Chemoenzymatic synthesis of enantiopure structured triacylglycerols and palm olein alcoholysis by lipase

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

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Abstract

In recent years enzymes have gained increased attention as practical biocatalysts with application both in the industry as well as lab-scale synthesis. Lipases have been studied widely, but our research focused on utilising them in the asymmetric synthesis of structured triacylglycerols (TAG) and palm olein alcoholysis.

A six step chemoenzymatic process was developed for synthesis of ABC type TAG. Starting from enantiopure solketal 1-*O*-benzylglycerol was synthesized. Pure stearic acid was then introduced to the primary hydroxyl group by immobilized *Candida antarctica* lipase (CAL), followed by catalytic hydrogenation of the benzyl protective group to afford a regioisomerically pure 1-MAG. A subsequent introduction of a second different saturated fatty acid (varying from acetic acid to palmitic acid), exclusively to the vacant primary hydroxyl group by CAL, afforded a non-symmetric regioisomerically pure 1,3-DAG that was finally acylated at the mid position with pure docosahexaenoic acid (DHA) by the EDCI coupling reaction. Both antipodes of the enantiopure TAG described above have been synthesized in good to excellent yields (63-97%) and fully characterised by traditional organic synthesis analysis.

Ten lipases were tested for their ability to catalyse ethanolysis of palm olein at a good conversion rate and selectivity towards saturated fatty acids that would enable separation of the saturated and unsaturated fatty acids in the oil. After some initial screening, two lipases were selected for further testing. A ^{1}H NMR based method was developed for determining the conversion and to provide further insight into the progress of the ethanolysis reaction.

Útdráttur

Undanfarið hefur áhugi efnafræðinga sífellt aukist á notkunarmöguleikum ensíma sem lífhvata bæði í iðnaði og í efnasmíðum. Lípasar hafa mikið verið rannsakaðir, en rannsóknir okkar beindust að því að nýta þá annars vegar við efnasmíðar á handhverfuhreinum stöðubundnum þríglýseríðum og hins vegar til að alkóhólrjúfa pálmaolíu.

Sex skrefa efnasmíð, sem byggir bæði á hefðbundnum lífrænum aðferðum og ensímhvötuðum, hefur nú verið þróuð til að smíða ósamhverf stöðubundin þríglýseríð af ABC gerð. Byrjað var á að smíða hendið 1-*O*-bensýlglýseról úr handhverfuhreinu sólketali, á endastæða hýdroxýlhópinn var síðan innleidd sterínsýra með aðstoð *Candida antarctica* lípasa (CAL) og bensýlhópurinn loks fjarlægður með hvataðri vetnun til myndunar á hendnu 1-mónóglýseríði. Því næst var sama lípasa beitt til að tengja aðra frábrugðna mettaða fitusýru (allt frá ediksýru til palmitínsýru) inn á lausa endastæða hýdroxýlhópinn til myndunar á ósamhverfu hendnu 1,3-díglýseríði sem að síðustu var asýlað í miðstöðu með hreinni dókósahexaensýru (DHA) með EDCI kúplunarhvarfi. Báðar handhverfur þeirra þríglýseríða sem hér hefur verið lýst hafa verið smíðaðar í allt frá góðri upp í ágætri nýtni (63-97%) og hefur bygging þeirra verið staðfest með hefðbundnum aðferðum.

Tíu lípasar voru prufaðir til að kanna hæfni þeirra til að hvata etanólrof á pálmaolíu á góðum umbreytingar hraða með sértækni gagnvart mettuðum fitusýrum sem myndi gera kleift að aðgreina mettuðu og ómettuðu fitusýrurnar í olíunni. Skimun á lípösunum tíu leiddi til þess að tveir þeirra voru valdir til frekari athugana. Þróuð var aðferð til að ákvarða umbreytinguna og öðlast betri innsýn í efnahvarfið er byggir á ^{1}H NMR litrófsgreiningum.

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Abbreviations

TAG – Triacylglycerol

DAG – Diacylglycerol

MAG – Monoacylglycerol

CAL – *Candida antarctica* lipase

DHA – Docosahexaenoic acid

EPA – Eicosapentaenioc acid

FA – Fatty acid

PUFA – Polyunsaturated fatty acid

SAFA – Saturated fatty acid

ME – Methyl ester

EE – Ethyl ester

EDCI - 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

DMAP - 4-dimethylaminopyridine

MP or m.p. – Melting point

NMR – Nuclear magnetic resonance

GC – Gas chromatography

TLC – Thin layer chromatography

ee – enantiomeric excess

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At last I would like to thank my family and friends for their support during my studies.

1 Introduction

This M.Sc. project is composed of two projects which both focus on using lipase in lipid modification. In the first project, *Candida antarctica* lipase (CAL) was employed in the chemoenzymatic synthesis of enantiopure structured triacylglycerols containing DHA at the mid position. The second project involved screening of ten different lipases for their ability to catalyse the ethanolysis of palm olein at a good conversion rate and high selectivity towards saturated fatty acids, but such a lipase could become useful in production of biodiesel with a side production of unsaturated triacylglycerol concentrates. A ^{1}H NMR method was developed to determine the conversion of ethanolysis and gain a better insight into the reaction mechanism.

1.1 Fatty acids

Fatty acids are carboxylic acids possessing a hydrocarbon chain. In nature they are synthesized by a fatty acid synthase complex by condensation of malonyl coenzyme A units.[1] The carbon nearest to the carboxyl carbon is denoted alfa (α), the second one is denoted beta (β) and so forth.[2] The terminal methyl end is called omega (ω).[3] Figure 1 illustrates the structure of a saturated fatty acid of the length of n carbons with denotations.

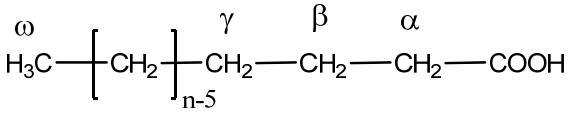

Figure 1. Structure of a saturated fatty acid possessing n carbons.

Fatty acids found in nature usually contain an even number of carbons, most often a chain length of 14-24 carbons.[4] Fatty acids are generally straight chains and if they contain double bonds they are in the *cis* configuration. Even though straight chains and *cis* double bonds are the most common structure, fatty acids can also be found branched, with odd number chains, trans double bonds or a variety of functional groups in nature. Fatty acids devoid of double bonds are called saturated, monounsaturated if they have one double bond and polyunsaturated (PUFA) if they contain more than one double bond. In animal tissue fatty acids can have one to six *cis* double bonds but higher plants and algae rarely contain more than three.[1] Fatty acids both have a systematic name originated in the IUPAC name system and a common name which often refers to their historical source.[2] Fatty acids can also be described by a shorthand designation on the form of X:Y, were X is the number of carbons in the chain and Y is the number of double bonds. Unsaturated fatty acids also have additional shorthand to denote the position of the double bonds on the form of (*n*-x), where x is the position of the first double bond counting from the ω-end. Using the ω end as a point to determine the position of the first double bond makes it easier to see

the relationship between unsaturated fatty acids and their precursor, rather than counting from the carboxyl carbon as is conventional. In polyunsaturated fatty acids the double bonds are usually separated by a methylene group and thus from the shorthand designation the position of all the double bonds can be predicted even though not formally stated. In Table 1, the names of some common fatty acids can be found, both their systematic and common names along with their shorthand designation.[1]

Table 1. The names and designation of some common fatty acids[1]

Systematic name	**Common name**	**Shorthand designation**
Ethanoic	Acetic	2:0
Butanoic	Butyric	4:0
Hexanoic	Caproic	6:0
Octanoic	Caprylic	8:0
Decanoic	Capric	10:0
Dodecanoic	Lauric	12:0
Tetradecanoic	Myristic	14:0
Hexadecanoic	Palmitic	16:0
Octadecanoic	Stearic	18:0
9-octadecenoic	Oleic	18:1 *n*-9
9,12-octadecadienoic	Linoleic	18:2 *n*-6
9,12,15-octadecatrienoic	α-Linolenic	18:3 *n*-3
5,8,11,14-eicosatetraenoic	Arachidonic	20:4 *n*-6
5,8,11,14,17-eicosapentaenoic	EPA	20:5 *n*-3
4,7,10,13,16,19-docosahexaenoic	DHA	22:6 *n*-3

Even though saturated fatty acids can have a wide range of conformations because of their free rotation about the C-C bonds, their fully extended shape is the most energy favourable. This allows them to pack closely together in crystals causing the higher saturated fatty acids to be solid at room temperature. As their unsaturation increases they can no longer pack as close together causing decrease in melting point, even one double bond can have the effect that results in the fatty acid to be liquid at room temperature, like 18:1 *n*-9. Also as their unsaturation increases their susceptibility to oxidation increases.[1,4]

The most common saturated fatty acids are stearic and palmitic acid but the most common unsaturated fatty acid is oleic acid. Figure 2 shows the structures for some of the common unsaturated fatty acids.

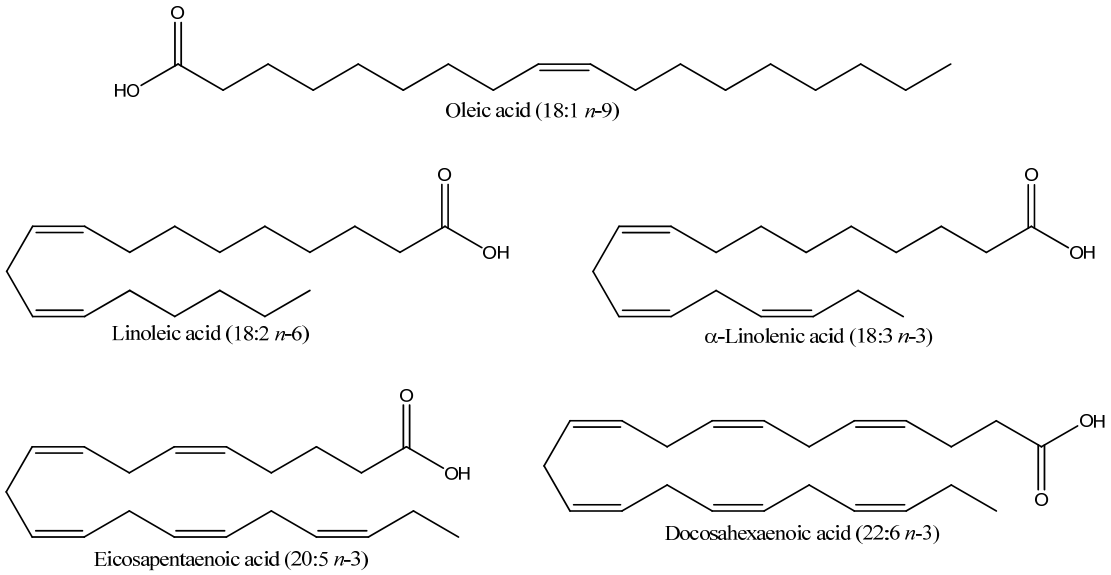

Figure 2. Structure of some common unsaturated fatty acids.

Linoleic acid and α-linolenic acid are the so-called essential fatty acids, which means that mammals cannot synthesize them by themselves but have to get them from their diet. These fatty acids are however found in plants.[4] Linoleic acid (18:2 *n*-6) is a precursor for other longer ω-6 fatty acids like arachidonic acid; it is an essential component of the phospholipid membrane and a precursor of eicosanoids. α-Linolenic acid (18:3 *n*-3) serves as a precursor for the ω-3 fatty acids like EPA and DHA. Even though our bodies can synthesize them, they are also essential dietary components and mainly found in fish. EPA is a precursor for prostanoids and both EPA and DHA are known to have beneficial health effects.[1,9] Fatty acids are rarely found in their free form in nature but rather esterified as a part of various lipids, like triacylglycerols, phospholipids or ether lipids.[3]

1.2 Triacylglycerols

Triacylglycerol (TAG) is a glycerol esterified with three fatty acids. If all the three fatty acids are of the same kind then it is said to be a simple triacylglycerol. If it contains two or three different fatty acids then it is said to be a mixed triacylglycerol, but they are far more common. Diacylglycerols (DAG, two fatty acids per glycerol) and monoacylglycerols (MAG, one fatty acid per glycerol) are usually only found in trace amount in animal and plant tissues. Figure 3 shows the structure of a triacylglycerol.

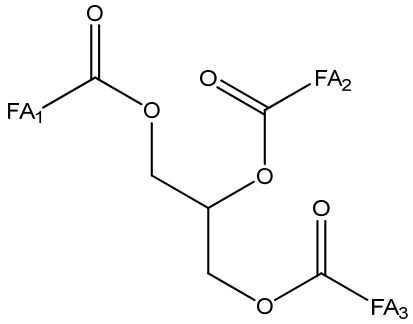

Figure 3. Structure of triacylglycerol

The role of triacylglycerols in animals is to serve as energy reserves. They are stored and synthesized in specialized cells called adipocytes which make up the adipose tissue. For warm-blooded animals living in cold environment, like whales, seals, penguins and polar bears, the adipose tissue serves as an important thermal insulator. Almost all commercial fats and oils are composed almost entirely of triacylglycerols.[1,3]

Glycerol is prochiral with a stereogenic centre about the central carbon. A stereospecific numbering (*sn*) system based on the Fischer projection of a natural L-glycerol is used to identify each of the positions as shown in Figure 4. The prefix *sn* is then used in the compound name, if not the compound is either racemic or its stereochemistry is unknown. When a group is introduced at the *sn*-1 or *sn*-3 position of the glycerol it becomes chiral possessing *S* or *R* configuration, respectively. If the same group is then introduced at the other end position the chirality is lost. However, if a different group is introduced at the other end the chirality is conserved. If the group at the *sn*-1 position has a higher priority than that at the *sn*-3 position then the glycerol derivative has *S* configuration, but *R* configuration if reversed.[1,5]

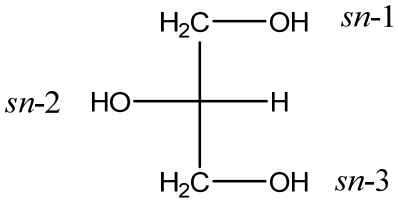

Figure 4. Stereospecific numbering of glycerol.

