica* lipase (46 mg), 4 Å molecular sieves (160 mg), dry dichloromethane (4.5 mL) and DHA acetoxime ester (177 mg, 0.46 mmol), as well as additional DHA acetoxime ester (109 mg, 0.28 mmol) and lipase (26 mg). The product (R)-7 was afforded as a clear oil (264 mg, 89% yield). Notebook reference: EKRII_11C. \(^1\)H-NMR (400 MHz,CDCl\(_3\)) \(\delta\) 5.45-5.28 (m, 12H, =CH), 4.19 (dd, \(J = 11.5, 4.4\) Hz, 1H, CH\(_2\)OCO), 4.13 (dd, \(J = 11.5, 6.2\) Hz, 1H, CH\(_2\)OCO), 4.03-3.95 (m, 1H, CH\(_2\)CH\(_2\)), 3.51-3.39 (m, 4H, CHCH\(_2\)O and OCH\(_2\)CH\(_2\)), 2.90-2.77 (m, 10H, =CHCH\(_2\)CH=), 2.46 (s (br), 1H, CHO\(_2\)H), 2.43-2.38 (m, 4H, CH\(_2\)CH\(_2\)COO), 2.08 (quintet, \(J = 7.2\) Hz, 2H, =CHCH\(_2\)CH\(_3\)), 1.57 (quintet (br), \(J = 6.9\) Hz, 2H, OCH\(_2\)CH\(_2\)), 1.35-1.16 (m, 26H, CH\(_2\)), 0.97 (t, \(J = 7.5\) Hz, 3H, CH\(_3\) in DHA), 0.88 (t (br), \(J = 6.8\) Hz, 3H, CH\(_3\) in 1-O-alky) ppm.

(R)-1-O-octadecyl-3-eicosapentaenoyl-sn-glycerol, 5.

A procedure identical to that described above for (R)-4 was followed using (S)-3-O-octadecyl-sn-glycerol (S)-2 (250 mg, 0.73 mmol), immobilized *Candida antarctica* lipase (60 mg), 4 Å molecular sieves (300 mg), dry dichloromethane (7 mL) and EPA acetoxime
ester (260 mg, 0.73 mmol), as well as additional EPA acetoxime ester (156 mg, 0.44 mmol) and lipase (50 mg). The product (R)-5 was afforded as a clear oil (387 mg, 85% yield). Notebook reference: EKRII_19B. \(^1\)H-NMR (400 MHz,CDCl\(_3\)) \(\delta\) 5.45-5.28 (m, 10H, =CH\(_2\)), 4.18 (dd, \(J = 11.5, 4.4\) Hz, 1H, CH\(_2\)OCO), 4.12 (dd, \(J = 11.5, 6.2\) Hz, 1H, CH\(_2\)OCO), 4.04-3.95 (m, 1H, CH\(_2\)CHCH\(_2\)), 3.51-3.40 (m, 4H, CHCH\(_3\)O and OCH\(_2\)CH\(_2\)), 2.88-2.77 (m, 8H, =CHCH\(_2\)CH=), 2.47 (s (br), 1H, CHO\(_2\)), 2.36 (t, \(J = 7.6\) Hz, 2H, CH\(_2\)COO), 2.14-2.04 (m, 4H, =CHCH\(_2\)CH\(_2\) and =CHCH\(_2\)CH\(_2\) in EPA), 1.72 (quintet, \(J = 7.5\) Hz, 2H, CH\(_2\)CH\(_2\)COO), 1.57 (quintet (br), \(J = 6.9\) Hz, 2H, OCH\(_2\)CH\(_3\)), 1.37-1.17 (m, 30H, CH\(_2\)), 0.97 (t, \(J = 7.5\) Hz, 3H, CH\(_3\) in EPA), 0.88 (t (br), \(J = 6.8\) Hz, 3H, CH\(_3\) in 1-O-alkyl) ppm.

(R)-1-O-octadecyl-3-docosahexaenoyl-sn-glycerol, 8.

A procedure identical to that described above for (R)-4 was followed using (S)-3-O-octadecyl-sn-glycerol (S)-2 (100 mg, 0.29 mmol), immobilized Candida antarctica lipase (30 mg), 4 Å molecular sieves (110 mg), dry dichloromethane (3 mL) and DHA acetoxime ester (110 mg, 0.29 mmol), as well as additional DHA acetoxime ester (68 mg, 0.18 mmol) and lipase (15 mg). The product (R)-8 was afforded as a clear oil (163 mg, 86% yield). Notebook reference: EKRII_1B. \(^1\)H NMR (400 MHz, CDC\(_3\)) \(\delta\) \(^1\)H-NMR (400 MHz,CDCl\(_3\)) \(\delta\) 5.44-5.28 (m, 12H, =CH\(_2\)), 4.19 (dd, \(J = 11.5, 4.4\) Hz, 1H, CH\(_2\)OCO), 4.13 (dd, \(J = 11.5, 6.2\) Hz, 1H, CH\(_2\)OCO), 4.03-3.96 (m, 1H, CH\(_2\)CHCH\(_2\)), 3.50-3.40 (m, 4H, CHCH\(_3\)O and OCH\(_2\)CH\(_2\)), 2.88-2.78 (m, 10H, =CHCH\(_2\)CH=), 2.47 (d (br), \(J = 2.4\) Hz, 1H, CHO\(_2\)), 2.43-2.38 (m, 4H, CH\(_2\)CH\(_2\)COO), 2.07 (quintet, \(J = 7.2\) Hz, 2H, =CHCH\(_2\)CH\(_2\)), 1.57 (quintet (br), \(J = 6.9\) Hz, 2H, OCH\(_2\)CH\(_3\)), 1.33-1.18 (m, 30H, CH\(_2\)), 0.97 (t, \(J = 7.5\) Hz, 3H, CH\(_3\) in DHA), 0.88 (t (br), \(J = 6.8\) Hz, 3H, CH\(_3\) in 1-O-alkyl) ppm.

(R)-1-O-cis-octadec-9-enyl-3-eicosapentaenoyl-sn-glycerol, 6.

To a mixture of (S)-3-O-cis-octadec-9-enyl-sn-glycerol (S)-3 (150 mg, 0.44 mmol), EPA acetoxime ester (188 mg, 0.53 mmol), immobilized Candida antarctica lipase (51 mg) was added and the flask containing the mixture connected to an oil vacuum pump system (10\(^{-2}\) mmHg) and the resulting mixture stirred over a hot plate at 30\(^{\circ}\)C for 2 hours. Then, the vacuum was disconnected, dichloromethane added and the lipase filtered off. The crude mixture was concentrated by evaporation and then purified on a 4% boric acid impregnated flash silica gel column with a gradient elution of petroleum ether/ethyle acetate (90:10 and alkyl) ppm.

(R)-1-O-cis-octadec-9-enyl-3-docosahexaenoyl-sn-glycerol, 9.

A procedure identical to that described above for (R)-6 was followed using (S)-3-O-cis-octadec-9-enyl-sn-glycerol (S)-3 (150 mg, 0.44 mmol), DHA acetoxime ester (209 mg, 0.54 mmol) and immobilized Candida antarctica lipase (60 mg). The product (R)-9 was afforded as a clear oil (261 mg, 91% yield). Notebook reference: EKRII_23B (spectra EKRII_19B). \(^1\)H-NMR (400 MHz,CDCl\(_3\)) \(\delta\) 5.45-5.28 (m, 14H, =CH\(_2\)), 4.19 (dd, \(J = 11.5,
4.4 Hz, 1H, CH$_2$OCO), 4.13 (dd, $J = 11.5$, 6.2 Hz, 1H, CH$_2$OCO), 4.03-3.96 (m, 1H, CH$_2$CH$_2$H), 3.51-3.40 (m, 4H, CHCH$_2$O and OCH$_2$CH$_2$ in DHA), 2.88-2.78 (m, 10H, =CHCH$_2$CH$_3$), 2.45 (d, $J = 4.6$ Hz, 1H, CHO$_2$), 2.43-2.38 (m, 4H, CH$_2$CH$_2$COO), 2.08 (quintet, $J = 7.2$ Hz, 2H, =CHCH$_2$CH$_3$ in DHA), 2.02 (quartet (br), $J = 6.4$ Hz, 4H, =CHCH$_2$ in 1-O-alkyl), 1.57 (quintet (br), $J = 6.9$ Hz, 2H, OCH$_2$CH$_2$), 1.38-1.22 (m, 22H, CH$_2$), 0.97 (t, $J = 7.5$ Hz, 3H, CH$_3$ in DHA), 0.88 (t (br), $J = 6.8$ Hz, 3H, CH$_3$ in 1-O-alkyl) ppm.