Recently so-called structured triacylglycerols have gained increased attention. Structured triacylglycerols are predesigned to have certain fatty acids at certain position of the glycerol backbone. They may find use as functional food or pharmaceuticals.[6,7]

1.3 Health effects of n-3 PUFA

Since the reports on Inuit of Greenland having a lower cardiovascular mortality rate than expected based on their high fat traditional diet of seal meat, whale blubber and fatty fish, many researchers have studied the health effects of long chain n-3 fatty acids (EPA and

DHA).[8] Numerous epidemiological studies have linked fish and fish oil consumption to lowered cardiovascular disease risk. Intervention and case-control studies have also shown this relationship between fish intake and cardiovascular diseases where it was shown that an intake of only one fatty fish meal per week caused 50% reduction in risk of cardiac arrest. Fish oils have been found to prevent cardiac arrhythmias, reduce blood triglycerides and decrease thrombosis. The n-3 fatty acids are known to have secondary protection effects, intervention studies on patients who have survived myocardial infarction have shown that significant reduction in recurrence and death if patients were given fish oil. DHA has also been associated with a reduced risk for coronary artery diseases because of its effect on HDL/LDL cholesterol ratio and total cholesterol/HDL ratio.[8,9,10]

The anti-inflammatory effects of n-3 PUFA are thought to contribute to some of their abilities to lower the risk of cardiovascular diseases.[8] Because of their anti-inflammatory effects they have been studied as possible therapy for some inflammatory conditions. Studies on the effects of n-3 fatty acids on inflammatory bowel disease, asthma and cystic fibrosis have been inconclusive, some reporting good effects while others showed no change in the conditions.[9] However, n-3 PUFA have shown some therapeutic effects on rheumatoid arthritis.[9,11]

Studies have shown that DHA is important for development of the brain and eyes of infants. Since DHA is found in breast milk women are encouraged to breastfeed and take DHA supplementary to ensure they get enough.[10,12] DHA is also important for normal brain function in adults. Learning disabilities and depression have been linked to deficiencies of DHA. High ratio of n-6/n-3 fatty acids has been linked to violent and aggressive behaviour.[10] EPA has shown to have beneficial effects on patients with schizophrenia.[13] Fish consumption and especially DHA have shown protective effects against dementia and Alzheimer's disease.[14,15]

1.4 Lipases

Lipases are serine hydrolases which catalyse the hydrolysis of lipids, but since the reaction is reversible they can also catalyse the formation of acylglycerols from glycerol and fatty acids. Most lipases have a lid that covers their catalytic site, when active their lid opens to make the binding site available for the substrate. Many lipases have a catalytic triad of serine, histidine and aspartic acid.[16,17] As for many enzymes, lipases have found their use in synthetic chemistry and many reports have been published over the last decades on that subject.[18,19] One of the reasons they have been found so useful in organic synthesis is due to their selectivity, but they can be regioselective, stereoselective or structure-specific. Many lipases are 1,3-regioselective towards glycerol, that is they prefer the primary hydroxyl groups at the *sn*-1 and *sn*-3 while the *sn*-2 position remains unchanged. Such lipases have been found useful in interesterification and transesterification reactions and also in the synthesis of structured triacylglycerols.[6,17] One such lipase, the *Candida antarctica* lipase (CAL), has been used with good results in the chemoenzymatic synthesis of symmetrically structured triacylglycerols of the MLM (medium – long – medium) type containing EPA or DHA at the mid position like the one shown in Figure 5.[7]

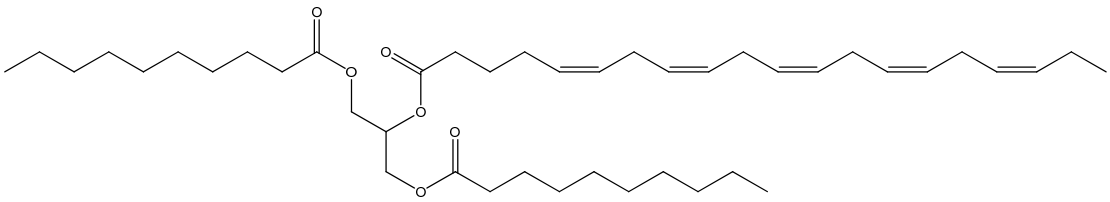

Figure 5. Symmetrically structured triacylglycerol 2-eicosapentaenoyl-1,3-didecanoylglycerol

Because the lipases offer mild reaction conditions, they have been found useful in synthesis involving polyunsaturated fatty acids. This results in reducing the risk of oxidation, double bond migration, *cis-trans* isomerisation and polymerization.[17,20] Lipases can also offer lower reaction temperatures that reduce the risk of an acyl migration side reaction, but this is important in the synthesis of structured lipids.[7] Many factors, such as water activity, immobilization support and quality of substrate interface, can lead to a change in enzyme activity and selectivity.[16] Even changing the reaction medium from water to an organic solvent can change in the efficiency and stereoselectivity of the enzyme.[19] Because of all the good properties of these enzymes they may yet to find more uses in organic synthesis, such as the synthesis of structured enantiopure lipids.

1.5 Acyl migration

Acyl migration is a spontaneous intramolecular rearrangement of acyl groups on partially acylated polyols such as glycerol. Numerous factors can speed up this process such as temperature, pH, presence of acid or base, type of solvent and the support material of immobilized lipases.[21] In the synthesis of structured triacylglycerols it is especially important that this side reaction is maintained at minimum. Figure 6 shows all possible reactions and intermediates in lipase catalyzed acylation of glycerol.[7]

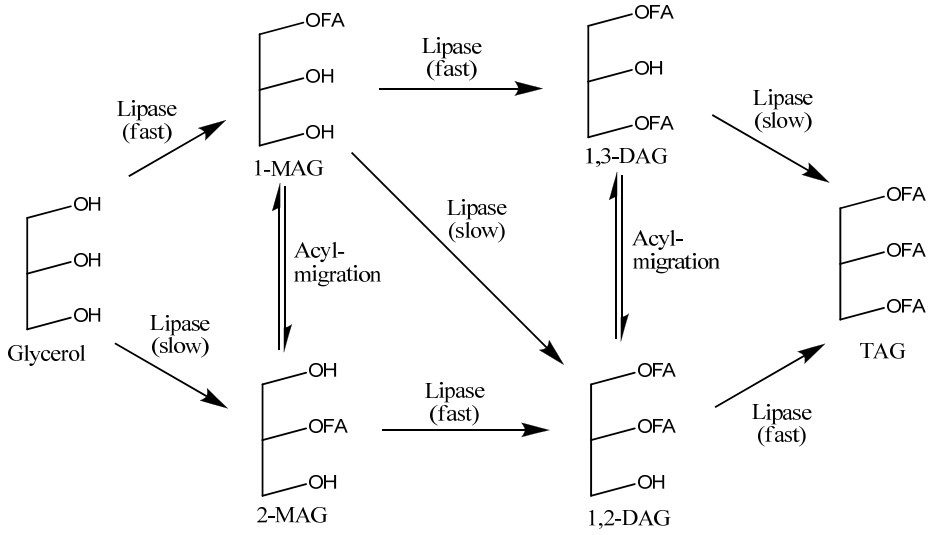

Figure 6. All possible reactions and intermediates in lipase catalyzed acylation of glycerol.

It is generally assumed that the acyl migration processes are significantly slower than the processes involving the lipase acting on the terminal positions, but still significantly faster than processes involving lipase acting on the mid position.[20] Investigation of the acyl migration process has revealed that at thermodynamic equilibrium the ratio between the two DAG regioisomers is approximately 70% 1,3-DAG and 30% 1,2-DAG[22] and the two MAG regiosiomers is approximately 90:10% 1-MAG:2-MAG.[23]

1.6 Chirality[5]

Enantiomers, although having a different absolute configuration, have identical chemical properties such as chromatographic (TLC, HPLC, GC) and spectroscopic (IR, NMR) behaviour and physical properties including melting point and solubility. However if the two enantiomers are mixed it results in a change in melting point and solubility but the chemical properties will remain the same. In order to distinguish between and separate the enantiomers based on their chemical properties it is necessary to introduce an external chiral influence, such as a chiral solvent (NMR) or chiral stationary phase (HPLC).

Even though being identical in their properties, enantiomers have one property that differentiates them and that is their optical activity. Chiral compounds are optically active which means that they have the ability to rotate the plane of plane-polarized light. If an enantiopure compound rotates the plane 20° clockwise (+) then the other enantiomer should rotate it 20° anticlockwise (-). Optical rotation is measured in a polarimeter and most often at a wavelength of 589 nm which corresponds to the wavelength of the sodium-D line emission. A sample of known concentration in an achiral solvent is put in a cell of known length. It is then placed in the polarimeter and the light of a sodium vapour lamp is passed through the cell. When the plane-polarized light passes through the sample it results in a non-equivalent right and left handed circularly polarised vectors which when summed give the overall rotation observed. The size of the rotation depends on the structure of the compound and to some degree the presence of a chromophore which can interact with the polarized light. If no chromophore is present in the chiral compound especially if it is completely non-polarized, such as saturated hydrocarbons, it may have extremely small optical rotation. The specific rotation is determined using the following equation:

$$[\alpha]_{\lambda}^{t} = \frac{100 * \alpha'}{l * c}$$

where α' is the observed rotation, l is the cell path length in dm (usually l = 1), c is the concentration in g per 100 cm^3 (100 mL) of solvent, t is the temperature in Celsius and λ is the wavelength of incident light in nm. When measuring samples one must take into an account the factors that can affect the measured rotation, which are the temperature, concentration, wavelength and solvent. So if two samples believed to be the same are to be compared these factors must be the same for both measurements for them to be comparable. It is estimated that errors in measured rotation are at least ±4% from the combined effects of temperature and concentration changes.

Optical rotation can be used to determine the enantiopurity of a chiral compound, if the specific rotation of an enantiopure compound is known then the optical purity (%) can be

calculated from the measured optical rotation of the sample. Enantiopurity is usually expressed as enantiomeric excess (ee) defined as:

% ee = % major enantiomer - % minor enantiomer

So if the ratio of the enantiomers is 85:15, then the ee is 70%. With advances in HPLC and NMR technologies and better chiral separation methods they have become more important in determining enantiopurity.

But why are asymmetric synthesis and the ability to distinguish and separate enantiomers so important? The building blocks of life (amino acids, carbohydrates, DNA) are chiral and only found in one enantiomeric form. When the chiral receptor site of an enzyme interacts with a biologically active chiral compound, like a drug, there is no wonder that the two enantiomers do not interact in the same way with the enzyme and can have different effects. These different effects can range from the one enantiomer being inactive to having serious health effects. The drug thalidomide is an example where both the enantiomers have a sedative effect but one of them, the (-)-enantiomer, also causes foetal deformities. The alkaloid (-)-levorphanol and its enantiomer (+)-dextrorphan are both used as drugs but have very different acvitity. (-)-Levorphanol is a narcotic analgesic 5-6 time stronger than morphine, while (+)-dextrophan is a cough suppressant used in cough medicine. The (+)-enantiomer of DOPA is inactive since the enzyme can't digest it but a build up of it can be dangerous. So even though the one enantiomer is inactive it may be necessary to be able to synthesis only the active one, even economical. Nowadays all isomers of new drugs are tested for their possible effects before being put on the market.

1.7 Biodiesel

With growing energy demand, decreasing fossil fuel supplies and more ecological awareness, a demand in alternative and renewable energy has risen. One of the alternative energy sources which have gained attention is biofuel, that is ethanol and biodiesel as substitutes for gasoline and petroleum diesel, respectively.[24,25] Biodiesel is defined as fatty acid mono-alkyl esters of vegetable oils and animal fats, with methyl and ethyl esters being most commonly used although propyl and butyl esters can also be used.[26,27]

Biodiesel is thought of as an environmentally friendly fuel because it is made from renewable resources like vegetable oil; it is biodegradable and is considered to be CO_2-neutral.[27] Exhaust emission of biodiesel is also considerably lower than for diesel.[26]

Industrially, biodiesel is produced with chemical catalysts such as NaOH, KOH and sodium methoxide. This method has some disadvantages such as the oil needs to be refined and there is formation of soaps and pigments. These by-products need to be removed because they reduce the quality of the biodiesel.[24] The disadvantages of this method have turned the interest of many researchers towards enzyme-catalysed production of biodiesel. In recent years many reports have been made on the use of enzyme-catalysed transesterification of oils.[24,27,28,29] A ^{1}H NMR method like the one described in this thesis could prove a quick way of determining the conversion of such enzyme-catalysed reactions. It might become especially useful when the goal is not to get 100% conversion, rather to get methyl or ethyl esters of certain fatty acids (saturated and less unsaturated)

and leave the remaining fatty acid (PUFA) on the glycerol backbone and thus only to get to a certain conversion level. The former may find use as biodiesel with the latter serving as a source of bioactive (PUFA) that might be further concentrated and purified.[17]

2 Chemoenzymatic synthesis of enantiopure structured triacylglycerols

A highly efficient two step chemoenzymatic synthesis of symmetrically structured triacylglycerols (TAG) of the MLM type (medium-long-medium) possessing pure eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) at the mid position has recently been described.[7] Such structured TAGs have gained increased attention of scientists as dietary and health supplements.[30,31,32] The primary goal of this project was to design a viable synthesis of asymmetrically labelled ABC type structured TAGs and utilize it to synthesize a variety of such enantiopure TAGs.

Enantiopure ABC type structured TAGs may find uses in various applications like screening for biological effects of individual fatty acids, possibly related to their position on the glycerol backbone. They may also be useful as chiral substrates to investigate lipase enantioselectivity towards TAG, as standards, fine chemicals, drug supplements, drug carriers, or even as drugs. The methodology could also be used to introduce isotopically labelled fatty acids into a predetermined position of TAG.

The ideal way to synthesize such enantiopure structured TAG would be to use very regio- and enantioselective lipases that would selectively or exclusively acylate one position, *sn*-1, *sn*-2 or *sn*-3. In that way one could synthesize these triacylglycerols with three different fatty acids as shown in Figure 7.

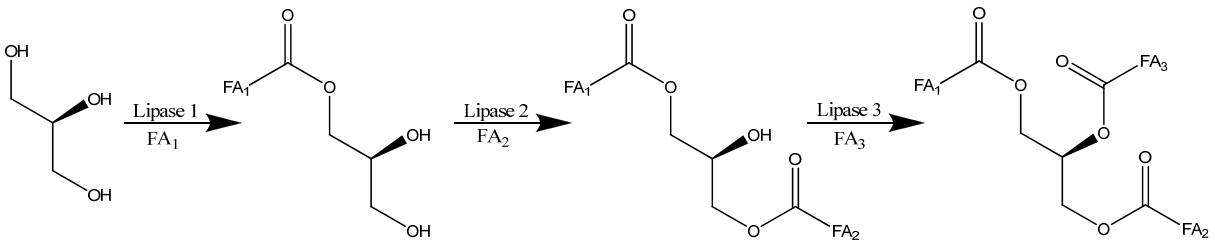

Figure 7. Ideal synthesis of triacylglycerols.

Lipase 1 would have to be highly regio- and enantioselective acting exclusively at the *sn*-1 (*sn*-3) position to afford an enantiopure (*S*)-1-MAG ((*R*)-3-MAG). The second lipase would then act regioselectively on the vacant primary hydroxyl group. No reports have been made on lipases of the first type[33,34], but lipases of the second type are well known and have been used to synthesize the structured MLM type TAG previously described.[7] Another route must therefore be taken to synthesize TAG of the ABC type. Using conventional organic chemistry methods would mean that many protection/deprotection

steps would be needed. However by using nature's own technique, the lipase, together with the classic organic synthesis methods we can reduce the number of protection/deprotection steps needed. By exploiting the 1,3 regioselective properties of the *Candida antarctica* lipase (CAL) we have now designed a six step chemoenzymatic process to synthesize triacylglycerols of the ABC type.[33] A series of triacylglycerols of the ABC type were synthesized with stearic acid and DHA as A and B, respectively, and C varying from acetic acid to palmitic acid. Figure 8 gives an overview of the synthesis and the compounds synthesized. A similar task was recently completed by Kristinsson and Haraldsson using EPA at the sn-2 position (unpublished results).

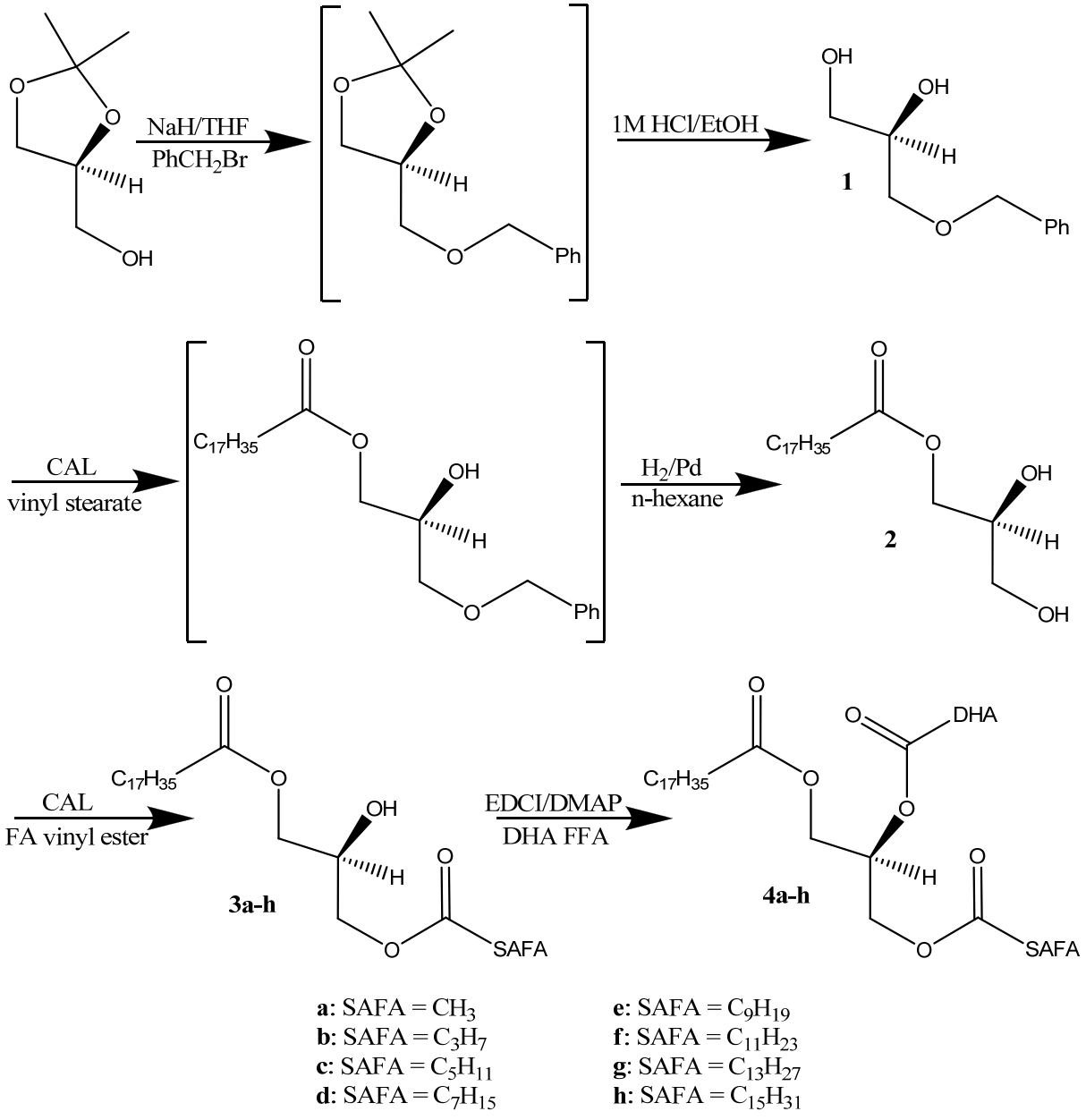

Figure 8. Overview of the synthesis of structured ABC type triacylglycerols.

Figure 8 shows the structure of the *S* enantiomer of 1-MAG, 1,3-DAG and TAG but both enantiomers of the described TAGs have been synthesized. All compounds were characterized with high-resolution ^{1}H and ^{13}C NMR, IR spectroscopy and accurate mass measurements. Optical rotation of all the compounds synthesized was measured at 20°C and 589 nm and their specific rotation determined using the equation given in chapter 1.6.

2.1 The synthesis

2.1.1 Benzylglycerols and 1-MAGs

The six-step chemoenzymatic synthesis of enantiopure structured triacylglycerols starts with a two-step one pot synthesis of chiral 1(3)-*O*-benzyl-*sn*-glycerol from enantiopure solketal. Using sodium hydride as a base in THF, a benzyl protective group was introduced to the vacant primary hydroxyl group of solketal by Williamson ether synthesis using benzyl bromide. Without isolation, the benzylated solketal was submitted to an acidic deprotection of the isopropylidene moiety yielding an enantiopure 1-*O*-benzylglycerol **1** isolated as a clear liquid by Kugelrohr distillation. (*S*)-**1** and (*R*)-**1** were obtained in 55 and 65% overall yields, respectively, as Table 2 shows. Specific rotation was obtained for (*S*)-**1** and (*R*)-**1** in dichloromethane at a concentration of 10g/100mL (Table 2). Both antipodes of enantiopure benzylglycerol are commercially available.[35]

Table 2. Yields, melting point and specific rotation of benzylglycerols and 1-MAGs.

Nr.	Compound	Yield (%)	MP (°C)	Solvent	c (g/100mL)	$[\alpha]_0^{D^2}$
(*S*)-**1**	1-*O*-benzylglycerol	55	-	CH_2Cl_2	10.10	-3.02
(*R*)-**1**	3-*O*-benzylglycerol	65	-	CH_2Cl_2	10.19	3.42
(*S*)-**2**	1-C_{18}-MAG	96	69.7-70.5	THF	6.04	2.43
(*R*)-**2**	3-C_{18}-MAG	96	74.1-75.4	THF	6.07	-2.32

The benzylglycerols, (*S*)-**1** and (*R*)-**1** were acylated at the vacant primary hydroxyl group using vinyl stearate and immobilized CAL in dichloromethane at room temperature. The reaction time was from 90 minutes up to 2 ½ hours. Without purification the acylated benzyl ether was deprotected with a palladium catalyzed hydrogenolysis in petroleum ether. The regioisomerically pure 1-octadecanoyl-*sn*-glycerols (*R*)-**2** and (*S*)-**2** were afforded as white crystals after recrystallization from petroleum ether. Excellent overall yields of 96% were obtained for both enantiomers (Table 2). Melting points of (*S*)-**2** and (*R*)-**2** were measured and as Table 2 shows the melting point of the *R* antipode is slightly higher. Specific rotation measurements in THF gave good and consistent results (Table 2).

Analysis of the ^{1}H NMR spectra of the benzylglycerols showed that the incorporation of a benzyl group had been successful and that the isopropylidene moiety had been removed. In the ^{1}H NMR spectra of the 1-MAGs the appearance of the characteristic peak pattern of the glycerol backbone of 1-MAG along with the disappearance of the aromatic protons confirmed a successful synthesis of 1-MAG. The ^{1}H NMR spectra of 1-MAG showed no traces of 2-MAG so it can be assumed that no acyl migration took place and thus no loss of

enantiopurity. Figure 12 in chapter 3.3.2 shows the ^{1}H NMR spectra of all the glyceryl moieties.

2.1.2 1,3-DAGs

(*S*)-**2** and (*R*)-**2** were acylated by the immobilized CAL at the vacant primary hydroxyl group using a second different SAFA varying from acetic acid to palmitic acid as their vinyl esters. The reaction was performed in dichloromethane and was completed after 2 ½ - 6 hours. The asymmetric 1,3-DAGs (*S*)-**3a-h** and (*R*)-**3a-h** were afforded as white crystals recrystallized from petroleum ether. Their yields and melting point are reported in Table 3. Again, the melting points of the *R* enantiomers are slightly higher, but the enantiomers should have identical melting points.[5] One explanation for this would be that some impurities were in all the 1,3-DAGs and 1-MAG of either of the enantiomers, but their ^{1}H NMR spectra showed no impurities so it seems unlikely. Another possible explanation is that they might have been measured on two different melting point apparatus. The two enantiomers were measured at different times and it is possible that a different apparatus was used each time because the chemistry department owns two apparatus of the same type.

Specific rotation of the 1,3-DAGs (*S*)-**3a-h** and (*R*)-**3a-h** was measured in dichloromethane, but because of their extremely low rotation values, as can be seen in Table 3, efforts were made to find a solvent that would give a higher rotation values. Benzene and a 1:1 mixture of ethanol/chloroform were tried, but the results were similar to those in dichloromethane. Ethyl acetate, diisopropyl ether and tert-butyl methyl ether didn't solve the DAGs properly. Because of this it was decided to stick with the first choice of solvent that is dichloromethane. Taking a closer look at the specific rotation of the 1,3-DAGs (*S*)-**3a-h** and (*R*)-**3a-h** presented in Table 3, it becomes clear that the rotation is very low and doesn't seem to follow much trend although in most cases the rotation of opposite enantiomers is in opposite directions. On both ends of the glycerol are saturated fatty acids so even though being chiral the difference between the ends is small. This small difference between the end acyl groups probably contributes to these extremely low specific rotation values. The observed rotation of (*S*)-**3a-h** and (*R*)-**3a-h** is usually on the last digit measurable by our instrument, that is specially designed to measure low optical rotation values, but this all combined probably causes the lack of trend.

Upon synthesis of (*S*)-**4a** and **b** a problem occurred with some impurities that showed up on the TLC and the ^{1}H NMR spectra. Efforts were made to separate them while doing the filtering column but that was not successful in acceptable yields. These impurities could be traced back to a faint impurity spot on the TLC of the corresponding DAGs which had been disregarded on the grounds that their ^{1}H NMR spectra were clean. Mass and ^{1}H NMR analysis of the isolated impurities revealed it to be caused by formation of symmetric 1,3-octadecanoyl-*sn*-glycerol in the synthesis of the asymmetric DAGs. Mass analysis of TAGs (*S*)-**4c-h** and (*R*)-**4c-h** showed that this happens to some extent in synthesis of all the DAGs. This caused the biggest problems in the synthesis of **3a-b** where this adduct was formed to the highest extent and where the differences in chemical properties are at the uttermost. However, this difference could be utilised to separate the impurities from the desired product.

*Table 3. Yields, melting point and specific rotation of 1,3-DAGs (S)-**3a-h** and (R)-**3a-h**.*

Nr.	$SAFA_1$	$SAFA_2$	Yield (%)	MP (°C)	c (g/100mL)	$[\alpha]_D^{20}$
(*S*)-**3a**	$-C_{17}H_{35}$	$-CH_3$	63	41.6-44.0	1.13	0.44
(*S*)-**3b**	$-C_{17}H_{35}$	$-C_3H_7$	81	50.8-51.7	1.19	0.34
(*S*)-**3c**	$-C_{17}H_{35}$	$-C_5H_{11}$	82	57.0-57.5	1.08	0.37
(*S*)-**3d**	$-C_{17}H_{35}$	$-C_7H_{15}$	80	59.1-59.8	1.08	0.28
(*S*)-**3e**	$-C_{17}H_{35}$	$-C_9H_{19}$	78	60.7-62.0	1.00	0.40
(*S*)-**3f**	$-C_{17}H_{35}$	$-C_{11}H_{23}$	78	59.7-60.2	1.03	-0.58
(*S*)-**3g**	$-C_{17}H_{35}$	$-C_{13}H_{27}$	88	63.6-64.5	1.28	0.23
(*S*)-**3h**	$-C_{17}H_{35}$	$-C_{15}H_{31}$	91	68.3-69.4	1.03	0.29
(*R*)-**3a**	$-CH_3$	$-C_{17}H_{35}$	60	45.6-46.3	0.77	-1.04
(*R*)-**3b**	$-C_3H_7$	$-C_{17}H_{35}$	85	54.0-54.7	1.04	-1.06
(*R*)-**3c**	$-C_5H_{11}$	$-C_{17}H_{35}$	92	57.3-58.2	1.02	-0.39
(*R*)-**3d**	$-C_7H_{15}$	$-C_{17}H_{35}$	83	60.7-62.0	1.02	-0.10
(*R*)-**3e**	$-C_9H_{19}$	$-C_{17}H_{35}$	87	63.6-64.4	1.01	-0.40
(*R*)-**3f**	$-C_{11}H_{23}$	$-C_{17}H_{35}$	84	65.2-66.0	0.82	-0.61
(*R*)-**3g**	$-C_{13}H_{27}$	$-C_{17}H_{35}$	90	68.2-69.6	1.25	0.00
(*R*)-**3h**	$-C_{15}H_{31}$	$-C_{17}H_{35}$	95	70.4-72.2	1.27	0.08

First, attempts were made to separate them by prep-TLC but separation was inadequate. While testing methanol as recrystallization solvent for the shorter chains (C_4 and C_2) instead of petroleum ether it became clear that the symmetric 1,3-DAG crystallized better and faster out than the shorter chain asymmetric DAGs. This enabled the separation of 1,3-DAG impurities from (*S*)-**3a-b** and (*R*)-**3a-b** by doing two recrystallizations in two different solvents: first in methanol to remove the impurities and secondly in petroleum ether to yield the pure asymmetric 1,3-DAGs desired. The amount of symmetric 1,3-DAG impurities is estimated to be 5% and 16% (mol percentage) for C_4 and C_2, respectively. At first it was thought that it might be possible that some vinyl octadecanoate or octadecanoic acid was present in the MAG used for synthesis, but both the ^{1}H NMR spectrum and the reaction condition of the MAG synthesis ruled out that possibility. We now think the most likely course of events leading to the formation of symmetric 1,3-DAGs to be lipase catalysed hydrolysis of the MAG to some extent affording a free FA and glycerol. The lipase will then in some cases introduce the free FA to the vacant primary hydroxyl group of MAG instead of the vinyl ester. It is possible that some loss of enantiopurity could have occurred during this reaction, but further analysis is needed to see to what extent that may have happened.

Analysis of the ^{1}H NMR spectra of the 1,3-DAGs showed that the characteristic peaks of the glyceryl moiety had changed from 1-MAG to 1,3-DAG revealing a successful acylation on the other end position. The spectra showed no signs of MAG, TAG or 1,2-DAG.

2.1.3 TAGs

Finally the 1,3-DAGs (*S*)-**3a-h** and (*R*)-**3a-h** were acylated at the mid position with pure DHA as free fatty acid using EDCI as a coupling reagent. The reaction was performed in

dichloromethane overnight at room temperature. The TAGs (*S*)-**4a-h** and (*R*)-**4a-h** were isolated using a short silica column as yellowish oils in good to excellent yields (63-97%). The lowest yields were obtained for (*S*)-**4a** and (*R*)-**4a** or 63 and 70%, respectively. More solvent (dichloromethane) was needed for their column chromatography than the other TAGs, but that could be a factor in their lower yields. A slight increase in the solvent polarity could help with that. Yields of individual TAGs can be found in Table 4 along with their specific rotation.

*Table 4. Yields and specific rotation of (S)-**4a-h** and (R)-**4a-h** TAGs.*

Nr.	$SAFA_1$	$SAFA_2$	Yield (%)	c (g/100mL)	$[\alpha]_D^{20}$
(*S*)-**4a**	$-C_{17}H_{35}$	$-CH_3$	63	9.90	-1.41
(*S*)-**4b**	$-C_{17}H_{35}$	$-C_3H_7$	76	10.12	-0.80
(*S*)-**4c**	$-C_{17}H_{35}$	$-C_5H_{11}$	91	8.01	-0.49
(*S*)-**4d**	$-C_{17}H_{35}$	$-C_7H_{15}$	87	9.08	-0.22
(*S*)-**4e**	$-C_{17}H_{35}$	$-C_9H_{19}$	94	10.02	-0.12
(*S*)-**4f**	$-C_{17}H_{35}$	$-C_{11}H_{23}$	89	10.01	-0.03
(*S*)-**4g**	$-C_{17}H_{35}$	$-C_{13}H_{27}$	96	10.02	-0.02
(*S*)-**4h**	$-C_{17}H_{35}$	$-C_{15}H_{31}$	80	10.16	0.00
(*R*)-**4a**	$-CH_3$	$-C_{17}H_{35}$	70	10.03	1.49
(*R*)-**4b**	$-C_3H_7$	$-C_{17}H_{35}$	83	10.18	0.84
(*R*)-**4c**	$-C_5H_{11}$	$-C_{17}H_{35}$	85	10.15	0.45
(*R*)-**4d**	$-C_7H_{15}$	$-C_{17}H_{35}$	93	10.36	0.15
(*R*)-**4e**	$-C_9H_{19}$	$-C_{17}H_{35}$	95	6.05	0.08
(*R*)-**4f**	$-C_{11}H_{23}$	$-C_{17}H_{35}$	94	3.30	0.06
(*R*)-**4g**	$-C_{13}H_{27}$	$-C_{17}H_{35}$	97	8.17	0.05
(*R*)-**4h**	$-C_{15}H_{31}$	$-C_{17}H_{35}$	97	10.31	0.01

To begin with the plan was to measure the specific rotation of the TAGs (*S*)-**4a-h** and (*R*)-**4a-h** in dichloromethane, but the optical rotation was observed to be very small in this solvent and in some cases the enantiomers were measured to have same direction of rotation. A better solvent therefore had to be found. Benzene, THF, 2-propanol, ethanol, ethyl acetate, tert-butyl metyl ether, diisopropyl ether and a 1:1 mixture of ethanol/chloroform were all tested to see if they would give better results than dichloromethane. Of all these solvents it was decided to use benzene because it gave slightly better results than the others and it also has a higher boiling point making the measurements easier. Table 4 shows the specific rotation of the TAGs (*S*)-**4a-h** and (*R*)-**4a-h** measured in benzene. When the results for the TAGs (*S*)-**4a-h** and (*R*)-**4a-h** in Table 4 are plotted against the SAFA chain length it becomes clear that the specific rotation increases as the difference in chain length increases. As more of the DHA in the mid position is exposed due to decrease in chain length the more is the difference between rotation of individual TAGs in the series. This is well displayed in Figure 9 where the curves take a steep drop at first when the chain length increases up to C_8 but after that the slope of the curve drastically levels out. It would be interesting to see if this series of TAG containing a saturated fatty acid or less unsaturated fatty acid, like oleic acid, at the mid position rather than DHA would give a similar trend in specific rotation.