Synthesis of the 1-O-alkyl-2,3-diacyl-sn-glycerols

(R)-1-O-hexadecyl-2,3-dieicosapentaenoyl-sn-glycerol, 4a. To a solution of (R)-3-O-hexadecyl-sn-glycerol (100 mg, 0.32 mmol), DMAP (71 mg, 0.63 mmol) and EDAC (182 mg, 0.95 mmol) in dry dichloromethane (3 mL) was added EPA as a free acid (210 mg, 0.69 mmol) and the resulting solution stirred at 35°C for 16 hours under nitrogen atmosphere. Then additional EPA FFA (23 mg, 0.08 mmol) was added and the reaction mixture stirred for further 3 hours. The reaction mixture was passed through a short silica gel column with diethyl ether/petroleum ether (5:95) as an eluent, affording the product as a yellowish oil after concentration (65 mg, 86% yield); Notebook reference: EKRII_50A. [α]$_{D}^{20}$ = -6.5 (c 1.02 benzene); IR (NaCl) 3013 (=C-H cis), 2924 (C-H), 1742 (C=O), 1724 (C=O)$_2$, 1654 (C=C), 1124 (C-O-C) cm$^{-1}$; HRMS (ESI) $m/z$ calcd for C$_{59}$H$_{96}$O$_5$ + Na: 907.7150; found 907.7168 amu. $^1$H-NMR (400 MHz,CDCl$_3$) $\delta$ 5.43-5.28 (m, 20H, =CH$_2$), 5.22-5.17 (m, 1H, CH$_2$CH$_2$H), 4.35 (dd, $J = 11.9$, 3.7 Hz, 1H, CH$_2$OCO), 4.16 (dd, $J = 11.9$, 6.4 Hz, 1H, CH$_2$OCO), 3.56-3.50 (2xd, $J = 10.6$, 5.3 Hz, 2H, CHCH$_2$O), 3.47-3.37 (2xd, $J = 9.4$, 7.3 Hz, 2H, OCH$_2$CH$_2$), 2.88-2.77 (m, 16H, =CHCH$_2$CH=), 2.34 (t, $J = 7.6$ Hz, 2H, CH$_2$COO, sn-2), 2.32 (t, $J = 7.6$ Hz, 2H, CH$_2$COO, sn-3), 2.15-2.02 (m, 8H, =CHCH$_2$CH$_2$ and =CHCH$_2$CH$_2$), 1.70 (quintet, $J = 7.4$ Hz, 2H, CH$_2$CH$_2$COO), 1.69 (quintet, $J = 7.4$ Hz, 2H, CH$_2$CH$_2$COO), 1.53 (quintet (br), $J = 6.9$ Hz, 2H, OCH$_2$CH$_2$), 1.35-1.18 (m, 26H, CH$_2$), 0.97 (t, $J = 7.5$ Hz, 6H, CH$_3$ in EPA), 0.88 (t (br), $J = 6.9$ Hz, 3H, CH$_3$ in 1-O-alkyl) ppm; $^{13}$C NMR (CDCl$_3$) $\delta$ 173.14 (α C=O), 172.84 (β C=O), 132.02 (2), 128.89, 128.87 (2), 128.86, 128.56 (2), 128.26 (2), 128.19, 128.18, 128.17 (2), 128.07 (2), 127.86 (2), 127.01 (2), 71.77 (OCH$_2$CH$_2$), 70.16 (CH$_2$CH$_2$CH$_2$), 68.92 (CH$_2$CH$_2$O), 62.85 (CH$_2$OCO), 33.72, 33.49, 31.92, 29.70 (5), 29.66, 29.65, 29.62, 29.56, 29.47, 29.36, 26.53, 26.51, 26.04, 25.63 (6), 25.54 (2), 24.83, 24.74, 22.69, 20.56 (2), 14.27 (2), 14.11 ppm.

(R)-1-O-hexadecyl-2-docosapentaenoyl-3-eicosapentaenoyl-sn-glycerol, 4b. To a solution of (R)-1-O-hexadecyl-3-eicosapentaenoyl-sn-glycerol (R)-5 (50 mg, 0.08 mmol), DMAP (12 mg, 0.11 mmol) and EDAC (27 mg, 0.14 mmol) in dry dichloromethane (2 mL) was added DHA as a free acid (43 mg, 0.13 mmol) and the resulting solution stirred at 35°C for 16 hours under nitrogen atmosphere. The reaction mixture was passed through a short silica gel column with ether/dichloromethane (10:90) as an eluent, affording the product as a yellowish oil after concentration (65 mg, 86% yield); Notebook reference: EKRII_50A. [α]$_{D}^{20}$ = -6.4 (c 1.08 benzene); IR (NaCl) 3013 (=C-H cis), 2924 (C-H), 1742 (C=O), 1654 (C-O-C) cm$^{-1}$; HRMS (ESI) $m/z$ calcd for C$_{61}$H$_{98}$O$_5$ + Na: 933.7306; found 933.7298 amu. $^1$H-NMR (400 MHz,CDCl$_3$) $\delta$ 5.45-5.28 (m, 22H, =CH), 5.22-5.17 (m, 1H, CH$_2$CH$_2$H), 4.35 (dd, $J = 11.9$, 3.7 Hz, 1H, CH$_2$OCO), 4.17 (dd, $J = 11.9$, 6.4 Hz, 1H, CH$_2$OCO), 3.57-3.50 (2xd, $J = 10.7$, 5.3 Hz, 2H, CHCH$_2$O), 3.47-3.37 (2xd, $J = 9.4$, 6.8 Hz, 2H, OCH$_2$CH$_2$), 2.88-2.77 (m, 18H, =CHCH$_2$CH=), 2.42-2.36 (m, 4H, CH$_2$CH$_2$COO in DHA), 2.32 (t, $J = 7.6$ Hz, 2H,
CH₂COO in EPA), 2.13-2.04 (m, 6H, =CHCH₂CH₃ in EPA and DHA, and =CHCH₂CH₂ in EPA), 1.69 (quintet, J = 7.4 Hz, 2H, CH₂CH₂COO in EPA), 1.60-1.48 (m, 2H, OCH₂CH₂), 1.35-1.17 (m, 26H, CH₂), 0.97 (t, J = 7.5 Hz, 6H, CH₃ in EPA and DHA), 0.88 (t (br), J = 6.8 Hz, 3H, CH₃ in 1-O-alkyl ppm); ¹³C NMR (CDCl₃) δ 173.14 (α C=O), 172.37 (β C=O), 132.02 (2), 129.31, 128.87 (2), 128.56 (2), 128.24, 128.18, 128.17, 128.10, 128.07 (2), 128.04, 127.86 (2), 127.79, 127.01 (2), 71.78 (OCH₂CH₂), 70.28 (CH₂CH₂CH₂), 68.89 (CH₂CH₂), 62.82 (CH₂O), 34.15, 33.50, 31.93, 29.70 (5), 29.66, 29.65, 29.62, 29.56, 29.48, 29.36, 26.53, 26.04, 25.63 (6), 25.60, 25.54 (2), 24.74, 22.72, 22.69, 20.56 (2), 14.27 (2), 14.12 ppm.

(R)-1-O-hexadecyl-2,3-didocosahexaenoyl-sn-glycerol, 7b.

A procedure identical to that described above for 4b was followed using (R)-1-O-hexadecyl-3-docosahexaenoyl-sn-glycerol (R-7 (44 mg, 0.07 mmol), DMAP (6 mg, 0.05 mmol), EDAC (17 mg, 0.09 mmol), dry dichloromethane (2 mL) and DHA FFA (24 mg, 0.07 mmol). After 16 hours additional DHA FFA (13 mg, 0.04 mmol) was added, then, after a further 3 hours, additional DMAP (6 mg, 0.05 mmol) and EDAC (12 mg, 0.06 mmol) were added and the mixture was stirred for 2 hours. The compound was then purified on a short silica gel column with dichloromethane as an eluent. The product 7b was afforded as a yellowish oil (57 mg, 87% yield); Notebook reference: EKRII_72A. [α]²⁰ₒ₋₆₋₇ (c 1.00 benzene); IR (NaCl) 3012 (=C-H cis), 2925 (C-H), 1742 (C=O), 1654 (C=C), 1124 (C-O-C) cm⁻¹; HRMS (ESI) m/z calcd for C₆₃H₁₀₀O₅ + Na: 959.7463; found 959.7509 amu. ¹H-NMR (400 MHz,CDCl₃) δ 5.43-5.28 (m, 24H, =CH), 5.22-5.17 (m, 1H, CH₂CH₂CH₂), 4.35 (dd, J = 11.9, 3.7 Hz, 1H, CH₂O), 4.18 (dd, J = 11.9, 6.4 Hz, 1H, CH₂O), 3.57-3.50 (2xdd, J =10.6, 5.3 Hz, 2H, CH₂O), 3.47-3.37 (2xdt, J = 9.4, 6.9 Hz, 2H, OCH₂CH₂), 2.90-2.75 (m, 20H, =CHCH₂CH=), 2.42-2.36 (m, 8H, CH₂CH₂COO), 2.08 (quintet, J = 7.4 Hz, 4H, =CHCH₂CH₂), 1.58-1.50 (m, 2H, OCH₂CH₂), 1.33-1.18 (m, 26H, CH₂), 0.97 (t, J = 7.5 Hz, 6H, CH₃ in DHA), 0.88 (t (br), J = 6.8 Hz, 3H, CH₃ in 1-O-alkyl ppm; ¹³C NMR (CDCl₃) δ 172.67 (α C=O), 172.37 (β C=O), 132.02 (2), 129.35, 129.31, 128.56 (2), 128.24 (2), 128.18 (2), 128.07 (2), 128.03, 128.02, 127.86 (2), 127.79 (2), 127.01 (2), 71.78 (OCH₂CH₂), 70.27 (CH₂CH₂CH₂), 68.88 (CH₂O), 62.90 (CH₂O), 34.15, 33.97, 31.92, 29.70 (5), 29.66 (2), 29.62, 29.56, 29.52, 29.47, 29.36, 26.04, 25.63 (6), 25.60, 25.54 (2), 22.72, 22.69, 22.66, 20.56 (2), 14.27 (2), 14.11 ppm.

(R)-1-O-hexadecyl-3-docosahexaenoyl-2-eicosapentaenoyl-sn-glycerol, 7a.

A procedure identical to that described above for 4b was followed using (R)-1-O-hexadecyl-3-docosahexaenoyl-sn-glycerol (R-7 (97 mg, 0.15 mmol), DMAP (18 mg, 0.16 mmol), EDAC (46 mg, 0.24 mmol), dry dichloromethane (3 mL) and EPA FFA (58 mg, 0.19 mmol). Additional EPA FFA (11 mg, 0.04 mmol) was added after 16 hours and the reaction mixture stirred for a further 3 hours. The product was purified on a short silica gel column using dichloromethane as an eluent. The product 7a was afforded as a yellowish oil (123 mg, 87% yield); Notebook reference: EKRII_53B. [α]²⁰ₒ₋₆₋₁ (c 0.97 benzene); IR (NaCl) 3013 (=C-H cis), 2923 (C-H), 1743 (C=O), 1653 (C=C), 1123 (C-O-C) cm⁻¹; HRMS (ESI) m/z calcd for C₆₃H₁₀₈O₈ + Na: 933.7306; found 933.7308 amu. ¹H-NMR (400 MHz,CDCl₃) δ 5.46-5.38 (m, 24H, =CH), 5.22-5.17 (m, 1H, CH₂CH₂CH₂), 4.35 (dd, J = 11.9, 3.7 Hz, 1H, CH₂O), 4.17 (dd, J = 11.9, 6.5 Hz, 1H, CH₂O), 3.57-3.50 (2xdd, J =10.6, 5.3 Hz, 2H, CH₂O), 3.47-3.37 (2xdt, J = 9.4, 7.2 Hz, 2H, OCH₂CH₂), 2.88-2.77 (m, 18H, =CHCH₂CH=), 2.41-2.37 (m, 4H, CH₂CH₂COO in DHA), 2.34 (t, J = 7.6 Hz, 2H, CH₂COO in EPA), 2.16-2.02 (m, 6H, =CHCH₂CH₃ in EPA and DHA, and
(R)-1-Octadecyl-2,3-dieicosapentaenoyl-sn-glycerol, 5a.