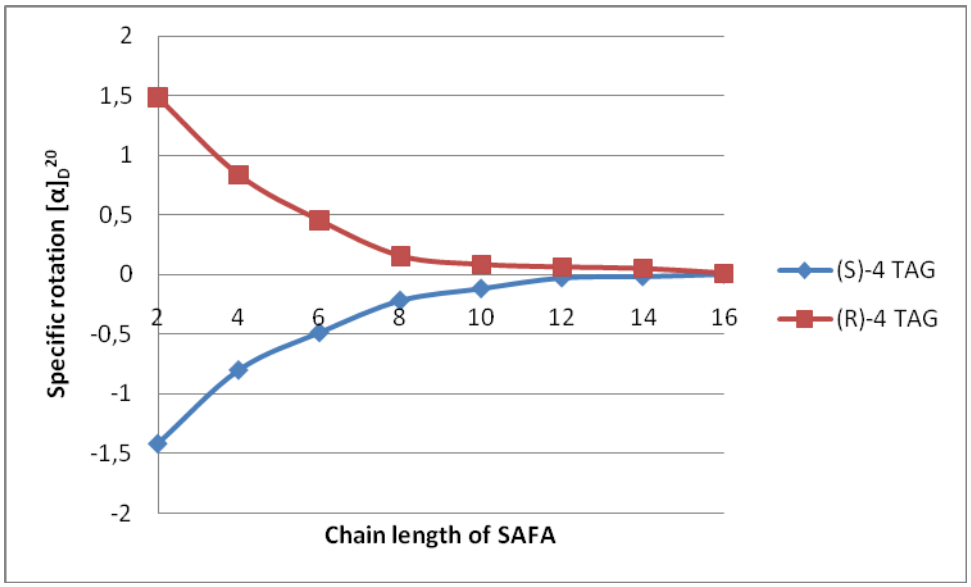

*Figure 9 Specific rotation plotted against the chain length of the varying FA of (S)-**4a-h** and (R)-**4a-h***

The ^{1}H NMR spectra of the TAGs displayed the characteristic pattern of TAG. Now present in the spectra were also peaks corresponding to the double bonds of DHA confirming successful incorporation of DHA. Figure 10 shows the ^{1}H NMR spectrum of one of the TAGs synthesized.

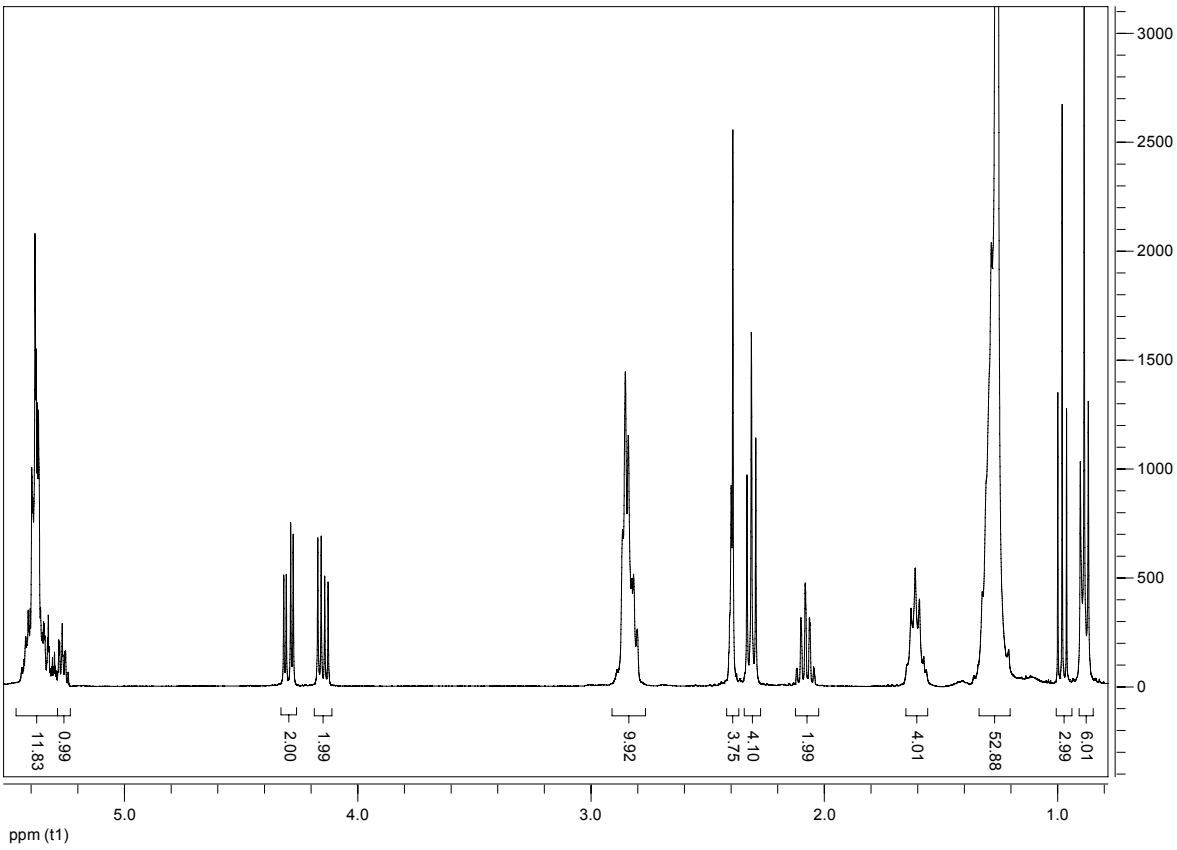

*Figure 10. ^{1}H NMR spetrum of (S)-**4h**.*

2.2 Summary

The benzylglycerols (*S*)-**1** and (*R*)-**1** were synthesized in good overall yields of 55 and 65%, respectively. Isolation was done by Kugelrohr distillation, but using column chromatography instead might improve the yields.

The 1-MAGs (*S*)-**2** and (*R*)-**2** were both isolated in the excellent overall yields of 96%. Investigation of the ^{1}H NMR spectra of the MAGs showed no signs of acyl migration in the reactions therefore no losses of enantiopurity should have occurred.

The 1,3-DAGs (*S*)-**3a-h** and (*R*)-**3a-h** were synthesized in good to excellent yields (60-95%). A side reaction occurred with a formation of symmetric 1,3-dioctadecanoyl-*sn*-glycerol. It caused the most problem in synthesis of the **3a-b** but was solved by using two different recrystallization methods to purify the **3a-b**. It is possible that some loss of enantiopurity may have occurred during this reaction, but further analysis is needed to see to what extent it happened. Specific rotation of the 1,3-DAGs (*S*)-**3a-h** and (*R*)-**3a-h**, measured in dichloromethane, is extremely small and doesn't follow much trend. Attempts were made to find a better solvent for optical rotation measurements but no solvent tested resulted in better outcomes.

The TAGs (*S*)-**4a-h** and (*R*)-**4a-h** were also isolated in good to excellent yields of 63-97%. After some testing for a good solvent for optical rotation measurements, benzene proved to be the best candidate. Its stability in terms of lower volatility made it easier to measure the rotation and it also gave higher rotation values. The specific rotation of the TAGs (*S*)-**4a-h** and (*R*)-**4a-h** follows an interesting trend when measured in benzene as is displayed in Figure 9.

On the whole, the synthesis was successful with good to excellent yields for all compounds. Some loss of enantiopurity might have occurred in the synthesis of DAGs but we believe it only to be minor. Enantiopurity of the compounds synthesized has yet to be confirmed by chiral HPLC analysis.

3 Lipase catalyzed alcoholysis of palm olein

In the summer of 2008 work began on a research project in collaboration with the Danish company Novozyme A/S. The main goal of this project was to find a lipase that could catalyse the ethanolysis of palm olein at a good conversion rate and with high selectivity towards saturated fatty acids that would enable separation of the saturated and unsaturated fatty acids in the oil. All the experiments aimed therefore at finding the right lipase and right conditions that would give high amounts of saturated ethyl esters at high conversion. In the palm olein used the most abundant fatty acids were palmitic acid (39.6%), stearic acid (4.3%), oleic acid (41.8%) and linoleic acid (11%). Saturated fatty acids therefore account for about 44% of the oil and unsaturated for about 53%. It was believed that the distribution of the fatty acids into positions of the glycerol backbone of the triacylglycerols was such that the bulk of the saturated fatty acids were located at the end positions with the bulk of the monounsaturated and polyunsaturated fatty acids located at the mid position.

The lipases were from a selection of nine immobilized lipase preparations provided by Novozyme and one lipase from Amano in Japan. It was anticipated that a 1,3-regioselective lipase displaying high preference toward the end positions of the glycerol backbone might become a suitable one, especially if that same lipase also displayed an expected preference for saturated fatty acids over unsaturated fatty acids. At the same time it was hoped that acyl migration side reactions[7] would be maintained at minimum. However, there were some concerns about the reaction temperature limited to the melting point of the oil being close to 35 °C and that temperature level might induce some acyl migration working against the regiocontrol of the reaction.

High resolution 400 MHz ^{1}H NMR spectroscopy was used to monitor the progress of the reactions and determine the extent of conversion. Two methods were developed to determine the conversion from NMR. The first one is based more on a subjective interpretation of each spectrum (see page 34). The second method was developed to make the integration more consistent and thus the results (see page 38). All the results in Chapter 3.1 were determined using the first method. One methanolysis reaction was also performed to see if these methods applied or were even better suited for conversion determination in methanolysis. Gas chromatography was then used to determine the fatty acid composition of the ethyl esters.

3.1 Results from ethanolysis.

3.1.1 Screening of lipases

The first part of the experiments involved screening of ten immobilized lipases for their ability to catalyse the ethanolysis of palm olein. Nine of the lipases were provided by

Novozymes and one was from Amano (see Table 28 in chapter 5.1). In all cases 10% dosage of lipase was used as based on the initial oil substrate weight with 2 molar equivalents of absolute ethanol and ethanol fortis. Eight lipases showed activity and they were all tested further using 20% lipase dosage and 2 molar equivalents of ethanol fortis. All these reactions were performed at 35°C and samples collected after 1, 2, 4, 8 and 24 hours. Table 5-Table 7 reveal the results from this part of the experiments showing only the extent of conversion and the ratio between saturated (S) and unsaturated (U) fatty acids in the ethyl ester product fraction. Because of the high conversion in Table 7 the ration between the saturated and unsaturated ethyl esters was only determined for the samples after 1 and 2 hours.

Table 5. Results when using 10% (wt) dosage of lipase preparation (as based on weight of TAG substrate) and 2 molar equivalents of absolute ethanol (0% water) as based on mol TAG

Time	1		2		4		8		24	
Lipase	Conv.	S/U	Conv.	S/U	Conv.	S/U	Conv.	S/U	Conv.	S/U
Lipase A	16.0	55/41	29.4	57/39	49.5	54/41	54.9	51/44	62.5	47/46
Lipase B	12.5	56/33	22.4	59/34	40.7	59/34	43.4	59/36	49.5	54/39
Lipase C	31.5	64/31	53.4	60/34	61.0	55/40	64.5	49/45	65.0	45/49
Lipase D	2.6	-	2.9	-	2.9	-	3.2	-	4.4	-
Lipase E	22.1	55/40	37.1	49/43	56.2	46/47	60.4	44/48	64.5	43/51
Lipase F	23.5	55/40	38.9	52/43	56.8	50/45	61.8	48/46	66.1	44/49
Lipase G	37.5	54/41	55.7	52/44	61.9	49/46	64.3	47/47	66.9	44/49
Lipase H	16.1	55/40	24.5	52/43	38.6	48/46	52.2	45/46	58.4	45/49
Lipase I	-	-	-	-	2.5	-	2.8	-	3.0	-
Lipase J	18.9	65/31	25.1	65/32	31.9	63/33	44.7	60/35	56.4	51/44

Table 6. Results for the same conditions as described in Table 5 when using 95% ethanol (ethanol fortis) instead of absolute ethanol.

Time	1		2		4		8		24	
Lipase	Conv.	S/U	Conv.	S/U	Conv.	S/U	Conv.	S/U	Conv.	S/U
Lipase A	17.5	54/39	32.2	55/38	49.6	52/39	53.4	49/41	57.9	45/44
Lipase B	18.8	51/31	30.9	54/32	43.4	55/33	44.9	55/34	49.4	52/38
Lipase C	47.5	58/35	62.6	53/40	65.7	46/45	65.6	43/48	66.0	41/48
Lipase D	3.0	-	3.6	-	4.4	-	7.5	-	11.3	-
Lipase E	6.3	48/46	7.9	49/43	12.3	47/40	34.1	47/44	58.7	41/47
Lipase F	20.9	51/41	43.8	49/43	55.0	47/44	57.2	46/44	61.8	42/46
Lipase G	41.4	50/43	56.8	49/45	62.2	47/45	64.3	45/46	65.8	41/48
Lipase H	8.0	51/42	10.8	50/40	17.2	48/41	31.2	46/45	47.9	44/47
Lipase I	-	-	-	-	2.6	-	2.8	-	2.9	-
Lipase J	23.8	57/34	30.0	56/35	43.4	55/36	49.6	54/38	61.2	48/44

Table 7. Results for the same conditions as described in Table 5 when using 20% (wt) dosage of lipase preparation and 2 molar equivalents of 95% ethanol (ethanol fortis).

Time	1		2		4	8	24
Lipase	Conv.	S/U	Conv.	S/U	Conv.	Conv.	Conv.
Lipase A	48.9	53/38	53.0	50/41	57.9	61.2	63.9
Lipase B	42.1	57/33	44.1	57/34	46.3	48.6	56.7
Lipase C	63.7	52/40	65.6	46/45	66.1	65.6	65.9
Lipase E	12.0	53/41	22.8	49/43	51.8	63.1	63.2
Lipase F	54.7	45/44	60.5	48/43	62.9	63.9	64.4
Lipase G	60.3	44/46	63.6	47/44	64.3	65.3	65.4
Lipase H	12.8	52/42	22.8	50/44	42.8	54.7	61.9
Lipase J	31.7	56/35	38.1	54/37	54.6	62.9	66.5

Based on the conversion, saturated FA/unsaturated FA ratio and the composition of the remaining acyl glycerides two lipases, A and C, were selected for further experiments. As can be noticed from Table 5 the best performance in terms of the sat./unsat. ratio in the ethyl esters was by lipase C, close to 65/30, however at very low conversion that is not practical. At higher and more practical conversion levels (50 – 60%) it is evident that lipases A and C were the most promising, offering a ratio of approximately 55/40.

From these results it was also decided to investigate 20% lipase dosage, absolute ethanol and to collect samples at shorter time intervals, i.e. 10, 30, 60, 90 and 120 minutes. The second part of these experiments was aimed at finding out whether the amount of ethanol used or water content of the system could affect the selectivity of the lipases towards saturated fatty acids.

3.1.2 Ethanol concentration

The first set of experiments in part 2 was aimed at comparing the effect of using 2 molar equivalents of absolute ethanol or ethanol fortis with 20% lipase dosage. The results are revealed in Table 8 and Table 9. When Table 8 and Table 9 are compared with the results for lipases A and C in Table 7 it can be noticed that after 1 and 2 hours the conversion and sat./unsat. ratio were very similar for both lipases. These results suggest that adding more water to the system does not affect the selectivity of the lipases.

Table 8. The results when using 20% (wt) dosage of lipase A (as based on weight of TAG substrate) and 2 molar equivalents of absolute ethanol as based on mol TAG. All reactions were performed at 35 °C.