A procedure identical to that described above for 4a was followed using (S)-3-Octadecyl-sn-glycerol (100 mg, 0.29 mmol), DMAP (68 mg, 0.61 mmol), EDAC (168 mg, 0.88 mmol), dry dichloromethane (4 mL) and EPA FFA (190 mg, 0.63 mmol), with additional EPA FFA (20 mg, 0.07 mmol) added to the solution after 16 hours. The product 5a was afforded as a yellowish oil (243 mg, 92% yield); Notebook reference: EKRII_36A. [α]D -6.6 (c 1.04 benzene); IR (NaCl) 3013 (C=H) cm⁻¹; HRMS (ESI) m/z calced for C₆₀H₁₀₀O₅ + Na: 935.7463; found 935.7425 amu. ¹H-NMR (400 MHz,CDCl₃) δ 5.43-5.28 (m, 20H, =CH₂), 5.22-5.17 (m, 1H, CH₂CH₂CH₂), 4.35 (dd, J = 11.9, 3.7 Hz, 1H, CH₂OOC), 4.16 (dd, J = 11.9, 6.4 Hz, 1H, CH₂OOC), 3.56-3.49 (2xdx, J = 10.7, 5.3 Hz, 2H, CH₂CH₂O), 3.47-3.37 (2xd, J = 9.4, 7.2 Hz, 2H, OCH₂CH₂), 2.88-2.76 (m, 16H, =CHCH₂CH=), 2.34 (t, J = 7.6 Hz, 2H, CH₂OOC), 2.32 (t, J = 7.6 Hz, 2H, CH₂OOC), 2.14-2.04 (m, 8H, =CHCH₂CH₂ and =CHCH₂CH₂), 1.70 (quintet, J = 7.4 Hz, 2H, CH₂CH₂COO), 1.69 (quintet, J = 7.4 Hz, 2H, CH₂CH₂COO), 1.53 (quintet (br), J = 6.8 Hz, 2H, OCH₂CH₂), 1.35-1.16 (m, 30H, CH₂), 0.97 (t, J = 7.5 Hz, 6H, CH₃ in EPA), 0.88 (t (br), J = 6.8 Hz, 3H, CH₃ in 1-O-alkyl) ppm; ¹³C NMR (CDCl₃) δ 173.15 (α C=O), 172.38 (β C=O), 132.01 (2), 128.89 (2), 128.86 (2), 128.56 (2), 128.26 (2), 128.18, 128.17, 128.16 (2), 128.07 (2), 127.85 (2), 127.00 (2), 71.76 (OCH₂CH₂), 70.16 (CH₂CH₂O), 68.92 (CH₂OOC), 33.71, 33.49, 31.92, 29.70 (6), 29.65 (3), 29.61, 29.56, 29.47, 29.36, 26.53, 26.51, 26.04, 25.62 (6), 25.54 (2), 24.82, 24.74, 22.69, 20.55 (2), 14.26 (2), 14.11 ppm.

(R)-1-Octadecyl-2-docosapentaenoyl-3-eicosapentaenoyl-sn-glycerol, 5b.

A procedure identical to that described above for 7b was followed using (R)-1-Octadecyl-3-eicosapentaenoyl-sn-glycerol (R)-6 (97 mg, 0.15 mmol), DMAP (17 mg, 0.15 mmol), EDAC (41 mg, 0.21 mmol), dry dichloromethane (3.5 mL) and DHA FFA (63 mg, 0.19 mmol). Additional DHA FFA (10 mg, 0.03 mmol), as well as DMAP (6 mg, 0.05 mmol) and EDAC (11 mg, 0.06 mmol) were added to the mixture to push the reaction towards completion. The product 5b was afforded as a yellowish oil (126 mg, 87% yield); Notebook reference: EKRII_54A. [α]D -6.1 (c 0.36 benzene); IR (NaCl) 3013 (C=H) cm⁻¹; HRMS (ESI) m/z calced for C₆₃H₁₀₀O₅ + Na: 961.7619; found 961.7625 amu. ¹H-NMR (400 MHz,CDCl₃) δ 5.44-5.28 (m, 22H, =CH₂), 5.22-5.17 (m, 1H, CH₂CH₂CH₂), 4.35 (dd, J = 11.9, 3.7 Hz, 1H, CH₂OOC), 4.17 (dd, J = 11.9, 6.5 Hz, 1H, CH₂OOC), 3.57-3.50 (2xdx, J =10.7, 5.3 Hz, 2H, CH₂CH₂O), 3.47-3.37 (2xdx, J = 9.4, 7.3 Hz, 2H, OCH₂CH₂), 2.88-2.76 (m, 18H, =CHCH₂CH=), 2.41-2.37 (m, 4H, CH₂CH₂COO in DHA), 2.32 (t, J = 7.6 Hz, 2H, CH₂COO in EPA), 2.14-2.04 (m, 6H, =CHCH₂CH₂ in EPA and DHA, and =CHCH₂CH₂ in EPA), 1.70 (quintet, J = 7.4 Hz, 2H, CH₂CH₂COO in EPA), 1.57-1.50 (m, 2H, OCH₂CH₂), 1.35-1.19 (m, 30H, CH₂), 0.97 (t, J = 7.5 Hz, 6H, CH₃ in EPA and DHA), 0.88 (t (br), J = 6.8 Hz, 3H, CH₃ in 1-O-alkyl) ppm; ¹³C NMR (CDCl₃) δ 173.15 (α C=O), 172.38 (β C=O),
132.03 (2), 129.31, 128.88, 128.87, 128.57 (2), 128.28 (3), 128.25, 128.18, 128.17, 128.10, 128.07 (2), 128.04, 127.87 (2), 127.79, 127.01 (2), 71.78 (OCH₃CH₂), 70.28 (CH₂CHCH₂), 68.89 (CH₃O), 62.82 (CH₂OCO), 34.15, 33.50, 31.93, 29.71 (6), 29.66 (2), 29.62, 29.56, 29.48, 29.36, 26.53, 26.04, 25.63 (6), 25.60, 25.54 (2), 24.74, 22.72, 22.69, 22.67, 20.56 (2), 14.27 (2), 14.12 ppm.

(R)-1-O-octadecyl-2,3-didocosahexaenoyl-sn-glycerol, 8b.

A procedure identical to that described above for 7b was followed using (R)-1-O-octadecyl-3-docosahexaenoyl-sn-glycerol (R)-8 (80 mg, 0.12 mmol), DMAP (13 mg, 0.12 mmol), EDAC (28 mg, 0.19 mmol), dry dichloromethane (2.5 mL) and DHA FFA (53 mg, 0.16 mmol). Additional DHA FFA (16 mg, 0.05 mmol), as well as DMAP (6 mg, 0.05 mmol) and EDAC (12 mg, 0.06 mmol) were added to the mixture to push the reaction towards completion. The product 8b was afforded as a yellowish oil (105 mg, 89% yield); Notebook reference: EKRII_74B. [α]D -6.8 (c 0.99 benzene); IR (NaCl) 3013 (C-H cis), 2924 (C-H), 1742 (C=O), 1654 (C=C), 1125 (C-O-C) cm⁻¹; HRMS (ESI) m/z calcd for C₅₉H₁₀₀O₈ + Na: 987.7766; found 987.7754 amu. ¹H-NMR (400 MHz, CDCl₃) δ 5.43-5.28 (m, 24H, =CH₂), 5.22-5.17 (m, 1H, CH₂CHCH₂), 4.35 (dd, J = 11.9, 3.7 Hz, 1H, CH₂OCO), 4.18 (dd, J = 11.9, 6.4 Hz, 1H, CH₂OCO), 3.57-3.50 (2xd, J =10.7, 5.3 Hz, 2H, CH₂CH₂O), 3.47-3.37 (2xd, J = 9.4, 6.8 Hz, 2H, OCH₂CH₂), 2.88-2.80 (m, 20H, =CH₂CH₂=), 2.43-2.34 (m, 8H, CH₂CH₂COO), 2.07 (quintet, J = 7.6 Hz, 4H, =CH₂CH₂CH₃), 1.54 (quintet (br), J = 6.8 Hz, 2H, OCH₂CH₂), 1.33-1.21 (m, 30H, CH₂), 0.97 (t, J = 7.5 Hz, 6H, CH₃ in DHA), 0.88 (t (br), J = 6.8 Hz, 3H, CH₃ in 1-O-alkyl) ppm; ¹³C NMR (CDCl₃) δ 172.67 (α C=O), 172.38 (β C=O), 132.03 (2), 129.36, 129.31, 128.56 (2), 128.28 (4), 128.24 (2), 128.10 (2), 128.07 (2), 128.04, 128.02, 127.87 (2), 127.79 (2), 127.01 (2), 71.78 (OCH₂CH₂), 70.27 (CH₂CHCH₂), 68.88 (CH₂CH₂O), 62.91 (CH₂OCO), 34.16, 33.98, 31.92, 29.71 (6), 29.66 (2), 29.62, 29.56, 29.48 (2), 29.36, 26.04, 25.63 (6), 25.60, 25.59, 25.54 (2), 22.72, 22.69, 22.66, 20.56 (2), 14.27 (2), 14.12 ppm.

(R)-1-O-octadecyl-3-docosahexaenoyl-2-eicosapentaenoyl-sn-glycerol, 8a.

A procedure identical to that described above for 7b was followed using (R)-1-O-octadecyl-3-docosahexaenoyl-sn-glycerol (R)-5 (80 mg, 0.12 mmol), DMAP (35 mg, 0.18 mmol), dry dichloromethane (2.5 mL) and EPA FFA (52 mg, 0.17 mmol). Additional EPA FFA (13 mg, 0.04 mmol), as well as DMAP (7 mg, 0.06 mmol) and EDAC (14 mg, 0.07 mmol) were added to the mixture to push the reaction towards completion. The product 8a was afforded as a yellowish oil (101 mg, 88% yield); Notebook reference: EKRII_76B. [α]D -6.1 (c 1.06 benzene); IR (NaCl) 3013 (C-H cis), 2924 (C-H), 1742 (C=O), 1653 (C=C), 1124 (C-O-C) cm⁻¹; HRMS (ESI) m/z calcd for C₆₅H₁₀₂O₂S + Na: 961.7619; found 961.7624 amu. ¹H-NMR (400 MHz, CDCl₃) δ 5.45-5.27 (m, 22H, =CH₂), 5.22-5.17 (m, 1H, CH₂CHCH₂), 4.35 (dd, J = 11.9, 3.7 Hz, 1H, CH₂OCO), 4.17 (dd, J = 11.9, 6.5 Hz, 1H, CH₂OCO), 3.57-3.50 (2xd, J =10.6, 5.3 Hz, 2H, CH₂CH₂O), 3.47-3.37 (2xd, J = 9.4, 7.3 Hz, 2H, OCH₂CH₂), 2.88-2.77 (m, 18H, =CH₂CH₂CH₃), 2.40-2.37 (m, 4H, CH₂CH₂COO in DHA), 2.34 (t, J = 7.6 Hz, 2H, CH₂COO in EPA), 2.14-2.04 (m, 6H, =CHCH₂CH₃ in EPA and DHA, and =CHCH₂CH₂ in EPA), 1.70 (quintet, J = 7.5 Hz, 2H, CH₂CH₂COO in EPA), 1.54 (quintet (br), J = 6.7 Hz, 2H, OCH₂CH₂), 1.35-1.19 (m, 30H, CH₂), 0.97 (t, J = 7.5 Hz, 6H, CH₃ in EPA and DHA), 0.88 (t (br), J = 6.8 Hz, 3H, CH₃ in 1-O-alkyl) ppm; ¹³C NMR (CDCl₃) δ 172.85 (β C=O), 172.67 (α C=O), 132.03 (2), 129.35, 128.89, 128.86, 128.57 (2), 128.29, 128.27 (2), 128.24, 128.19, 128.17, 128.10, 128.07 (2), 128.02, 127.86 (2), 127.79, 127.01 (2), 71.78 (OCH₂CH₂), 70.14 (CH₂CHCH₂), 68.92 (CH₃O), 62.95 (CH₂OCO), 33.97, 33.72,

(R)-1-O-cis-octadec-9--enyl-2,3-dieicosapentaenoyl-sn-glycerol, 6a.
A procedure identical to that described above for 4a was followed using (S)-3-O-cis-octadec-9- enyl-sn-glycerol (79 mg, 0.23 mmol), DMAP (53 mg, 0.47 mmol), EDAC (142 mg, 0.74 mmol), dry dichloromethane (3 mL) and EPA FFA (162 mg, 0.53 mmol), with additional EPA FFA (16 mg, 0.05 mmol) added after 16 hours. Furthermore additional DMAP (4 mg, 0.04 mmol) and EDAC (10 mg, 0.05 mmol) were added, after 3 hours, and the reaction mixture stirred for another 2 hours. The material was then purified on a short silica gel column using dichloromethane as an eluent. The product 6a was afforded as a yellowish oil (182 mg, 87% yield); Notebook reference: EKRII_44A. [α]$_{D}^{20}$ -6.4 (c 0.99 benzene); IR (NaCl) 3012 (s =C–H), 2924 (C–H), 1742 (C=O), 1654 (C=C), 1125 (C=O) cm$^{-1}$; HRMS (ESI) m/z calcd for C$_{61}$H$_{89}$O$_{5}$ + Li: 917.7569; found 917.7592 amu. H-1 NMR (400 MHz,CDCl$_{3}$) δ 5.43-5.28 (m, 22H, =CH), 5.22-5.17 (m, 1H, CH$_{3}$CH$_{2}$CH$_{2}$), 4.35 (dd, J = 11.9, 3.7 Hz, 1H, CH$_{2}$OCO), 4.16 (dd, J = 11.9, 6.4 Hz, 1H, CH$_{2}$OCO), 3.57-3.50 (2xddd, J = 10.7, 5.3 Hz, 2H, CH$_{2}$CH$_{2}$), 3.47-3.37 (2xdt, J = 9.4, 6.8 Hz, 2H, OCH$_{2}$CH$_{2}$), 2.88-2.77 (m, 16H, =CHCH$_{2}$CH$_{2}$ =, 2.34 (t, J = 7.6 Hz, 2H, CH$_{2}$COO), 2.32 (t, J = 7.6 Hz, 2H, CH$_{2}$COO), 2.14-2.06 (m, 8H, =CHCH$_{2}$CH$_{2}$ and =CHCH$_{2}$CH$_{3}$), 2.02 (quart, br, J = 6.5 Hz, 4H, =CHCH$_{2}$ in 1-O-alkyl), 1.70 (quintet, J = 7.4 Hz 2H, CH$_{2}$CH$_{2}$COO), 1.69 (quintet, J = 7.4 Hz 2H, CH$_{2}$CH$_{2}$COO), 1.54 (quintet, br, J = 6.9 Hz 2H, OCH$_{2}$CH$_{2}$), 1.38-1.18 (m, 22H, CH$_{2}$), 0.97 (t, J = 7.5 Hz, 6H, CH$_{3}$ in EPA), 0.88 (t, br, J = 6.8 Hz, 3H, CH$_{3}$ in 1-O-alkyl) ppm; $^{13}$C NMR (CDCl$_{3}$) δ 173.12 (α, C=O), 172.82 (β, C=O), 132.01 (2), 129.92, 129.80, 128.88, 128.86 (2), 128.56 (2), 128.26 (2), 128.18, 128.16 (3), 128.06 (3), 127.85 (2), 127.00 (2), 71.75 (OCH$_{2}$CH$_{2}$), 70.15 (CH$_{2}$CH$_{2}$), 68.92 (CH$_{2}$CH$_{2}$O), 62.85 (CH$_{2}$O), 33.71, 33.49, 31.90, 29.76 (2), 29.55, 29.52, 29.51, 29.44, 29.31 (2), 29.28, 27.21 (2), 26.52, 26.50, 20.53 (2), 25.53 (2), 24.82, 22.73, 22.67, 20.55 (2), 14.26 (2), 14.10 ppm.

(R)-1-O-cis-octadec-9-enyl-2-docosapentaenoyl-3-eicosapentaenoyl-sn-glycerol, 6b.
A procedure identical to that described above for 7a was followed using (R)-1-O-cis-octadec-9-enyl-3-eicosapentaenoyl-sn-glycerol (R)-6 (100 mg, 0.16 mmol), DMAP (15 mg, 0.13 mmol), EDAC (43 mg, 0.22 mmol), dry dichloromethane (3 mL) and DHA FFA (59 mg, 0.18 mmol) as well as additional DHA FFA (20 mg, 0.06 mmol). The compound was purified on a silica gel column with diethyl ether/dichloromethane (10:90) as an eluent. The product 6b was afforded as a yellowish oil (138 mg, 92% yield); Notebook reference: EKRII_80A. [α]$_{D}^{20}$ -6.8 (c 0.89 benzene); IR (NaCl) 3012 (=C–H), 2925 (C–H), 1742 (C=O), 1653 (C=C), 1125 (C=O–C) cm$^{-1}$; HRMS (ESI) m/z calcd for C$_{63}$H$_{100}$O$_{5}$ + Na: 959.7463; found 959.7459 amu. H-1 NMR (400 MHz,CDCl$_{3}$) δ 5.43-5.28 (m, 24H, =CH), 5.22-5.17 (m, 1H, CH$_{2}$CH$_{2}$CH$_{2}$), 4.35 (dd, J = 11.9, 3.7 Hz, 1H, CH$_{2}$OCO), 4.17 (dd, J = 11.9, 6.4 Hz, 1H, CH$_{2}$OCO), 3.57-3.50 (2xddd, J = 10.7, 5.3 Hz, 2H, CH$_{2}$CH$_{2}$O), 3.47-3.37 (2xdt, J = 9.4, 6.8 Hz, 2H, OCH$_{2}$CH$_{2}$), 2.88-2.77 (m, 18H, =CHCH$_{2}$CH$_{2}$ =), 2.42-2.36 (m, 4H, CH$_{2}$CH$_{2}$COO in DHA), 2.32 (t, J = 7.6 Hz, 2H, CH$_{2}$COO in EPA), 2.13-2.04 (m, 6H, =CHCH$_{2}$CH$_{3}$ in EPA and DHA, and =CHCH$_{2}$CH$_{2}$ in EPA), 2.02 (quart, br, J = 5.8 Hz, 4H, =CHCH$_{2}$ in 1-O-alkyl), 1.69 (quintet, J = 7.4 Hz, 2H, CH$_{2}$CH$_{2}$COO in EPA), 1.54 (quintet, br, J = 6.8 Hz, 2H, OCH$_{2}$CH$_{2}$), 1.38-1.21 (m, 22H, CH$_{2}$), 0.97 (t, J = 7.5 Hz, 6H, CH$_{2}$ in EPA and DHA), 0.88 (t, br, J = 6.8 Hz, 3H, CH$_{3}$ in 1-O-alkyl) ppm; $^{13}$C NMR (CDCl$_{3}$) δ 173.13 (α, C=O), 172.36 (β, C=O), 132.03 (2), 129.93, 129.81, 129.31, 128.87, 128.57 (2), 128.27 (3), 128.25, 128.18, 128.17, 128.10, 128.07 (3), 128.04, 127.86 (2),
127.79, 127.01 (2), 71.77 (OCH₂CH₂), 70.28 (CH₂CHCH₂), 68.90 (CH₂CH₂O), 62.82 (CH₃O), 34.15, 33.50, 31.90, 29.77 (2), 29.56, 29.52 (2), 29.45, 29.32 (2), 29.29, 27.22 (2), 26.53, 26.04, 25.63 (6), 25.60, 25.54 (2), 24.74, 22.72, 22.68, 20.56 (2), 14.27 (2), 14.11 ppm.