T (min)	**Conv. (%)**	**TAG (%)**	**1,2-DAG (%)**	**1,3-DAG (%)**	**1-MAG (%)**	**2-MAG (%)**	**Glycerol (%)**	**S/U**
10	12.9	75.6	13.6	-	-	8.3	2.5	54 / 41
30	27.6	49.2	23.6	-	1.7	22.8	2.7	56 / 39
60	46.4	23.9	35.0	-	4.8	27.1	9.2	55 / 39
90	50.9	19.0	40.1	-	7.6	21.0	12.2	52 / 41
120	55.3	20.1	37.9	2.2	8.9	13.4	17.4	50 / 43

Table 9. The results using lipase C and the same experimental conditions as in Table 8.

T (min)	Conv. (%)	TAG (%)	1,2-DAG (%)	1,3-DAG (%)	1-MAG (%)	2-MAG (%)	Glycerol (%)	S/U
10	13.3	76.0	12.9	-	4.5	3.0	3.5	70 / 24
30	48.6	41.3	23.3	-	10.7	3.6	21.1	61 / 33
60	59.5	30.8	22.4	5.6	14.0	2.8	24.5	54 / 39
90	62.2	25.4	19.7	11.3	16.9	2.8	23.9	51 / 43
120	65.2	22.8	17.7	12.7	20.2	2.5	24.2	47 / 46

Next was to investigate whether higher ethanol concentration might affect the selectivity of the two selected lipases. As before 20% lipase dosage was used but this time with 4 molar equivalents of absolute ethanol. As can be seen in Table 10 and Table 11 the activity of both lipases decreased dramatically. Lipase C seems however to tolerate this high ethanol concentration better than lipase A, since it offered conversion between 6.6-26.9% over those 2 hours compared with only 3.0-11.7% when lipase A was used.

Table 10. Results when using 20% (wt) dosage of lipase A (as based on weight of TAG substrate) and 4 molar equivalents of absolute ethanol as based on mol TAG. The reaction was performed at 35 °C.

T (min)	Conv. (%)	TAG (%)	1,2-DAG (%)	1,3-DAG (%)	1-MAG (%)	2-MAG (%)	Glycerol (%)
10	3.0	93.6	4.7	-	0.9	-	0.8
30	4.5	90.1	6.3	-	1.8	1.8	-
60	5.7	87.9	7.9	-	1.8	1.8	0.6
90	8.7	82.7	10.7	-	0.8	4.1	1.7
120	11.7	77.5	13.2	-	0.8	6.1	2.4

Table 11. Results using of lipase C and the same experimental conditions as in Table 10.

T (min)	Conv. (%)	TAG (%)	1,2-DAG (%)	1,3-DAG (%)	1-MAG (%)	2-MAG (%)	Glycerol (%)
10	6.6	86.5	8.7	-	2.6	0.9	1.3
30	12.3	75.1	15.1	-	4.5	3.7	1.6
60	18.8	65.5	18.3	-	6.6	5.9	3.7
90	25.2	57.0	20.0	-	9.1	8.5	5.5
120	26.9	54.9	20.8	-	9.3	8.8	6.2

3.1.3 Drying of lipases

Water content of the system is one of the parameters that may influence the fatty acid selectivity of the lipases.[16] In order to test this, three different means were tried to eliminate water from the two selected lipases. First, incubating the lipases three times with excess oil before performing the reactions using 20% lipase dosage and 4 molar equivalents of ethanol used (Table 12 and Table 13). Secondly, mixing the lipase and oil

together and drying the resulting suspension under vacuum. The oil was then replaced with new dried oil before reactions (Table 14 and Table 15). Finally, the lipase was dried separately under vacuum and dried oil used in the reactions (Table 16 and Table 17).

In the incubation reactions 4 molar equivalents of ethanol were used. As can be noticed in Table 10 and Table 11 that did not provide good results, but as the results in Table 12 indicate lipase A seems to handle these conditions better after incubation. Lipase C on the other hand works even worse under these conditions after incubation. As the results in Table 13 show it can almost be concluded that this approach does not work at all.

Table 12. Results when using 20% (wt) dosage of lipase A (as based on weight of TAG substrate). The lipase was incubated 3 times with excess oil before reaction using 4 molar equivalents of absolute ethanol as based on mol TAG . The reaction was performed at 35 °C.

T (min)	Conv. (%)	TAG (%)	1,2-DAG (%)	1,3-DAG (%)	1-MAG (%)	2-MAG (%)	Glycerol (%)
10	8.3	84.1	10.0	-	0.9	2.5	2.5
30	10.0	79.7	12.0	-	2.4	4.7	1.2
60	14.4	73.1	14.6	-	1.5	8.1	2.8
90	21.0	61.5	18.5	-	1.8	15.4	2.8
120	26.1	53.2	20.7	-	2.1	20.7	3.1

Table 13. Results using lipase C and the same experimental conditions as in Table 12.

T (min)	Conv. (%)	TAG (%)	1,2-DAG (%)	1,3-DAG (%)	1-MAG (%)	2-MAG (%)	Glycerol (%)
10	8.7	86.5	6.9	-	1.7	-	4.8
30	8.2	85.5	8.5	-	2.6	-	3.4
60	8.0	85.1	8.5	-	3.4	0.9	2.1
90	8.6	84.9	8.5	-	2.5	0.9	3.2
120	8.3	83.5	10.1	-	4.1	0.8	1.5

Since experiments with 4 molar equivalents of ethanol did not provide any promising results, 2 molar equivalents were used in the reactions with lipase that had been dried under vacuum. When the results in Table 14 were compared with those in Table 4 it was noticed that lipase A offered a similar conversion, although slightly lower in Table 14, but the sat./unsat. ratio had not improved at all. Lipase C displayed almost no activity after this drying treatment as can be noticed from the results in Table 15. The results in Table 13 and Table 15 also indicate that lipase C could only be used once, but maybe if some water was brought back into the system it could be reused. Another thing that could also help explain these results is the fact that the oil sticks a lot to the carrier material of the lipase.

Table 14. Results when using 20% (wt) dosage of lipase A (as based on weight of TAG substrate). The lipase together with oil was first dried under vacuum, that oil was then replaced with new dried oil. 2 molar equivalents of absolute ethanol as based on mol TAG were used. All reactions were performed at 35 °C.

T (min)	Conv. (%)	TAG (%)	1,2-DAG (%)	1,3-DAG (%)	1-MAG (%)	2-MAG (%)	Glycerol (%)	S/U
10	10.4	84.3	7.6	-	0.8	1.7	5.6	-
30	19.1	67.5	15.5	-	2.0	10.1	4.9	54/41
60	26.3	56.4	19.3	-	3.3	15.0	6.1	54/41
90	42.8	36.1	28.6	-	4.5	18.0	12.7	54/42
120	46.8	27.4	35.9	-	8.5	15.3	12.9	52/43

Table 15. Results using lipase C and the same experimental conditions as in Table 14.

T (min)	Conv. (%)	TAG (%)	1.2-DAG (%)	1.3-DAG (%)	1-MAG (%)	2-MAG (%)	Glycerol (%)
10	4.2	89.3	9.8	-	-	-	0.9
30	4.1	90.5	7.3	-	1.8	-	0.5
60	6.0	86.2	9.5	-	3.4	0.9	-
90	6.6	78.5	15.0	-	7.1	6.3	-
120	7.9	83.0	11.7	-	3.4	0.8	1.2

When taking look at the results for the reaction when the lipase and oil were dried separately before use, it can be noticed that the activity of lipase A (Table 16) is only about one half of what it was in Table 8 and Table 14. When comparing Table 14 and Table 16 it was interesting to notice that even though the lipase went through a drying process before the reaction in both cases the conversion rate in Table 14 was higher than in Table 16. One way to explain this is that some water was still present in the system, meaning that the lipase didn't dry sufficiently when mixed with oil and then dried. If this is the case and the lipase was only truly dry in the reaction in Table 16, then some water is required to give good conversion rate. The results for lipase C (Table 17) on the other hand again imply that this lipase requires some water in order to work adequately.

Table 16. The results when using 20% (wt) dosage of lipase A (as based on weight of TAG substrate). The lipase was first dried under vacuum, the oil that was used was dried separately under vacuum. 2 molar equivalents of absolute ethanol as based on mol TAG were used. All reactions were performed at 35 °C.

T (min)	Conv. (%)	TAG (%)	1,2-DAG (%)	1,3-DAG (%)	1-MAG (%)	2-MAG (%)	Glycerol (%)
10	5.7	89.7	6.3	-	0.9	0.9	2.3
30	10.2	80.9	11.4	-	0.8	4.1	2.9
60	18.7	66.8	17.4	-	1.3	10.0	4.5
90	24.7	56.5	22.6	-	2.3	13.0	5.6
120	28.4	50.9	26.0	-	1.6	14.5	7.0

Table 17. The results using lipase C and the same experimental conditions as in Table 16.

T (min)	Conv. (%)	TAG (%)	1.2-DAG (%)	1.3-DAG (%)	1-MAG (%)	2-MAG (%)	Glycerol (%)
10	3.1	91.7	6.5	-	2.7	-	0.0
30	4.8	88.4	8.0	-	3.5	0.9	0.0
60	6.6	82.5	12.4	-	4.9	2.5	0.0
90	8.3	79.1	14.3	-	5.5	3.2	0.0
120	10.3	78.0	14.1	-	4.7	2.4	0.8

3.1.4 Titration of FFA

Final samples obtained after 24 hours from the four most promising lipases, A, C, H and J, in the experiments described in Table 5 and Table 6 were selected for titration to estimate the extent of hydrolysis side reaction for these lipases. As can be noticed from Table 18 the amount of FFA remained between 1.1-2.4% (wt.) when absolute ethanol was used. As expected the extent of hydrolysis increased when ethanol fortis was used, when the FFA levels increased to 2.4-3.3% (wt.). It is assumed that the hydrolysis levels remained constant throughout in all reactions.

Table 18. Titration of 24 hour samples from selected lipases in Table 5 and Table 6.

Lipase	Weight % of FFA	
	Abs. EtOH	EtOH fortis
Lipase A	2.4	3.2
Lipase C	1.2	3.0
Lipase H	2.0	2.4
Lipase J	1.1	3.3

3.1.5 Fatty acid composition in the acyl glyceride fraction

Fatty acid analyses were performed on all four acylglycerol fractions obtained after 4 hour reaction time in the experiments described in Table 5 for the four selected lipases (lipases A, C, H and J). This was intended to investigate how well they fit to the results obtained for the fatty acid composition of the ethyl ester fractions from these reactions. The acylglycerol fractions were obtained after separation from the ethyl esters using preparative TLC and were subsequently methylated and analysed by GLC. Based on the conversion, results from the GLC analyses of both the ethyl esters and acylglycerols the amount of saturated and unsaturated fatty acids was calculated and compared to the fatty acid composition of the initial oil. As Table 19 shows these calculated values are almost the same as the sat./unsat. ratio in the initial oil, but that was 44/53.

Table 19. Results from fatty acid analyses of the acylglycerol samples obtained after 4 hour reaction time in the experiments described in Table 5 for the four selected lipases. The acylglycerol (AG) fractions were separated from the ethyl esters (EE) by preparative TLC and subsequently analysed by GLC after methylation.

		GC analysis Area %		Calculated from conversion		
		Sat. FA	Unsat. FA	Conv.	Sat. FA	Unsat. FA
Lipase A	EE	54	41	49.5	44	53
	AG	34	66			
Lipase C	EE	55	39	61.0	44	52
	AG	27	73			
Lipase H	EE	48	46	38.6	44	53
	AG	42	58			
Lipase J	EE	63	33	31.9	44	55
	AG	35	65			

3.2 Results from methanolysis

A methanolysis was also performed on the palm olein using lipase A. The same experimental conditions were used as described in Table 5; 10% dosage of lipase based on initial oil substrate weight with 2 molar equivalents of methanol, reaction temperature was 35°C and samples collected after 1, 2, 4, 8 and 24 hours. The main purpose of this investigation was to see if the methods designed to determine the conversion of the ethanolysis reaction could be applied for methanolysis.

Table 20. Methanolysis. Results when using 10% (wt) dosage of lipase A (as based on weight of TAG substrate) and 2 molar equivalents of methanol as based on mol TAG.

T (min)	Conv. (%)	TAG (%)	1.2-DAG (%)	1.3-DAG (%)	1-MAG (%)	2-MAG (%)	Glycerol (%)
1	12.7	78.1	10.2	1.3	1.1	5.2	4.1
2	17.4	71.4	12.0	0.6	2.1	8.6	5.2
4	26.3	58.6	17.6	0.2	2.2	13.6	7.8
8	34.8	46.0	24.5	0.2	2.3	16.8	10.3
24	40.2	32.0	37.0	1.3	10.0	9.4	10.4

3.3 The use of ^{1}H NMR spectroscopy to monitor the progress of lipase catalyzed alcoholysis of triacylglycerol oil.

3.3.1 Details of the alcoholysis process

In lipase catalyzed alcoholysis of triacylglycerol oils it is assumed that most lipases act preferably at the less bulky primary alcoholic terminal positions rather than the more

hindered secondary mid-position of the glycerol backbone. This means that the first product in TAG alcoholysis is 1,2-DAG rather than the less preferred 1,3-DAG.[17] The 1,2-DAG may undergo a second fast lipase promoted alcoholysis to form 2-MAG or, alternatively, a slower temperature induced acyl migration to form the thermodynamically more stable 1,3-DAG. At thermodynamic equilibrium the ratio between the two DAG regioisomers is approximately 70% 1,3-DAG and 30% 1,2-DAG.[22] Figure 11 illustrates all potential intermediates and products formed in lipase catalyzed alcoholysis of triacylglycerols such as palm olein.

Once formed, the 1,3-DAG is anticipated to undergo fast lipase promoted alcoholysis to produce 1-MAG that subsequently undergoes a second fast alcoholysis by the lipase to form glycerol. Alternatively, the 1-MAG may also undergo slow acyl migration to form the thermodynamically less stable 2-MAG. At equilibrium the ratio between the two MAG regiosiomers is approximately 90% 1-MAG and 10% 2-MAG.[23] Once the 2-MAG forms it is believed to undergo acyl migration to form 1-MAG on which the lipase acts much faster than 2-MAG.

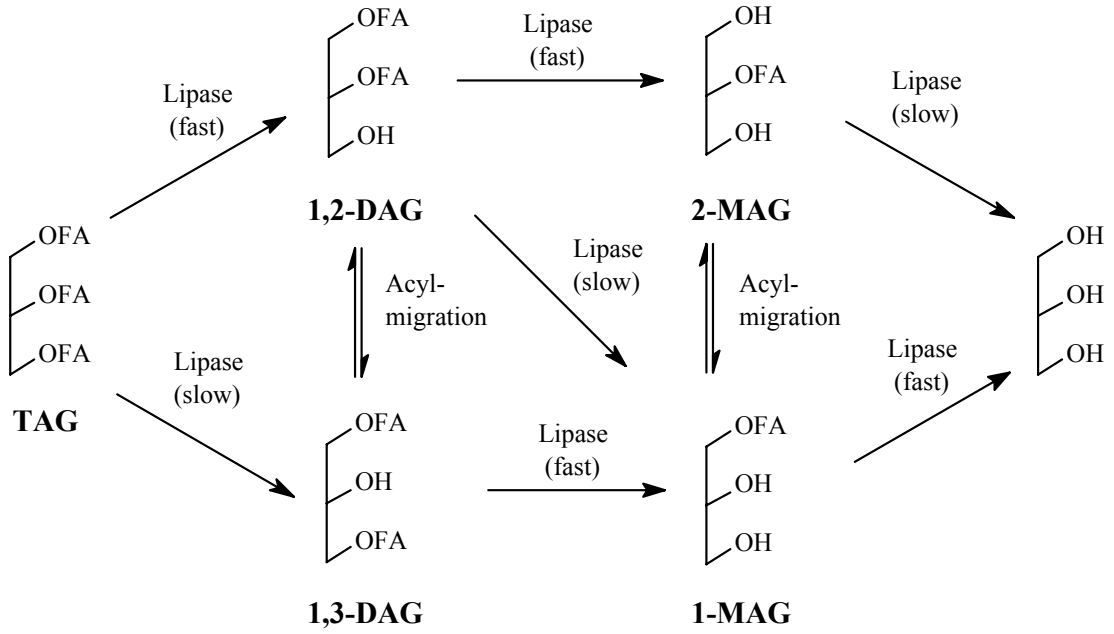

Figure 11. All potential intermediates and products formed in the ethanolysis of palm olein triacylglycerols

It is generally assumed that the acyl migration processes are significantly slower than the processes involving the lipase acting on the terminal positions, but still significantly faster than processes involving lipase acting on the mid position.[20] All this should be reflected in the results from these reactions. During the first hours the 1,2-DAG regioisomer should build up and dominate in the reaction mixture together with the initial TAG. Then, the build-up of 2-MAG is anticipated when there is still plenty of alcohol available. Towards the end of the reaction, when most of the alcohol has been consumed, 1-MAG, as formed from 2-MAG by acyl migration, is expected to take over. At that stage the 1,3-DAG also becomes noticeable in the reaction mixture. It is formed by acyl migration of 1,2-DAG that also starts diminishing in the reaction mixture.

This behaviour is pretty well reflected in Table 21 showing details for lipase A. After 2h 29% conversion into ethyl esters was reached with 48% TAG, 22% 1,2-DAG and 25% 2-MAG present. It is striking that some glycerol had already formed at such an early stage of the reaction. After 4h the conversion had reached 50% with 21% TAG, 35% 1,2-DAG, 26% 2-MAG and 11% 1-MAG present. At that stage 10% glycerol had formed as based on the initial TAG. The presence of 1,3-DAG was not detected until after 24h (6%) at 63% conversion with 25% each of TAG and 1,2-DAG present.

Table 21. The results from lipase A in the ethanolysis reaction described in Table 5, the first NMR method used for determination of conversion and glyceryl moieties.

T (h)	Conv. (%)	TAG (%)	1,2-DAG (%)	1,3-DAG (%)	1-MAG (%)	2-MAG (%)	Glycerol (%)
1	16.0	69.7	16.0	-	0.7	11.2	2.4
2	29.4	48.0	22.2	-	1.9	24.5	3.4
4	49.5	20.7	34.6	-	8.7	26.1	9.9
8	54.9	21.9	37.1	-	10.8	13.0	17.2
24	62.5	25.3	25.3	6.3	15.8	3.2	24.2

The results presented in Table 21 are used to demonstrate the details of how 400 MHz ^{1}H NMR spectroscopy was used to quantify the extent of conversion, individual acylglycerols and glycerol as the ethanolysis reaction proceeded when using 10% dose of lipase A in the presence of 2 molar equivalents of ethanol at 35 °C.

3.3.2 Quantification of individual acylglycerols

All individual acylglycerol adducts potentially involved in the reaction, 1-MAG and 2-MAG, 1,3-DAG and 1,2-DAG, as well as TAG, display quite characteristic ^{1}H NMR spectra in their glyceryl moiety proton region (5.30-3.50 ppm). This makes it quite straightforward to track down all individual acylglycerol constituents present in the reaction mixture at a time and to quantify them by reasonable or good accuracy. And, thereby, this becomes an excellent tool to monitor the progress of the reaction as it proceeds as well as monitoring the regiocontrol of the reactions.

The chemical shifts (δ) of the glyceryl protons depend very much upon whether the attached hydroxyl groups are free (lower) or acylated (higher) and also whether these protons are located at the mid-position (higher) or the end-positions (lower) of the glycerol backbone. This is particularly prominent for the proton at the mid-position undergoing a down-field shift (higher chemical shift value) of nearly 1.5 ppm upon acylation. As a general rule a proton located at the more substituted carbon at the mid-position resonates at a lower field (higher chemical shift) as compared to protons belonging to the less substituted terminal carbons. Acylation is also observed to result in a significant down-field shift of the protons of an adjacent vicinal carbon, i.e. the carbon next to the carbon possessing the acylated hydroxyl group. Also, the profile of the spectra is obviously much dependent upon the symmetry of the acylglycerol. All this is illustrated in Figure 12 for the glyceryl proton segments of the spectra that were obtained from reference samples of the corresponding palmitates of each of the acylglycerols.

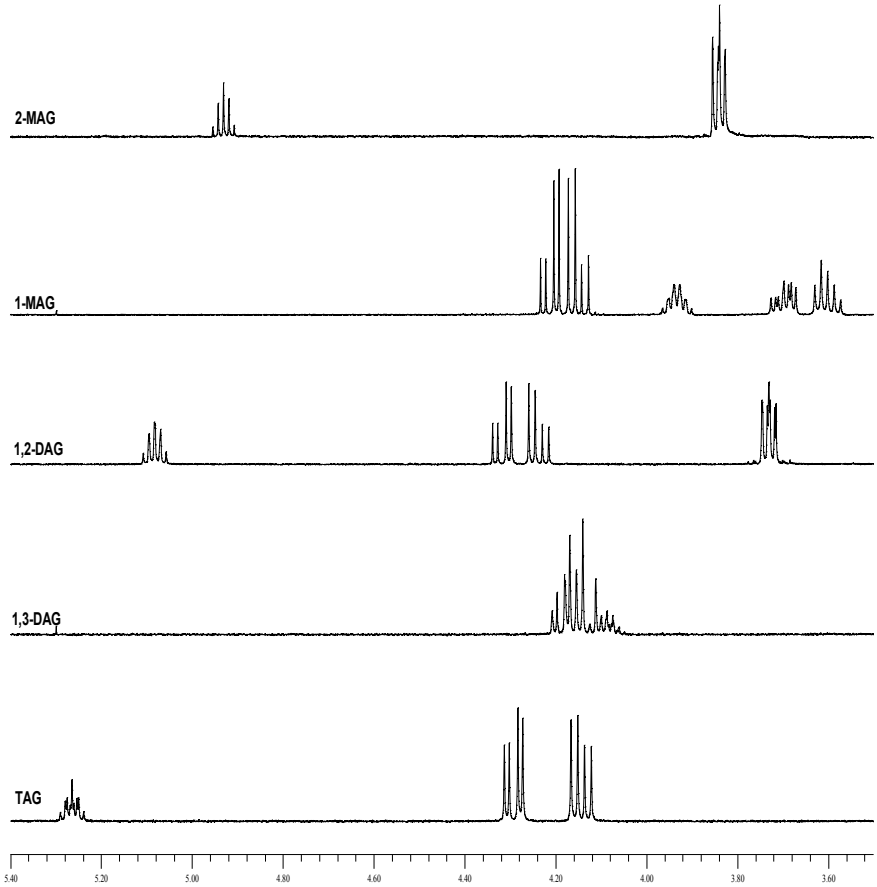

Figure 12. The glyceryl proton region of 400 MHz ^{1}H NMR spectra of individual acylglycerols: 2-MAG (top), 1-MAG (second from top), 1,2-DAG (third from top), 1,3-DAG (second from bottom) and TAG (bottom).

In the 1,3-DAG all five glyceryl protons resonated as a multiplet at 4.21-4.03 ppm. Here both end-positions are acylated with the mid-position free. In all other cases the central proton resonated as a multiplet at a distinct region well separated from the remaining protons at 4.93, 3.97-3.89, 5.11-5.06 and 5.29-5.24 ppm respectively for the 2-MAG, 1-MAG, 1,2-DAG and TAG (see Table 22). Apart from 1,3-DAG different proton type signals were quite well dispersed and well apart and obeyed a first order behaviour in all cases. Therefore, they were readily assigned and it was easy to quantify the constituents by integration of the peaks and accounting for how many protons belong to individual peaks. In 2-MAG all four methylene end-protons resonated as a multiplet at 3.86-3.83 ppm. The less symmetric 1-MAG adduct had the acylated proton pair resonating as two doublets of doublets at 4.21 and 4.15 ppm with the free-end protons resonating as two multiplets well apart at 3.72-3.67 and 3.62-3.57 ppm. The 1,2-DAG adduct similarly had two doublets of doublets at 4.32 and 4.24 for the acylated end, well separated from the non-acylated end-protons resonating as a multiplet at 3.75-3.72 ppm. Finally, the TAG adduct had its characteristic two doublets of doublets at 4.29 and 4.14 ppm. The details of the assignment are revealed in Table 22.

In a mixture comprised of several or even all constituents there is certainly some overlap between these peaks. Despite this it is relatively easy to quantify each constituent in the mixture by its ^{1}H NMR spectrum. The protons that belong to the mid-position of individual

acylglycerols, apart from the 1,3-DAG, are very well dispersed and easily quantified by integration. In TAGs comprised of unsaturated fatty acids the protons attached to the carbons of the double bonds resonate slightly downfield to the proton at the mid-position of the TAG and there is usually some overlap there. There is usually some partial overlap between some of the protons located at the end-positions of individual acylglycerols. That is relatively easy to deal with and correct for by estimation based on the peaks that belong to the protons at the mid-position or other accessible protons within the glycerol moiety. The quantification limits for individual acylglycerols by 400 MHz ^{1}H NMR spectroscopy are estimated below 0.25% as based on careful studies.[36]

Table 22. Assignment of the glycerol proton segment of the ^{1}H NMR of various acylglycerols

Acylglycerol	**Assignment**
1-MAG	4.21 (dd, J = 11.6 Hz, J = 4.8 Hz, 1H, OCO-CH_2-), 4.15 (dd, J = 11.6 Hz, J = 6.0 Hz, 1H, OCO-CH_2-), 3.97-3.90 (m, 1H, -CH_2-C*H*-CH_2-), 3.73-3.67 (m, 1H, -C*H*$_2$-OH), 3.63-3.57 (m, 1H, -C*H*$_2$-OH) ppm.
2-MAG	4.93 (quintet, J = 4.8 Hz, 1H, -CH_2-C*H*-CH_2-), 3.86-3.83 (m, 4H, -C*H*$_2$-OH) ppm.
1,2-DAG	5.11-5.06 (m, 1H, -CH_2-C*H*-CH_2-), 4.32 (dd, J = 12.0 Hz, J = 4.8 Hz, 1H, OCO-CH_2-), 4.24 (dd, J = 12.0 Hz, J = 5.6 Hz, 1H, OCO-CH_2-), 3.75-3.72 (m, 2H, -C*H*$_2$-OH) ppm.
1.3-DAG	4.21-4.06 (m, 5H, -CH_2-CH-CH_2-) ppm.
TAG	5.29-5.24 (m, 1H, -CH_2-C*H*-CH_2-), 4.29 (dd, J = 12.0 Hz, J = 4.4 Hz, 2H, -C*H*$_2$-CH-C*H*$_2$-), 4.14 (dd, J = 12.0 Hz, J = 6.0 Hz, 2H, -C*H*$_2$-CH-C*H*$_2$-) ppm.

The glycerol end-product warrants a special comment; since it is not soluble in the deuterated chloroform solvent in which the spectra are recorded and very little of it enters the reaction solution during the reactions. The glycerol content may be estimated from the extent of conversion in relation to the amount of individual acylglycerols and free fatty acids as determined by titration. Here it must be taken into account that the DAGs contribute one equivalent of ethyl esters, whereas the MAGs contribute two and the TAGs none.

3.3.3 Initial oil and determination of conversion in the ethanolysis reactions

The ^{1}H NMR spectrum of the initial palm olein oil is revealed in Figure 13. There are good agreements between the NMR spectrum and the fatty acid composition as determined by GLC analysis. The protons attached directly to the carbon-carbon double bonds resonated

as a multiplet at 5.29-5.24 ppm and their integration fits quite well to the 41.8% $C_{18:1}$ and 11.0% $C_{18:2}$ fatty acid composition results from GLC. This is further supported from the peak at 2.86-2.78 that corresponds to the methylene protons (CH_2) located in between the two double bonds in linoleic acid as well as the peak at 2.14-2.04 corresponding to protons in linoleic and oleic acids located on carbons allylic to one double bond. The integration values appear underneath the baseline of the spectrum and are roughly normalized to match number of proton equivalents underneath each peak. Detailed assignment of the oil spectrum is provided in Table 23.

Table 23. Assignment of the ^1H NMR spectrum of the palm olein TAG oil

Assignment
δ 5.42-5.33 (m, =CH in oleic and linoleic acids), 5.32-5.23 (m, 1H, CH_2-C*H*-CH_2, TAG), 5.11-5.06 (m, 1H, CH_2-C*H*-CH_2, 1,2-DAG impurity), 4.29 (dd, J=12.0 Hz, J=4.4 Hz, 2H, C*H*$_2$-CH-C*H*$_2$, TAG), 4.21–4.06 (m, 5H, -CH_2-CH-CH_2-, 1,3-DAG impurity as estimated by integration), 4.14 (dd, J=12.0 Hz, J=6.0 Hz, 2H, C*H*$_2$-CH-C*H*$_2$, TAG), 3.75-3.72 (m, -C*H*$_2$-OH, 1,2-DAG impurity), 2.86-2.78 (m, =C-CH_2-C=, linoleic acid), 2.35-2.30 (m, CH_2-COO), 2.14-2.04 (m, =C-C*H*$_2$ in oleic and linoleic acids), 1.64-1.55 (m, C*H*$_2$-CH_2-COO), 1.34-1.20 (m, -CH_2-) and 0.88 (t, J=6.8 Hz, -CH_3) ppm.

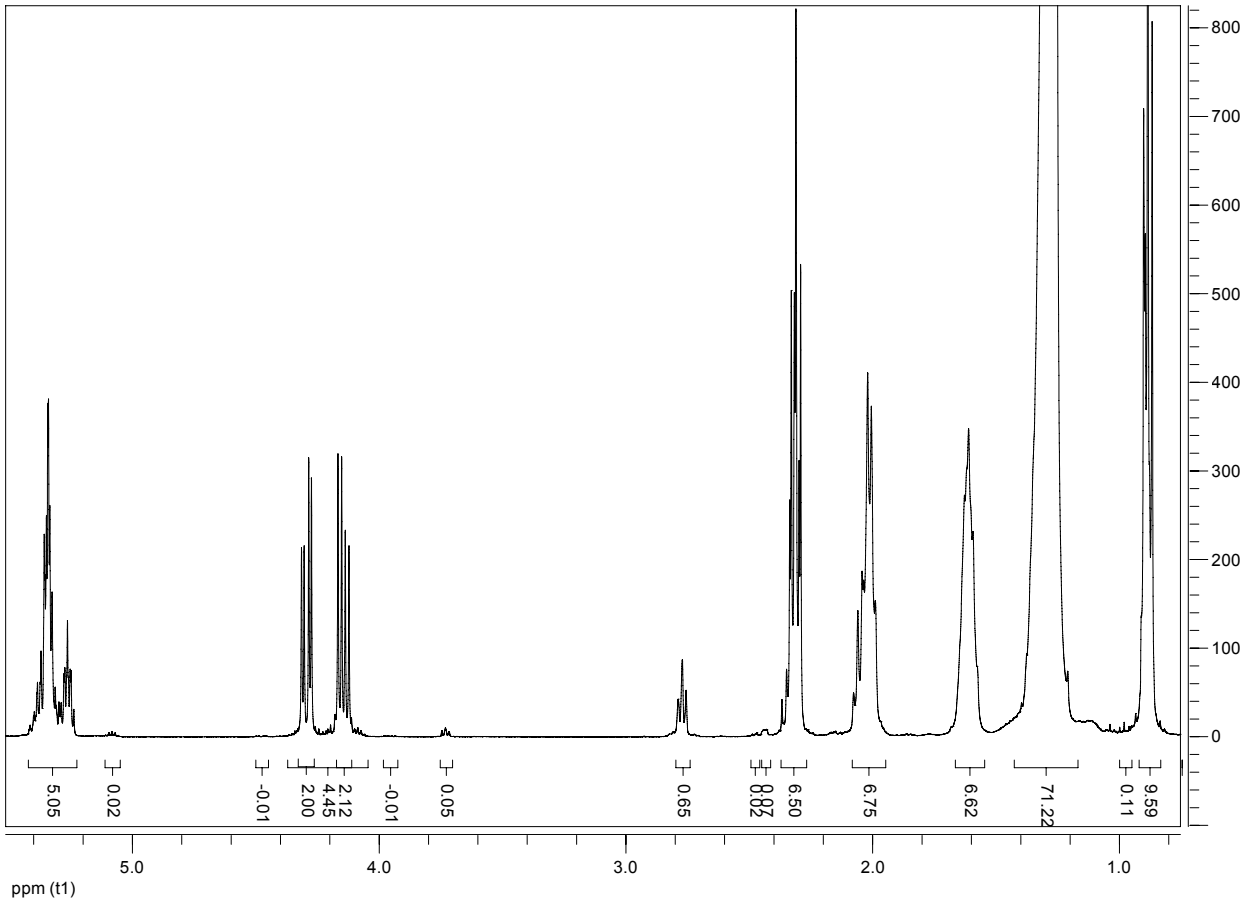

Figure 13. ^{1}H NMR spectrum of the oil used in all the reactions

It can be noticed from the spectrum that there are impurities of lower acylglycerols in the initial oil. This is better clarified in Figure 14 where the glyceryl segment of the spectrum (5.5 – 3.6 ppm) has been expanded. From calculations based on integration there are indications of the presence of 2-2.5% 1,2-DAG which is rather easy to determine from the spectrum. Assuming an acyl-migration equilibrium with 1,3-DAG this would imply the presence of approximately 6% 1,3-DAG which is supported by integration and comparison with the two major doublet of doublets corresponding to the two methylene groups of the glycerol moiety of the TAGs. All five 1,3-DAG glycerol protons resonate under one of these doublets, that resonating at higher field (lower chemical shift) at 4.14 ppm. In other words, the oil is approximately 92% pure in terms of TAG.