(R)-1-O-cis-octadec-9-ethyl-2,3-didocosahexaenoyl-sn-glycerol, 9b.

A procedure identical to that described above for 6a was followed using (S)-3-O-cis-octadec-9-ethyl-sn-glycerol (79 mg, 0.23 mmol), DMAP (54 mg, 0.48 mmol), EDAC (144 mg, 0.75 mmol), dry dichloromethane (3 mL) and DHA FFA (188 mg, 0.57 mmol), with additional DHA (20 mg, 0.06 mmol) as well as additional DMAP (10 mg, 0.09 mmol) and EDAC (20 mg, 0.10 mmol). The product 9b was afforded as a yellowish oil (193 mg, 87% yield); Notebook reference: EKRII_46A. [α]²⁰₀D -6.4 (c 1.02 benzene); IR (NaCl) 3013 (ν=CH cis), 2925 (C-H), 1742 (C=O), 1654 (C=C), 1124 (C-O-C) cm⁻¹; HRMS (ESI) m/z calcd for C₆₅H₁₀₂O₇ + Li: 969.7882; found 969.7874 amu. ¹H-NMR (400 MHz,CDCl₃) δ 5.43-5.28 (m, 26H, =CH), 5.22-5.17 (m, 1H, CH₂CHCH₂), 4.35 (dd, J = 11.9, 3.7 Hz, 1H, CH₂OCO), 4.17 (dd, J = 11.9, 6.4 Hz, 1H, CH₂OCO), 3.57-3.50 (2xdd, J = 10.7, 5.3 Hz, 2H, CH₂CH₂O), 3.47-3.37 (2xdt, J = 9.4, 6.8 Hz, 2H, OCH₂CH₂), 2.90-2.78 (m, 20H, =CH₂CH₂CH=), 2.41-2.36 (m, 8H, CH₂CH₂COO), 2.07 (quintet, J = 7.5 Hz, 4H, =CH₂CH₂CH=), 2.02 (quartet (br), J = 6.5 Hz, 4H, =CH₂CH₂ in 1-O-alkyl), 1.59-1.49 (m, 2H, OCH₂CH₂ ), 1.38-1.20 (m, 22H, CH₃), 0.97 (t, J = 7.5 Hz, 6H, CH₃ in DHA), 0.88 (t (br), J = 6.8 Hz, 3H, CH₃ in 1-O-alkyl) ppm; ¹³C NMR (CDCl₃) δ 172.67 (α, C=O), 172.37 (β, C=O), 132.03 (2), 129.93, 129.82, 129.36, 129.32, 128.57 (2), 128.28 (3), 128.25 (2), 128.10 (2), 128.08 (3), 128.04, 128.03, 128.77 (2), 127.79 (2), 127.01 (2), 71.78 (OCH₂CH₂), 70.27 (CH₂CHCH₂), 68.90 (CH₂CH₂O), 62.91 (CH₂OCO), 34.16, 33.98, 31.91, 29.78 (2), 29.57, 29.53 (2), 29.45, 29.32 (2), 29.30, 27.22 (2), 26.05, 25.64 (6), 25.61 (2), 25.55 (2), 24.72, 22.69, 22.67, 20.56 (2), 14.27 (2), 14.11 ppm.

(R)-1-O-cis-octadec-9-ethyl-3-docosahexaenoyl-2-eicosapentaenoyl-sn-glycerol, 9a.

A procedure identical to that described above for 7a was followed using (R)-3-O-cis-octadec-9-ethyl-1-docosahexaenoyl-sn-glycerol (R)-9 (70 mg, 0.11 mmol), DMAP (11 mg, 0.10 mmol), EDAC (30 mg, 0.16 mmol), dry dichloromethane (2.5 mL) and EPA FFA (41 mg, 0.14 mmol), as well as additional EPA FFA (10 mg, 0.03 mmol). The compound was purified on a silica gel column with diethyl ether/dichloromethane (10:90) as an eluent. The product 9b was afforded as a yellowish oil (86 mg, 86% yield); Notebook reference: EKRII_55B. [α]²⁰₀D -6.3 (c 0.97 benzene); IR (NaCl) 3012 (=C-H cis), 2925 (C-H), 1742 (C-O), 1653 (C=C), 1125 (C-O-C) cm⁻¹; HRMS (ESI) m/z calcd for C₆₅H₁₀₀O₁₃ + Na: 959.7463; found 959.7437 amu. ¹H-NMR (400 MHz,CDCl₃) δ 5.43-5.28 (m, 24H, =CH), 5.22-5.17 (m, 1H, CH₂CHCH₂), 4.35 (dd, J = 11.9, 3.7 Hz, 1H, CH₂OCO), 4.17 (dd, J = 11.9, 6.5 Hz, 1H, CH₂OCO), 3.57-3.50 (2xdd, J =10.6, 5.3 Hz, 2H, CH₂CH₂O), 3.47-3.37 (2xdt, J = 9.4, 7.3 Hz, 2H, OCH₂CH₂), 2.88-2.77 (m, 18H, =CH₂CH₂CH=), 2.41-2.37 (m, 4H, CH₂CH₂COO in DHA), 2.34 (t, J = 7.6 Hz, 2H, CH₂COO in EPA), 2.14-2.03 (m, 6H, =CH₂CH₂CH= in EPA and DHA, and =CH₂CH₂CH= in EPA), 2.02 (quartet (br), J = 6.5 Hz, 4H, =CH₂CH₂ in 1-O-alkyl), 1.70 (quintet, J = 7.4 Hz, 2H, CH₂CH₂COO in EPA), 1.54 (quintet (br), J = 6.8 Hz, 2H, OCH₂CH₂), 1.38-1.21 (m, 22H, CH₂H), 0.97 (t, J = 7.5 Hz, 6H, CH₃ in EPA and DHA), 0.88 (t (br), J = 6.8 Hz, 3H, CH₃ in 1-O-alkyl) ppm; ¹³C NMR (CDCl₃) δ 172.84 (β C=O), 172.66 (α C=O), 132.03 (2), 129.93, 129.81, 129.35, 128.89, 128.86, 128.57 (2), 128.29, 128.27 (2), 128.24, 128.19, 128.17, 128.10, 128.07 (2), 128.02, 127.87 (2), 127.79, 127.01 (2), 71.76 (OCH₂CH₂), 70.14 (CH₂CH₂CH₂), 68.93 (CH₂OCO), 62.95 (CH₂OCO), 33.97, 33.72, 31.90, 29.77 (2), 29.56, 29.52 (2), 29.45, 29.32 (2), 29.29,

**Synthesis of the diacylglycerols**

1,3-Dieicosapentaenoylglycerol.

To a mixture of glycerol (35 mg, 0.38 mmol), EPA acetoxime ester (302 mg, 0.84 mmol) and 4 Å molecular sieves (90 mg) in dry dichloromethane (2 mL), immobilised *Candida antarctica* lipase (50 mg) was added and the resulting mixture stirred for 4 hours at 0-4°C under nitrogen atmosphere. The lipase was then filtered away, the solvent removed and the compound purified on a 4% boric acid impregnated silica gel column using chloroform/ethyl acetate (95:5) as an eluent, affording the product as clear oil (205 mg, 82% yield). Notebook reference: EKRII_90A (spectra EKRIII_90A).

**1H-NMR (400 MHz, CDCl₃) δ**

5.44–5.28 (m, 20H, =C=CH), 4.21–4.04 (m, 5H, =CH₂C₃H₃), 2.88–2.77 (m, 16H, =CHC₂H₃=C=), 2.37 (t (br), J = 7.6 Hz, 5H, CH₂COO and CHOH), 2.16–2.03 (m, 8H, =CHCH₂C₃H₃), 1.72 (quintet, J = 7.5 Hz, 4H, =CHC₃H₃), 0.97 (t, J = 7.5 Hz, 6H, CH₃) ppm.

1,3-Didocosahexaenoylglycerol.

A procedure identical to that described above for 1,3-Dieicosapentaenoylglycerol was followed using glycerol (33 mg, 0.36 mmol), DHA acetoxime ester (329 mg, 0.85 mmol), 4 Å molecular sieves (96 mg), dry dichloromethane (2 mL) and immobilised *Candida antarctica* lipase (53 mg). The product was afforded as clear oil (203 mg, 79% yield). Notebook reference: EKRII_88A (spectra EKRIII_88A).

**1H-NMR (400 MHz, CDCl₃) δ**

5.46–5.28 (m, 24H, =CH), 4.21–4.06 (m, 5H, CH₂CHCH₂), 2.88–2.80 (m, 16H, =CHCH₂CH=), 2.44–2.37 (m, 9H, CH₂CH₂COO and CHOH), 2.08 (quintet, J = 7.3 Hz, 4H, CH₂CH₂COO), 0.97 (t, J = 7.5 Hz, 6H, CH₃) ppm.

3.2.2 **Synthesis of the polyunsaturated fatty acids α-linolenic acid and docosapentaenoic acid**

The total synthesis of the two polyunsaturated fatty acids consisted of separate syntheses of the polyunsaturated tails and the head group of the PUFAs. Thus, the first part of the synthesis involved creating the long tails via copper mediated coupling. The second part involved creating the protected head groups of ALA and DPA, respectively. The reactions used in the synthesis are described in detail and the structures and purity of all the compounds confirmed by 1H-NMR analysis. The six products formed in synthesis of the polyunsaturated tails had all previously been produced, so no validation aside from 1H-NMR, was needed to confirm their structure. The products formed in the synthesis of the head parts of ALA and DPA were further validated using 13C-NMR analysis and HRMS to confirm their exact mass. As mentioned above, the methods used to synthesise the aforementioned compounds and their analysis follows, with the exception of the last coupling reactions, i.e. coupling of the polyunsaturated tail and the head parts, which were omitted from the experimental part due to both a low quantity of the product as well as low quality of NMR-spectra of the crude compounds. The two compounds synthesised using the alternative route of synthesis of ALA were only authenticated using 1H-NMR, with the latter of the two consisting of a crude product with residues of the starting material.
Synthesis of the polyunsaturated tails

Octa-2,5-diyn-1-ol, 10.
Freshly ground K₂CO₃ (1.538 mg, 26.25 mmol), CuI (5.220 mg, 35.51 mmol) and NaI (8.092 mg, 55.85 mmol) were dissolved in dry DMF (30 mL) and the resulting mixture stirred for 15 min under nitrogen atmosphere. Then 1-bromopent-2-yn (3.990 mg, 27.14 mmol) and prop-2-yn-1-ol (1.528 mg, 27.26 mmol) were added to the solution, with additional DMF (10 mL), and the resulting mixture stirred overnight. Then, the solution was quenched with saturated aqueous NH₄Cl, extracted with diethyl ether and the solvent removed. The crude product, a red oil (3.14 g, 95% yield), was further purified on a silica gel column using ethyl acetate/petroleum ether (3:7) as an eluent, affording the product 10 as an orange oil (2.76 g, 83%). Notebook reference: EKRIII_31A. ¹H-NMR (400 MHz, CDCl₃): δ 4.26 (t, J = 2.0 Hz, 2H, HOCH₂), 3.19 (quintet, J = 2.0, 2.2 Hz, 2H, C≡CCH₂C≡C), 2.18 (quartet of triplets, J = 7.5, 2.2 Hz, 2H, C≡CCH₂CH₃), 1.60 (s (br), 1H, HOCH₂), 1.12 (t, J = 7.5 Hz, 3H, CH₃) ppm. ¹³C-NMR (CDCl₃): δ 82.45, 80.86, 78.34, 72.64, 51.32, 13.81, 12.34, 9.81 ppm.

1-Bromoocta-2,5-diyn, 11.
A mixture of triphenylphosphine (PPh₃) (13.922 mg, 53.08 mmol) and carbontribromide (CBr₃) (8.868 mg, 26.74 mmol) dissolved in dichloromethane (200 mL) was chilled to 0°C and stirred for 15 minutes under nitrogen atmosphere. Then, 10 (octa-2,5-diyn-1-ol) (2.503 mg, 20.49 mmol) dissolved in dichloromethane (50 mL) was slowly added to mixture and the resulting solution stirred for 1 hour at 0°C, followed by 1 hour at room temperature. The solvent was removed and the product passed through a silica gel platter using ethyl acetate/petroleum ether (3:7) as an eluent to remove any salts formed and then solvents removed, affording the product 11 as a yellowish oil (1.591 mg, 80% yield). Notebook reference: EKRIII_33A. ¹H-NMR (400 MHz, CDCl₃): δ 3.92 (t, J = 2.4 Hz, 2H, BrCH₂), 3.21 (quintet, J = 2.4 Hz, 2H, C≡CCH₂C≡C), 2.18 (quartet of triplets, J = 7.5, 2.4 Hz, 2H, C≡CCH₂CH₃), 1.12 (t, J = 7.5 Hz, 3H, CH₃) ppm.

6-(Trimethylsilyl)hexa-2,5-diyn-1-ol, 12.
Freshly ground K₂CO₃ (2.491 mg, 18.89 mmol), CuI (3.419 mg, 17.95 mmol) and NaI (5.360 mg, 35.76 mmol) were dissolved in dry DMF (15 mL) and the resulting mixture stirred for 15 min under nitrogen atmosphere. Then (3-bromoprop-1-ynyl)trimethylsilane (3.488 mg, 17.99 mmol) and prop-2-yn-1-ol (1.057 mg, 18.89 mmol), with additional dry DMF (10 mL), were added to the solution and the resulting mixture stirred overnight. The solution was then quenched with saturated aqueous NH₄Cl, extracted with diethyl ether, and finally the solvent removed, affording the product 12 as a red oil (2.870 mg, 96% yield). Notebook reference: EKRIII_27A. ¹H-NMR (400 MHz, CDCl₃): δ 4.26 (t (br), J = 2.2 Hz 2H, HOCH₂CC), 3.25 (t, J = 2.2 Hz, 2H, C≡CCH₂C≡), 1.64-1.61 (m, 1H, HOCH₂), 0.18 (s, 9H, Si(CH₃)₃) ppm.

Hexa-2,5-diyn-1-ol, 13.
To a mixture of methanol, water and dichloromethane (4:1:7, 50 mL), 12 (6-(trimethylsilyl)hexa-2,5-diyn-1-ol) (2.708 mg, 16.28 mmol)] and AgNO₃ (1.383 mg, 8.14 mmol) were added and the resulting mixture stirred overnight under nitrogenous atmosphere. The reaction mixture was then quenched with NH₄Cl-solution, extracted with diethyl ether during an aqueous workup and the solvent removed. The product was then purified on a silica gel column using ethyl acetate/petroleum ether (3:7) as an eluent and...
the solvents removed. The product 13 was afforded as a orange to red oil (720 mg, 47% yield). Notebook reference: EKRIII_29A. \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta 4.27\) (t, \(J = 2.1\) Hz, 2H, HOCH\(_2\)), 3.22 (dt, \(J = 2.1, 2.6\) Hz, 2H, C≡CH\(_2\)C≡C), 2.08 (t, \(J = 2.6\) Hz, 1H, CCH\(_1\)), 1.69 (s, 1H, HOCH\(_2\)) ppm.

Tetradeca-2,5,8,11-tetraynol, 14.
A solution of CsCO\(_3\) (1.118 mg, 3.43 mmol), CuI (658 mg, 3.45 mmol) and NaI (847 mg, 5.85 mmol) in dry DMF (6 mL) was stirred for 15 min under nitrogen atmosphere. Then 11 (1-bromoocta-2,5-diyne) (737 mg, 3.98 mmol) and 13 (hexa-2,5-diyn-1-ol) (250 mg, 2.66 mmol) were added to the solution, with additional 6 mL of dry DMF, and the resulting mixture stirred overnight. The solution was then quenched with saturated aqueous NH\(_4\)Cl, extracted with diethyl ether and the solvent removed affording a crude product, a dark oil (445 g, 84% yield), that was purified on a silica gel column using ethyl acetate/petroleum ether (3:7) as an eluent. This afforded the product 14 as an orange to brown oil (232 g, 44%). Notebook reference: EKRIII_44A. \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta 4.26\) (t, \(J = 2.0\) Hz, 2H, HOCH\(_2\)), 3.22-3.18 (m, 2H, HOCH\(_2\)C≡CCH\(_2\)), 3.18-3.13 (m, 4H, C≡CCH\(_2\)C≡CCH\(_2\)=C), 2.18 (quartet of triplets, \(J = 7.5, 2.3\) Hz, 2H, CH\(_2\)CH\(_3\)), 1.12 (t, \(J = 7.5\) Hz, 3H, CH\(_3\)) ppm.

1-bromotetradecan-2,5,8,11-tetrayne, 15.
A mixture of triphenylphosphate (PPh\(_3\)) (662 mg, 2.52 mmol) and carbontetrabromide (CBr\(_4\)) (388 mg, 1.17 mmol) dissolved in dichloromethane (16mL) was chilled to 0°C and stirred for 15 minutes under nitrogenous atmosphere. Then 14 (tetradeca-2,5,8,11-tetraynol) (180 mg, 0.90 mmol) dissolved in dichloromethane (4 mL) was slowly added to the mixture and the resulting solution stirred for 1 hour at 0°C followed by 1 hour at room temperature. The solvent was removed and the product passed through a silica gel platter using ethyl acetate/petroleum ether (3:7) as an eluent to remove any salts formed and then solvents removed, affording the product 15 as a brown oil (151 mg, 64% yield). Notebook reference: EKRIII_46A. \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta 3.91\) (t, \(J = 2.3\) Hz, 2H, BrCH\(_2\)), 3.23 (quintet, \(J = 2.3\) Hz, 2H, BrCH\(_2\)C≡CCH\(_2\)), 3.17-3.12 (m, 4H, C≡CCH\(_2\)C≡CCH\(_2\)=C), 2.18 (quartet of triplets, \(J = 7.5\), 2.3 Hz, 2H, CH\(_2\)CH\(_3\)), 1.12 (t, \(J = 7.5\) Hz, 3H, CH\(_3\)) ppm.

Synthesis of \(\alpha\)-linolenic acid (ALA)

(3-Methyloxetan-3-yl)methyl 8-bromooctanoate, 16.
To a solution of 3-methyl-3-oxetanemethanol (113 mg, 1.11 mmol), EDAC (233 mg, 1.21 mmol) and DMAP (15 mg, 0.12 mmol) dissolved in dry dichloromethane (2.5 mL), 8-bromooctanoic acid (247 mg, 1.11 mmol) was added and the resulting mixture then stirred for 4 hours at room temperature under nitrogen atmosphere. When the starting materials had been consumed, according to TLC analysis, the resulting reaction mixture was passed through silica gel, after partial removal of solvent, using a mixture of diethyl ether/petroleum ether (1:1) as an eluent. The product 16 was afforded as a clear oil after removal of solvent by rotary evaporation in vacuo (340 mg, 98% yield). Notebook reference: EKRIII_1D. HRMS (ESI) \(m/z\) calced for C\(_{13}\)H\(_{23}\)BrO\(_3\) + Na: 329.0723; found 329.0716 amu. \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta 4.52\) (d, \(J = 6.0\) Hz, 2H, OCH\(_2\)C), 4.39 (d, \(J = 6.0\) Hz, 2H, OCH\(_2\)), 4.16 (s, 2H, C\(_3\)H\(_2\)O), 3.40 (t, \(J = 6.9\) Hz, 2H, CH\(_2\)Br), 2.36 (t, \(J = 7.5\) Hz, 2H, COCH\(_2\)), 1.85 (quintet, \(J = 7.3, 6.9\) Hz, 2H, CH\(_2\)CH\(_2\)Br), 1.65 (quintet (br), \(J = 7.4\) Hz, 2H, COCH\(_2\)H\(_2\)), 1.44 (quintet (br), \(J = 7.3, 6.9\)Hz, 2H, CH\(_2\)CH\(_2\)CH\(_2\)Br), 1.37-
1.30 (m, 4H, COCH₂CH₂CH₂H), 1.33 (s, 3H, CH₃) ppm. \(^{13}C\)-NMR (CDCl₃): \( \delta \) 173.75, 79.57 (2), 68.47, 39.09, 34.12, 33.83, 32.67, 28.91, 28.38, 27.95, 24.82, 21.20 ppm.