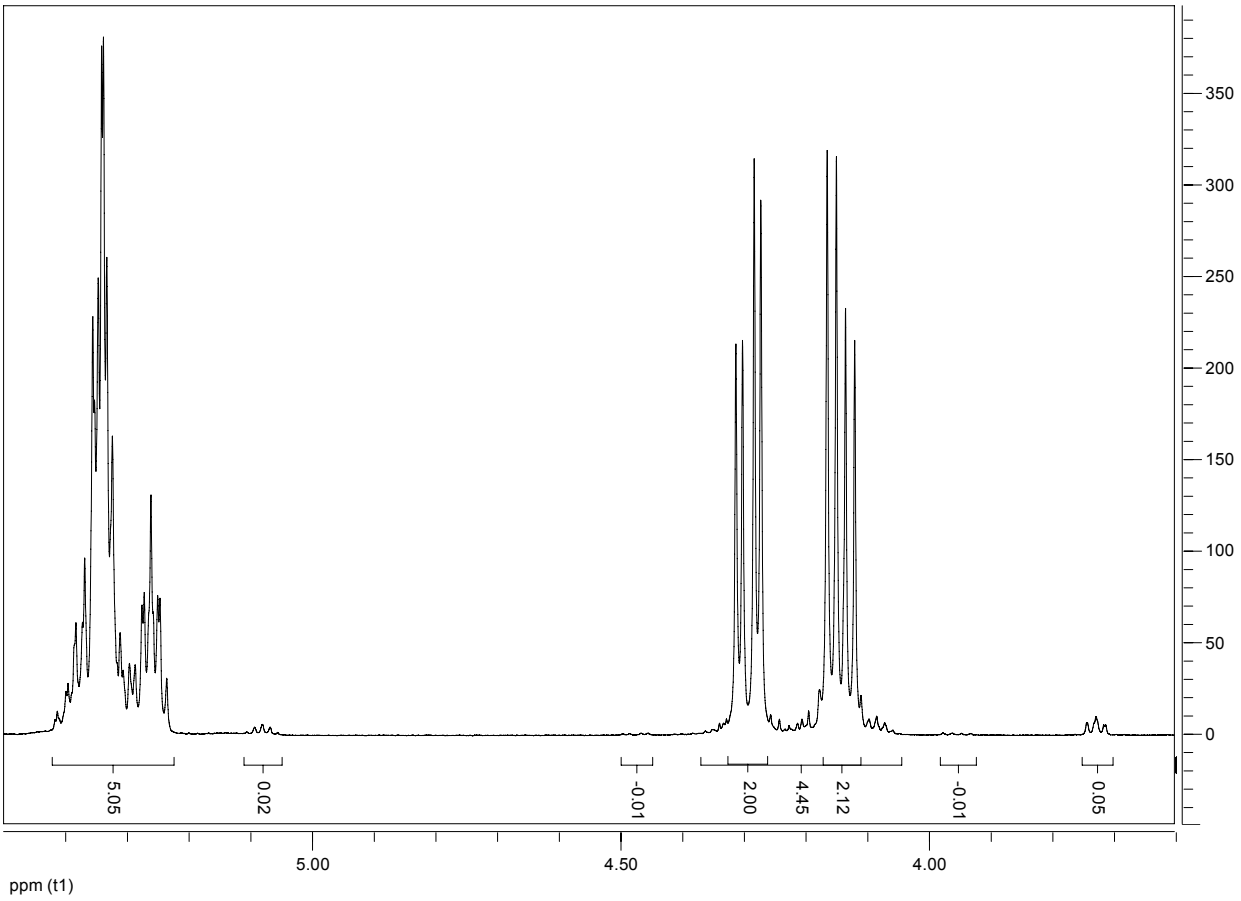

Figure 14. The ^{1}H NMR spectrum of the oil used in all the reactions, expanded between 5.5 and 3.6 ppm.

There are several peaks in the oil spectrum that may be utilized when determining the extent of conversion of the ethanolysis reactions. One possibility is to base it on integration of the methylene protons (CH_2) located α to the carbonyl group (2.35-2.30 ppm), another using the methylene protons located β to the carbonyl group (1.64-1.55 ppm) and the third the methyl protons (CH_3) located at the terminal carbon of all fatty acyl groups present (0.88 ppm). These peaks are all well separated in the spectrum and do not change much when the fatty acids are split off the TAGs and converted into ethyl esters. In this work the conversion was based on an average between all three.

The extent of conversion was determined as molar percentage of ethyl esters produced as the reaction proceeded. The conversion is based on the total number of equivalents of fatty acyl groups present in the initial TAGs (three-fold stoichiometric equivalents of TAGs being used). As mentioned earlier DAGs have contributed one ethyl ester equivalent, MAGs two and glycerol three, but TAGs none. Harmony must remain between the free hydroxyl groups in the reaction product mixture and ethyl esters formed in terms of molar equivalents. Usually ethyl esters are easily recognized in ^{1}H NMR spectra through their alcoholic ethyl group possessing two methylene protons (CH_2) resonating as a sharp quartet at 4.15 ppm and three terminal methyl protons (CH_3) resonating as a sharp triplet at 1.21 ppm.

The methyl protons are mostly hidden underneath the huge peak from the bulk of repeated methylene groups of the hydrocarbon chains of the fatty acids (1.34-1.20 ppm) and they are therefore virtually useless in this respect. What is left is the methylene protons. There is certainly an interference between these methylene protons and protons that belong to the glycerol moiety of the acylglycerols, including two of the terminal protons of the TAGs, all five protons of the 1,3-DAGs and two terminal protons of the 1-MAGs. One might think that this excludes the 1,3-DAGs from being detected, but that is not the case since it is possible to estimate the contribution of the 1,3-DAGs to that region from the remaining glyceryl protons from the other acylglycerols.

The first NMR method

For this project a method was developed for determining the conversion of ethanolysis by high-resolution ^{1}H NMR. This method is based on the methylene protons of the ethyl ester (EE) and the three peaks which remain the same regardless of whether they belong to the fatty acid bound to the glyceryl backbone or EE, that is the α, β or methyl protons. A conversion is determined for each of these three peaks and an average of them then gives the overall conversion at each time point. The mol percentage of each of the glyceryl moieties was also determined from ^{1}H NMR to aid further insight into the details of the ethanolysis reaction. All the results described in Chapter 3.1 are based on this method.

To calculate the conversion and determine the percentage of each glyceryl moiety present, the first thing to do was deciding which peak to use as a reference peak for integration of the spectra. Depending on the acylglycerol composition and amount of ethyl esters seen on NMR spectra, one of the following peaks was chosen as the reference peak: the multiplet for the mid proton of 1,2-DAG at 5.11-5.06 ppm set as one proton, the more down field doublet of doublets for the end protons of TAG at 4.29 ppm set as two protons or the quartet for the methylene protons of the alcoholic ethyl group at 4.15 ppm set as two protons. Figure 15-Figure 17 provide examples of cases where each of these peaks were used as reference.

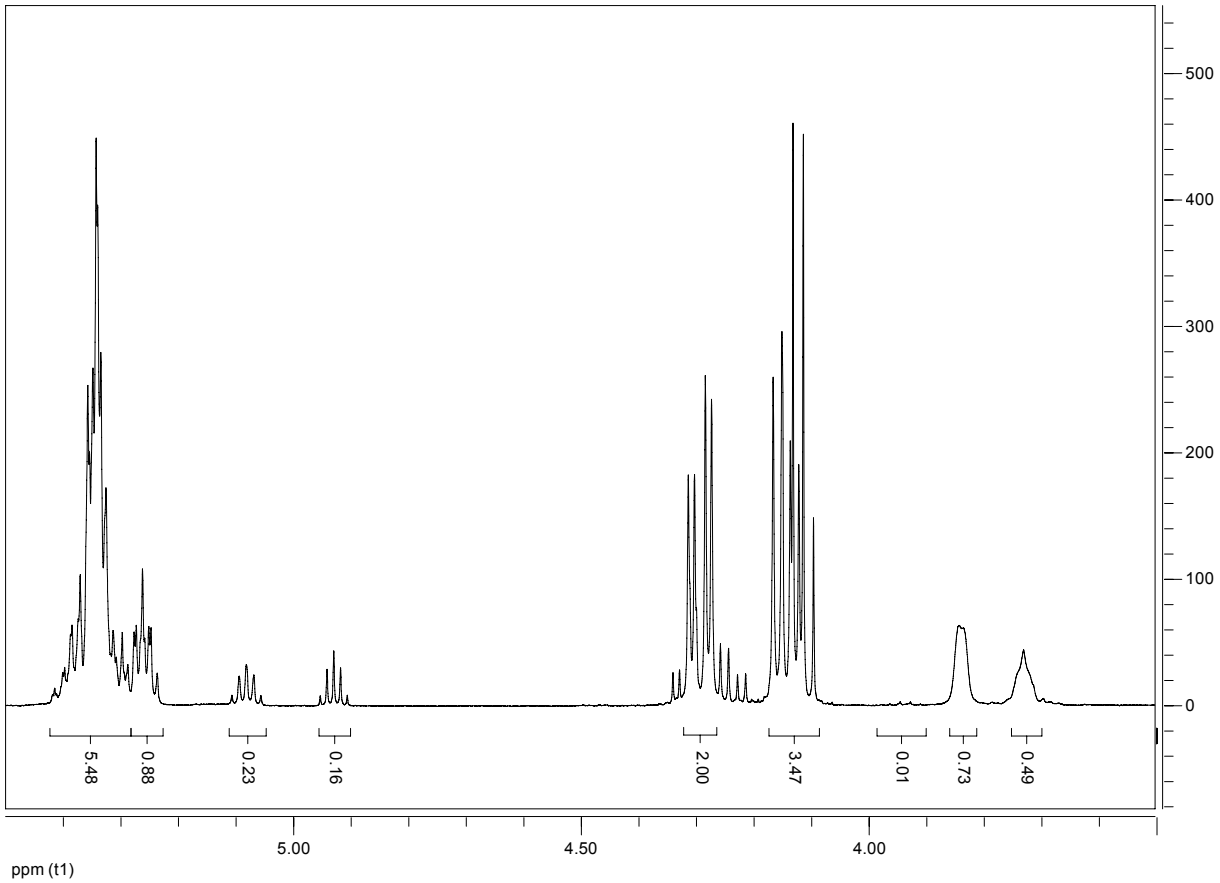

Figure 15. ^{1}H NMR expanded between 5.5 and 3.5 ppm. TAG used as reference

Choosing TAG as the reference peak is based on TAG being the major acylglycerol component of the glyceryl mixture at that time point. In Figure 15 the TAG doublet of doublets at 4.29 ppm has been chosen based on that and the fact that both the doublets of doublets are still distinguishable from other peaks with similar chemical shifts. As the reaction continues and more 1,2-DAG is formed from TAG it becomes more difficult and inaccurate to use the TAG peak at 4.29 because of its overlapping with the two doublets of doublets of 1,2-DAG. In cases like the one in Figure 16 where the TAG´s upper field doublet of doublets is still interfering with the EE peak and the overlapping of the TAG and 1,2-DAG has become too extensive to use the TAG peak, it is best to choose the 1,2-DAG peak at 5.11-5.06 ppm as a reference.

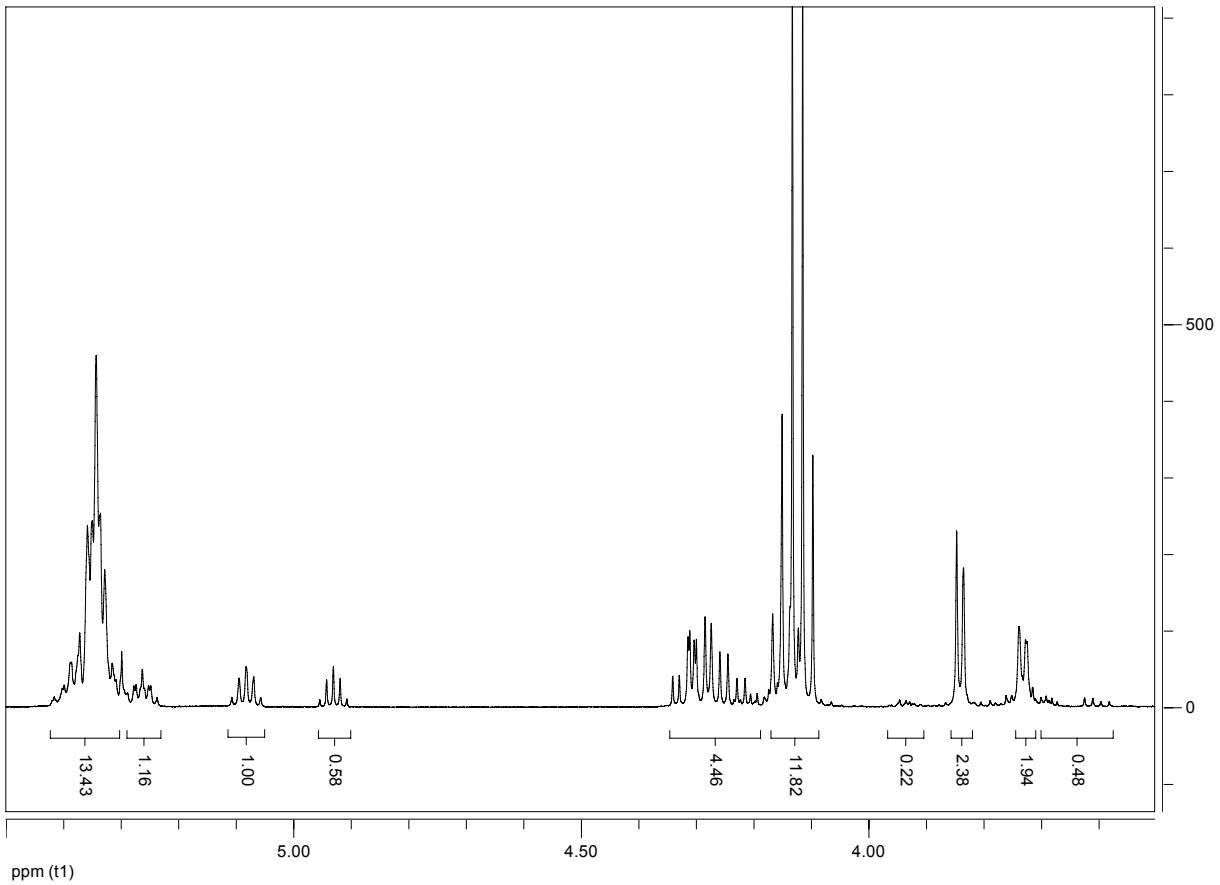

Figure 16. ^{1}H NMR expanded between 5.5 and 3.5 ppm. 1,2-DAG used as reference

Using either the TAG or the 1,2-DAG peaks as reference, the integral of the EE methylene peak must be calculated. For the TAG peak this is simple because all that needs to be done is to subtract 2 from the integral of the overlapping EE and TAG peaks. In Figure 15 the EE integral would therefore be 3.47-2 = 1.47. If any 1-MAG is present an integral accounting for two 1-MAG protons must also be subtracted. In the example in Figure 15 this was not done due to the very low amount of 1-MAG. When determining the integral of the EE peak in the example in Figure 16 the integral of TAG must first be determined by subtracting 2 from the integrals of the peaks between 4.34-4.21 ppm, which gives a TAG integral of 2.46. The EE integral is then calculated as: EE = 11.82 – TAG – 1-MAG = 11.82 – 2.46 – 0.48 = 8.88.