1-(7-Bromoheptyl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, 17.

To a stirred solution of 16 [(3-methyloxetan-3-yl)methyl-8-bromoocatanoate] (846 mg, 2.75 mmol) dissolved in dry dichloromethane (12 mL), under nitrogen atmosphere at -15°C, BF₃·etherate (173 µL, 1.37 mmol) was slowly added. The resulting mixture was kept at -15°C for 9 hours or until the starting material had been consumed, according to TLC analysis. The reaction was then quenched with triethyl amine (TEA) (383 µL, 2.75 mmol), diluted with diethyl ether and the solution filtered through a filter paper. Next the solvent was removed on a rotary evaporator and the residue re-dissolved in dichloromethane, passed through a short 5% TEA impregnated silica gel using dichloromethane as an eluent and the solvent removed. The product 17 was afforded as a clear semisolid compound (676 mg, 80% yield). Notebook reference: EKRIII_5E. HRMS (ESI) \( m/z \) calcd for C\(_{18}\)H\(_{23}\)BrO\(_3\) + Na: 329.0723; found 329.0716 amu. \(^1\)H-NMR (400 MHz, CDCl₃): \( \delta \) 3.89 (s, 6H, CCH\(_2\)O), 3.39 (t, \( J = 6.9 \) Hz, 2H, CH\(_2\)Br), 1.84 (quintet (br), \( J = 7.3, 7.0 \) Hz, 2H, CH\(_2\)CH\(_2\)Br), 1.67-1.63 (m, 2H, OCCCH\(_2\)), 1.47-1.38 (m, 4H, CH\(_2\)CH\(_2\)Br and OCCH\(_2\)CH\(_2\)), 1.34-1.25 (m, 4H, CH\(_2\)CH\(_3\)), 0.79 (s, 3H, CH₃) ppm. \(^{13}C\)-NMR (CDCl₃): \( \delta \) 109.02, 72.68 (3), 36.64, 33.97, 32.81, 30.21, 29.24, 28.59, 28.05, 23.02, 14.56 ppm.

Trimethyl(9-(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)non-1-ynyl)silane, 18.

To a stirred solution of HMPA (1.03 mL, 5.92 mmol) and THF (6 mL), ethynyl-trimethylsiline (498 mg, 5.07 mmol) was added and the resulting mixture stirred at -78°C for 15 minutes. Then, n-BuLi (2.96 mL, 2 M, 5.92 mmol) was added to the solution and it stirred for 3 hours at -78°C. Finally 17 (1-(7-bromoheptyl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane) (518 mg, 1.69 mmol) dissolved in THF (3 mL) was added slowly to the mixture and it stirred for 1 ½ hours at the same temperature before being slowly allowed to reach room temperature while stirred overnight. After TLC analysis had confirmed that the starting material had been consumed the reaction mixture was quenched with 0.05 M NaHCO₃ solution and the compound extracted with diethyl ether during an aqueous workup. The solvents were removed and the product 18 afforded as a slightly yellow oil (206 mg, 95% yield). Notebook reference: EKRIII_7G. HRMS (ESI) \( m/z \) calcd for C\(_{18}\)H\(_{22}\)O\(_3\)Si + Na: 347.1301; found 347.1297 amu. \(^1\)H-NMR (400 MHz, CDCl₃): \( \delta \) 3.89 (s, 6H, CCH\(_2\)O), 2.10 (t, \( J = 7.2 \) Hz, 2H, C\(\equiv\)CCH\(_3\)) 1.67-1.63 (m, 2H, OCCCH\(_2\)), 1.53-1.46 (m, 2H, C\(\equiv\)CCH\(_2\)CH\(_2\)), 1.45-1.41 (m, 2H, OCCCH\(_2\)CH\(_2\)), 1.39-1.34 (m, 2H, C\(\equiv\)CCH\(_2\)CH\(_2\)H), 1.30-1.26 (m, 4H, CH\(_2\)CH\(_2\)), 0.79 (s, 3H, CH₃), 0.14 (s, 9H, Si(CH\(_3\))\(_3\)) ppm. \(^{13}C\)-NMR (CDCl₃): \( \delta \) 109.05, 107.76, 84.19, 72.58 (3), 36.70, 33.21, 29.33, 28.90, 28.66, 28.61, 23.06, 19.83, 14.57, 0.18 (3) ppm.

4-methyl-1-(non-8-ynyl)2,6,7-trioxatricyclo[2.2.2.2]octane, 19.

To a solution of freshly ground K\(_2\)CO\(_3\) (325 mg, 2.35 mmol) dissolved in dry methanol (10 mL), 18 (trimethyl(9-(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)non-1-ynyl)silane) (381 mg, 1.17 mmol) dissolved in dry methanol (5 mL) was added and the resulting mixture stirred for 4 hours under nitrogen atmosphere. The reaction mixture was then quenched with a basic solution, 0.05 M NaHCO₃, and extracted with diethyl ether during an aqueous workup. After removal of the solvent the product 19 was afforded as a yellow to brown oil (389 mg, 94 % yield). Notebook reference: EKRIII_21A. HRMS (ESI) \( m/z \) calcd for C\(_{18}\)H\(_{23}\)O\(_3\) + Na: 275.1618; found 275.1625 amu. \(^1\)H-NMR (400 MHz, CDCl₃): \( \delta \) 3.89 (s, 6H, CCH\(_2\)O), 2.16 (dt, \( J = 2.6, 7.1 \) Hz, 2H, HC\(\equiv\)CCH\(_2\)), 1.92 (t, \( J = 2.6 \) Hz, 1H, 20.94 ppm. 47
**HC=CH\(_2\), 1.68-1.63 (m, 2H, OCCH\(_2\)), 1.55-1.47 (m, 2H, HC=CH\(_2\)CH\(_2\)), 1.45-1.34 (m, 4H, OCCH\(_2\)CH\(_2\) and HC=CH\(_2\)CH\(_2\)CH\(_2\)), 1.30-1.27 (m, 4H, CH\(_2\)CH\(_2\)), 0.79 (s, 3H, CH\(_3\)) ppm.**

\(^{13}\)C-NMR (CDCl\(_3\)): δ 109.05, 84.80, 72.58 (3), 68.00, 36.69, 30.21, 29.34, 28.93, 28.64, 28.47, 23.08, 18.37, 14.57 ppm.

Trimethyl(12-(4-methyl-2-6-7-trioxabicyclo[2.2.2]octan-1-yl)dodeca-1,4-diynyl)silane, **22.**

A solution of Cs\(_2\)CO\(_3\) (513 mg, 1.59 mmol), Cul (151 mg, 0.79 mmol) and NaI (297 mg, 1.98 mmol) in dry DMF (12 mL) was stirred for 15 min under nitrogen atmosphere. Then (3-bromoprop-1-ynyl)trimethylsilane (151 mg, 0.79 mmol) and **19** 4-methyl-1-(non-8-ynyl)2,6,7-trioxatricyclo[2.2.2]octane (100 mg, 0.39 mmol) were added to the solution, with additional dry DMF (8 mL), and the resulting mixture stirred over a night. The solution was then quenched with 0.05 M NaHCO\(_3\), extracted with diethyl ether during an aqueous workup. The workup caused the formation of emulsion which was dealt with using gravity filtration. Finally the solvent was removed, affording the crude product as brown liquid (114 mg, 80% yield), that was purified on a basic alumina column using petroleum ether/ethyl acetate (7:3) as eluent. This afforded the product **22** a brown oil (74 mg, 52%). Notebook reference: EKRIII_84A. \(^1\)H-NMR (400 MHz, CDCl\(_3\)): δ 3.89 (s, 6H, CCH\(_2\)), 3.18 (t, J = 2.4 Hz, 2H, CCH\(_2\)CC), 2.13 (tt, J = 2.4, 7.1 Hz, 2H, CCH\(_2\)CH\(_2\)), 1.67-1.63 (m, 2H, CCH\(_2\)), 1.50-1.41 (m, 4H, CCH\(_2\)CH\(_2\)CH\(_2\)), 1.39-1.33 (m, 2H, CCH\(_2\)CH\(_2\)), 1.31-1.25 (m, 4H, CH\(_2\)CH\(_2\)), 0.79 (s, 3H, CH\(_3\)), 0.16 (s, 9H, Si(CH\(_3\))\(_3\)) ppm.

1-(dodeca-8,11-diynyl)- 4-methyl-2-6-7-trioxabicyclo[2.2.2]octane, **23.**

To a solution of **22** (trimethyl(12-(4-methyl-2-6-7-trioxabicyclo[2.2.2]octan-1-yl)dodeca-1,4-diynyl)silane) (70 mg, 19.3 mmol) in THF (4 mL), under inert condition at -78°C, chilled TBAF (193 µL, 19.3 mmol) in THF (2 mL) was slowly added. The resulted mixture was stirred at -78 to -50°C for 6-7 hours, or until the starting materials had been consumed according to TLC analysis. The crude mixture was then immediately passed through basic alumina column using petroleumether/diethyl ether (1:1) that had been cooled to -15°C as an eluent. At last the solvent was removed affording the crude product **23** as a dark compound (54 mg, 96% yield). Notebook reference: EKRIII_86A. \(^1\)H-NMR (400 MHz, CDCl\(_3\)): δ 3.89 (s, 6H, CCH\(_2\)), 3.15 (quartet, J = 2.5 Hz, 2H, CCH\(_2\)C), 2.13 (tt, J = 2.4, 7.1 Hz, 2H, CCH\(_2\)CH\(_2\)), 2.05 (t, J = 2.7 Hz, 1H, HCCH\(_2\)), 1.67-1.63 (m, 2H, CCH\(_2\)), 1.51-1.41 (m, 4H, CCH\(_2\)CH\(_2\)CH\(_2\)), 1.38-1.33 (m, 2H, CCH\(_2\)CH\(_2\)), 1.29-1.26 (m, 4H, CH\(_2\)CH\(_2\)), 0.79 (s, 3H, CH\(_3\)) ppm.

**Synthesis of docosapentaenoic acid**

(3-Methyloxetan-3-yl)methyl 6-bromohexanoate, **24.**

To a solution of 3-methyl-3-oxetanemethanol (135 mg, 1.32 mmol), EDAC (278 mg, 1.45 mmol) and DMAP (18 mg, 0.15 mmol) dissolved in dry dichloromethane (3 mL), 6-bromohexanoic acid (257 mg, 1.32 mmol) was added and then stirred for 3 hours at room temperature under nitrogen atmosphere, or until the starting materials had been consumed. The resulting reaction mixture was then, after partial removal of solvent, passed through silica gel using a mixture of petroleum ether and diethyl ether (1:1) as an eluent. The product **24** was afforded as clear an oil after removal of solvent by rotary evaporation and vacuo (618 mg, 86% yield). Notebook reference: EKRIII_38B. HRMS (ESI) m/z calcd for C\(_{11}\)H\(_{19}\)BrO\(_2\) + Na: 301.0410; found 301.0419 amu. \(^1\)H-NMR (400 MHz, CDCl\(_3\)): δ 4.51 (d, J = 6.0 Hz, 2H, OCH\(_2\)C), 4.39 (d, J = 6.0 Hz, 2H, OCH\(_2\)C), 4.17 (s, 2H, CCH\(_2\)O), 3.41 (t, J = 6.7 Hz, 2H, CH\(_2\)Br), 2.38 (t, J = 7.4 Hz, 2H, COCH\(_2\)H), 1.88 (quintet (br), J = 7.5, 6.8 Hz,
ing material had been consumed, according to TLC analysis.

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\text{Product (49 mg, 0.16 mmol) in dry methanol (1.5 mL) was added and the resulting mixture stirred for 10 hours or until the starting material had been consumed, according to TLC analysis. The reaction was then quenched with TEA (419 µL, 3.01 mmol), diluted with diethyl ether and the solution filtered through a filter paper. Then, the solvent was removed on a rotory evaporator and the residue redissolved in dichloromethane, passed through a short 5% TEA impregnated silica gel using dichloromethane as an eluent and the solvent removed.}
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The product was afforded as a clear oil (335 mg, 80% yield). Notebook reference: EKRIII_56A. HRMS (ESI): m/z calcd for \( \text{C}_{13}\text{H}_{19}\text{BrO} + \text{Na}: 301.0410; \) found 301.0409 amu.

1H-NMR (400 MHz, CDC13): \( \delta 3.89 \) (s, 6H, \( \text{CC}H_2O \)), 3.40 (t, \( J = 6.9 \) Hz, 2H, \( \text{CH}_2\text{Br} \)), 1.84 (quintet (br), \( J = 7.0 \) Hz, 2H, \( \text{CH}_2\text{Br} \)), 1.69-1.65 (m, 2H, \( \text{OCCH}_2\text{Br} \)), 1.50-1.39 (m, 4H, \( \text{CH}_2\text{Br} \) and \( \text{OCCH}_2\text{Br} \)), 0.80 (s, 3H, \( \text{CH}_3 \)) ppm. 13C-NMR (CDCl3): \( \delta 108.91, 72.58 (3), 36.44, 33.78, 32.69, 30.23, 27.99, 22.36, 14.55 \) ppm.

Trimethyl(7-(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)hept-1-ynyl)silane, 26.

To a stirred solution of HMPA (320 µL, 1.84 mmol) and THF (3 mL), ethynyl-trimethylsilane (156 mg, 1.59 mmol) was added and the resulting mixture stirred at -78°C for 30 minutes. Then, \( n\)-BuLi (921 µL, 2 M, 1.84 mmol) was added to the solution and it stirred for 3 hours at -78°C. Finally 25 \( (1-(7\text{-bromopentyl})-4\text{-methyl-2,6,7-trioxabicyclo}[2.2.2]\text{octane}) \) (147 mg, 0.53 mmol) dissolved in THF (1 mL) was added slowly to the mixture and it stirred at -78°C for additional 1 ½ hours before being slowly allowed to reach room temperature while stirred overnight. After TLC analysis had confirmed that the starting material had been consumed, the reaction mixture was quenched with 0.05 M NaHCO3 solution, the product extracted with diethyl ether during an aqueous workup and the solvent removed. This afforded the product 26 as a slightly yellow oil (149 mg, 95% yield). Notebook reference: EKRIII_56A. HRMS (ESI) m/z calcd for \( \text{C}_{13}\text{H}_{20}\text{O}_3\text{Si} + \text{Na}: 319.1700; \) found 319.1706 amu. 1H-NMR (400 MHz, CDC13): \( \delta 3.89 \) (s, 6H, \( \text{CC}H_2O \)), 2.20 (t, \( J = 7.1 \) Hz, 2H, \( \text{C}=\text{CCH}_3 \)), 1.69-1.65 (m, 2H, \( \text{OCCH}_2\text{H} \)), 1.54-1.49 (m, 2H, \( \text{C}=\text{CCH}_2\text{H} \)), 1.47-1.42 (m, 2H, \( \text{OCCH}_2\text{H} \)), 1.41-1.35 (m, 2H, \( \text{C}=\text{CCH}_2\text{H} \)), 0.80 (s, 3H, \( \text{CH}_3 \)), 0.13 (s, 9H, \( \text{Si(CH}_3)_3 \)) ppm. 13C-NMR (CDCl3): \( \delta 108.99, 107.66, 84.26, 72.58 (3), 36.58, 30.22, 28.66, 28.53, 22.69, 19.76, 14.56, 0.17 (3) \) ppm.

1-(hept-6-ynyl) 4-methyl-2,6,7-trioxatricyclo[2.2.2]octane, 27.

To a solution of freshly ground K2CO3 (44 mg, 0.32 mmol) dissolved in dry methanol (1 mL), 26 (trimethyl(7-(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)hept-1-ynyl)silane) (49 mg, 0.16 mmol) in dry methanol (1.5 mL) was added and the resulting mixture stirred for 4 hours under nitrogen atmosphere. The reaction mixture was then quenched with a basic solution, 0.05 M NaHCO3, and extracted with diethyl ether during an aqueous workup. After removal of the solvent, the product 27 was afforded as a yellow to brown oil (35 mg, 94% yield). Notebook reference: EKRIII_56A. HRMS (ESI) m/z calcd for \( \text{C}_{13}\text{H}_{20}\text{O}_3\text{Na} + \text{Na}: 246.1305; \) found 247.1303 amu. 1H-NMR (400 MHz, CDC13): \( \delta 3.89 \) (s, 6H, \( \text{CC}H_2O \)), 2.18 (dt, \( J = 2.6, 7.1 \) Hz, 2H, \( \text{HC} \equiv \text{CCH}_2 \)), 1.92 (t, \( J = 2.6 \) Hz, 1H, \( \text{HC} \equiv \text{CCH}_2 \)), 1.69-1.65 (m, 2H, \( \text{OCCH}_2\text{H} \)), 1.56-1.49 (m, 2H, \( \text{HC} \equiv \text{CCH}_2\text{H} \)), 1.47-1.36 (m,
4H, OCCH$_2$CH$_2$ and HC≡CCH$_2$CH$_2$H), 0.80 (s, 3H, CH$_3$) ppm. $^{13}$C-NMR (CDCl$_3$): $\delta$
108.98, 84.68, 72.58 (3), 68.05, 36.56, 30.22, 28.58, 28.37, 22.66, 18.29, 14.56 ppm
4 Conclusions

Twelve ether lipids of the 1-O-alkyl-2,3-diacyl-sn-glycerol type were synthesised using pure saturated or monounsaturated 1-O-alkyl-sn-glycerols and long chain n-3 polyunsaturated fatty acids. Their synthesis exploited both enzymatic and chemical acylation. First, six adducts of 1-O-alkyl-3-acyl-sn-glycerol were formed using enzymatic reaction, where an immobilized lipase, CAL, and the activated form of the corresponding PUFA were used to acylate alkyl glyceryl ethers. The reactions resulted in pure compounds in very good to excellent yields. The mid positions of these intermediate compounds were then esterified, with the same or the other PUFA, with chemical acylation, resulting in the twelve 1-O-alkyl-2,3-diacyl-sn-glycerols. The method employed used EDAC as a coupling agent and DMAP as a catalytic base and resulted in twelve pure compounds in very good to excellent yields. The compounds were all isolated and characterized by traditional organic chemistry methods. The products are chiral so the specific rotation of the twelve compounds was also determined.

The total syntheses of the two polyunsaturated fatty acids, ALA and DPA, were conducted in a similar manner. They have comparable structures, since both are comprised of a long alkyl chain separating the carboxyl acid end from the polyunsaturated tail. Their synthesis consisted of protection of the carboxyl acid end of the alkyl chains, followed by an alkylation that resulted in a terminal acetylene. The idea was to couple this intermediate compound to a polyunsaturated tail that was synthesised using a series of copper mediated coupling reactions. Unfortunately, due to complications in the synthesis and in the purification process, the last coupling reaction did not afford the desired product in either acceptable purity or yield. As a result, the last steps of the proposed route could not be completed, i.e. the partial hydrogenation of the triple bonds and the removal of the carboxyl acid protection group. An alternative route could entail elongation of the terminal acetylene with a propargyl halide, which could then in turn be elongated until either it had reached its optimal length or had been coupled to a shorter polyyne tail. All the compounds synthesised for the part of the project were isolated and characterized by traditional organic chemistry methods, most of them in good yield. The alternative route looks promising, and thus, it is likely that changes in the complete synthesis of ALA and DPA would result in the desired products.
References