As the reaction proceeds even further it becomes best to use the EE methylene peak itself as the reference peak, taking care in integrating the quartet as tightly as possible, setting it as 2, like is done in Figure 17.

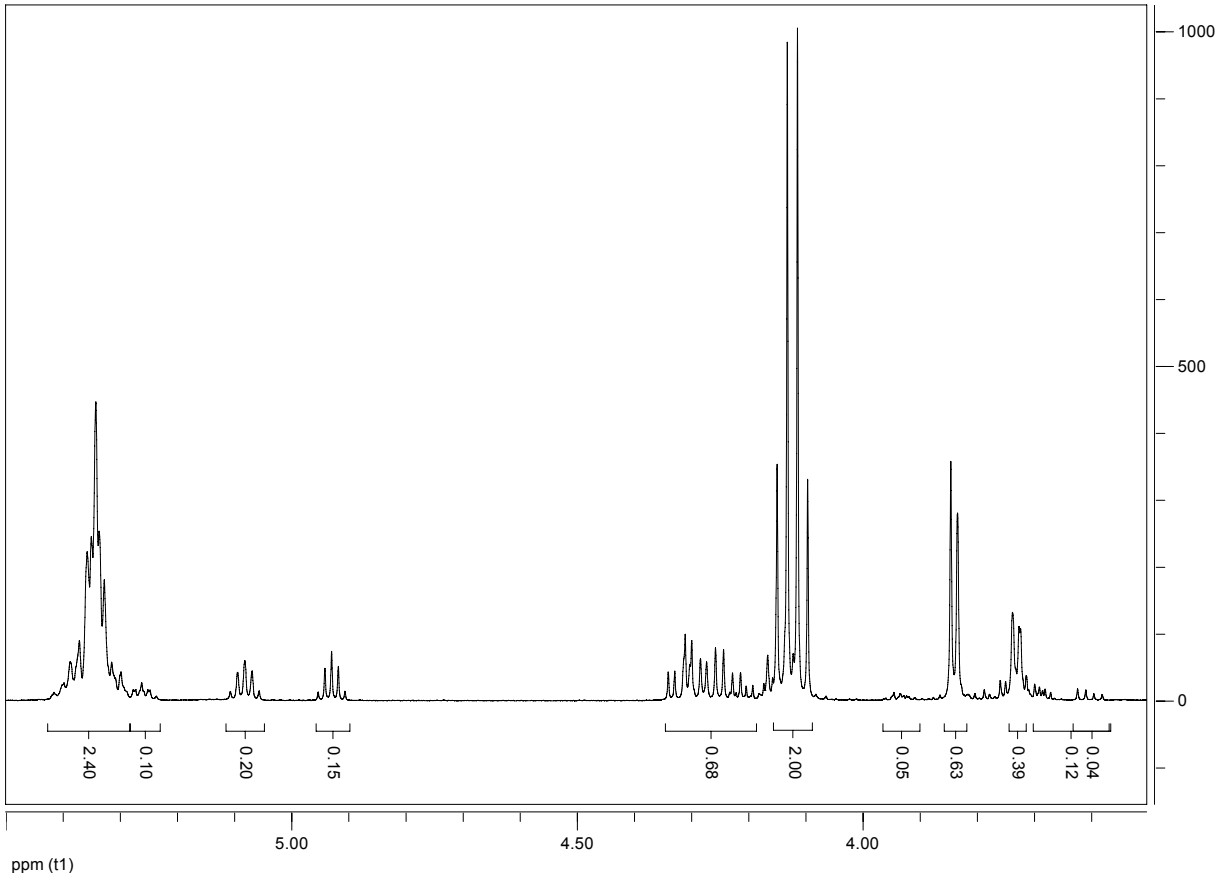

Figure 17. ^{1}H NMR expanded between 5.5 and 3.5 ppm. EE used as reference

The conversion is then determined based on the integration of the α protons (2.35-2.30 ppm), β protons (1.64-1.55 ppm) and the methyl protons (0.88 ppm). An average between all three is then determined giving the conversion of the reaction. Figure 18 shows the full ^{1}H NMR spectrum of the expanded area in Figure 16. From it the conversion is determined in the following way:

(EE / α protons) * 100% = (8.88/22.66)*100% = 39.2%

(EE / β protons) * 100% = (8.88/23.65)*100% = 37.5%

((EE*3) / (methyl protons*2)) * 100% = ((8.88*3) / (33.23*2))*100% = 40.1%

An average of these three then gives a conversion of 38.9%. Mol percentages of TAG, 1,2-DAG, 1,3-DAG, 1-MAG and 2-MAG are then determined from their integration, making sure that the integrals all represent the same number of protons. The amount of glycerol is determined by calculating the conversion from the glyceryl moieties in the following way:

$$\text{Calculated conversion} = \frac{\%1,2\text{-DAG} + \%1,3\text{-DAG} + (2 * \%1\text{-MAG}) + (2 * \%2\text{-MAG})}{3}$$

and subtracting it from the conversion determined by EE. The percentages of all the glyceryl moieties must then be corrected accordingly.

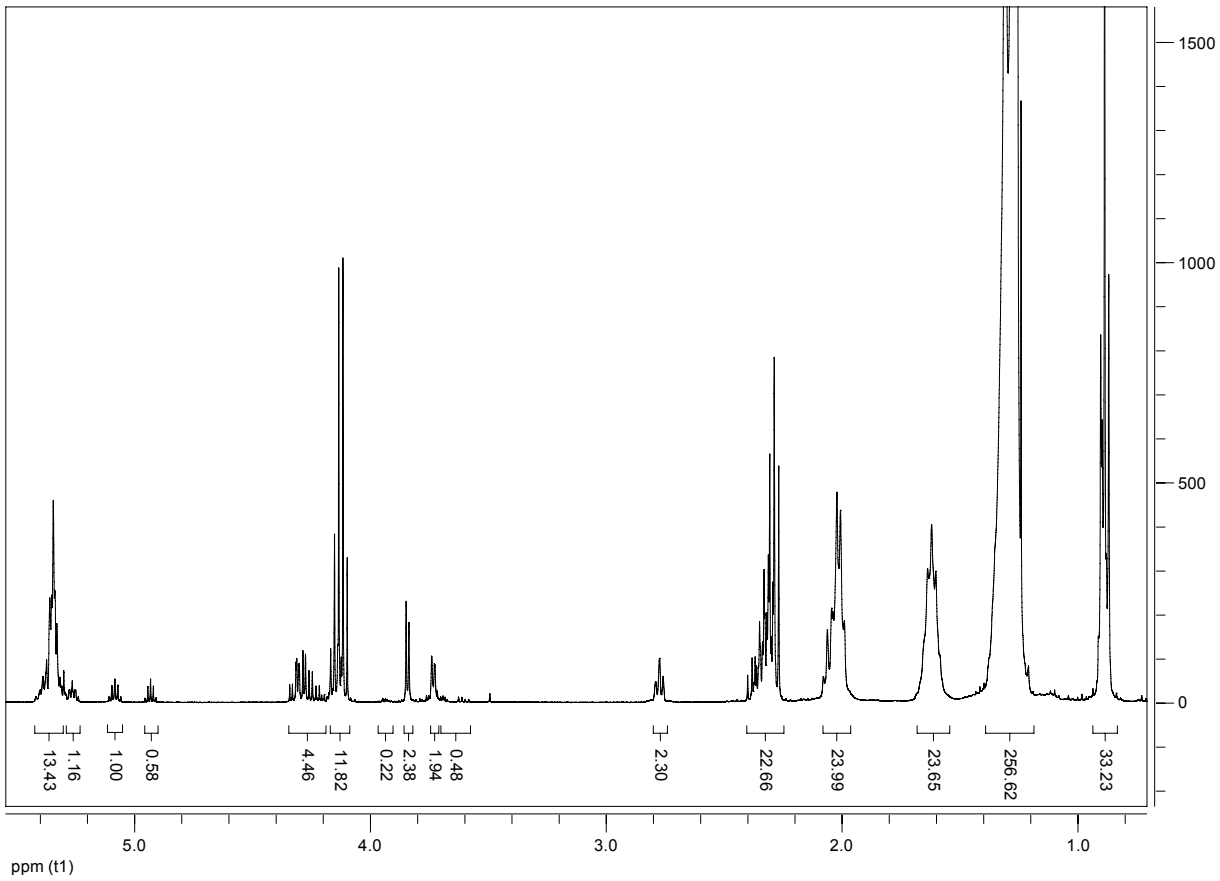

Figure 18. ^{1}H NMR spectrum of the ethanolysis after 2 hour reaction. Conversion: 38.9%.

The second NMR method

The first method requires subjective interpretation of each spectrum which could lead to different results. A more consistent and easily applied method was therefore needed. After the initial project work was finished it was thus decided to develop a method which fitted those criteria.. This second method is based on setting the α protons at 2.400-2.247 ppm as a reference peak defined as six protons (peak K in Table 24). The remaining peaks are then integrated by the defined areas shown in Table 24.

Table 24. Areas for integration in the second NMR calculation method for ethanolysis

Code	Integration area (ppm)	Peak components
A	5.286-5.228	TAG (1H)
B	5.112-5.050	1,2-DAG (1H)
C	4.958-4.900	2-MAG (1H)
D	4.344-4.206	TAG (2H) and 1,2-DAG (2H)
E	4.180-4.080	EE (2H), TAG (2H) and 1-MAG (1H)
F	4.206-4.047	EE (2H), TAG (2H), 1-MAG (2H) and 1,3-DAG (5H)
G	3.953-3.902	1-MAG (1H)
H	3.856-3.824	2-MAG (4H)
I	3.744-3.710	1,2-DAG (2H)
J	3.701-3.574	1-MAG (2H)
K	2.400-2.247	α protons of FA and EE (6H), reference peak

This ensures that all the ^{1}H NMR spectra are integrated in exactly the same way every time by these defined areas and always using the same reference peak. The integrals found for each area in Table 24 are then put into the following equations to determine the integral for each component:

TAG = (A + ((D/2) - B) / 2

1,2-DAG = (B + (I/2)) / 2

1,3-DAG = (F – E – 1-MAG) / 5

1-MAG = (G + (J/2)) / 2

2-MAG = (C + (H/4)) / 2

EE (2H) = E – 2*TAG – 1-MAG

Glycerol = (EE (2H) – (2*1,2DAG) – (2*1,3-DAG) – (4*1-MAG) – (4*2-MAG)) / 6

From this are then calculated the mol percentage of each glyceryl moiety. It must be noted that the integral for the EE is an equivalent of two protons while the other represent one proton. The conversion is calculated as following:

% conversion = (EE / K) * 100%

If the equations given are put in an Excel spreadsheet the conversion and amount of glyceryl moieties is calculated as integrals are typed in, both saving time and making the analysis easier. This was not possible with the first method because of the three different possibilities of reference peaks, making it difficult to always apply the same equations for all cases. Table 25 shows the results from the same reaction as in Table 21 using the second method for calculations.

Table 25. The results from lipase A in the ethanolysis reaction described in Table 5, the second NMR method used for determination of conversion and glyceryl moieties.

T (h)	Conv. (%)	TAG (%)	1,2-DAG (%)	1,3-DAG (%)	1-MAG (%)	2-MAG (%)	Glycerol (%)
1	18.4	65.6	15.9	0.4	1.0	11.8	5.3
2	32.7	41.5	22.2	0.1	3.7	24.9	7.6
4	47.2	18.2	32.5	0.2	8.3	24.2	16.5
8	54.0	15.3	30.6	0.8	8.5	11.5	33.3
24	62.0	17.4	17.4	2.4	9.2	2.1	51.4

Comparison of the results in Table 21 and Table 25 shows that the conversion is similar, implying that this method can be used as well. On the other hand, comparing the glyceryl moieties the amount of glycerol is much higher when using the second method. The most likely cause is that the errors in the integrals are multiplied up causing the glycerol to be overestimated. However, if the glycerol part in the second method is calculated in the same

manner as in the first method, that is using the percentages of DAGs, MAGs and conversion, the overall results become very similar to those in Table 21 as Table 26 shows.

Table 26. The results from lipase A in the ethanolysis reaction described in Table 5, the second NMR method used for determination of conversion and glyceryl moieties in combination with the first method.

T (h)	Conv. (%)	TAG (%)	1,2-DAG (%)	1,3-DAG (%)	1-MAG (%)	2-MAG (%)	Glycerol (%)
1	18.4	66.8	16.2	0.4	1.1	12.0	3.5
2	32.7	43.2	23.1	0.1	3.8	25.9	3.8
4	47.2	20.2	36.0	0.2	9.2	26.8	7.5
8	54.0	19.4	38.8	1.1	10.7	14.5	15.5
24	62.0	27.0	27.0	3.7	14.3	3.3	24.7

This combined method could thus prove to be a useful and quick tool to determine the conversion.

3.3.4 Determination of conversion in the methanolysis reaction

Conversion in the methanolysis reaction was determined with the second method for ethanolysis adapted to the difference in the methyl ester (ME) and EE peak position. Methyl esters are easily recognized in ^{1}H NMR spectra through their alcoholic methyl group possessing three methyl protons (CH_3) resonating as a sharp singlet at 3.66 ppm.

Table 27. Areas for integration in the second NMR calculation method for methanolysis

Code	Integration area (ppm)	Peak components
A	5.286-5.228	TAG (1H)
B	5.112-5.050	1,2-DAG (1H)
C	4.958-4.900	2-MAG (1H)
D	4.344-4.206	TAG (2H) and 1,2-DAG (2H)
E	4.180-4.080	TAG (2H) and 1-MAG (1H)
F	4.206-4.047	TAG (2H), 1-MAG (2H) and 1,3-DAG (5H)
G	3.953-3.902	1-MAG (1H)
H	3.856-3.824	2-MAG (4H)
I	3.744-3.710	1,2-DAG (2H)
J	3.701-3.650	ME (3H) and 1-MAG (1H)
K	2.400-2.247	α protons of FA and EE (6H), reference peak

The integrals found for each area are then put into the following equations to determine the integral for each component:

TAG = (A + ((D/2) - B) / 2

1,2-DAG = (B + (I/2)) / 2

1,3-DAG = (F – E – G) / 5

1-MAG = G

2-MAG = (C + (H/4)) / 2

ME (3H) = J - G

From this are then calculated the mol percentage of each glyceryl moiety, the amount glycerol is calculated in the same way as in the first method for ethanolysis. It must be noted that the integral for the ME is an equivalent of three protons while the other represent one proton. The conversion is calculated as follows:

% conversion = ((ME*2) / (K*3)) * 100%

The results from these calculations are reported in Table 20. Figure 19 shows the ^{1}H NMR spectrum of the methanolysis at a reaction time of 4h. The main difference between the ME and EE is the position of their distinctive ^{1}H NMR peaks. The methyl peak of ME is only overlapping with 1-MAG while the EE methylene protons overlap with TAG, 1,3-DAG and 1-MAG. For this reason, the conversion determination from ME might be a little more accurate than for EE.

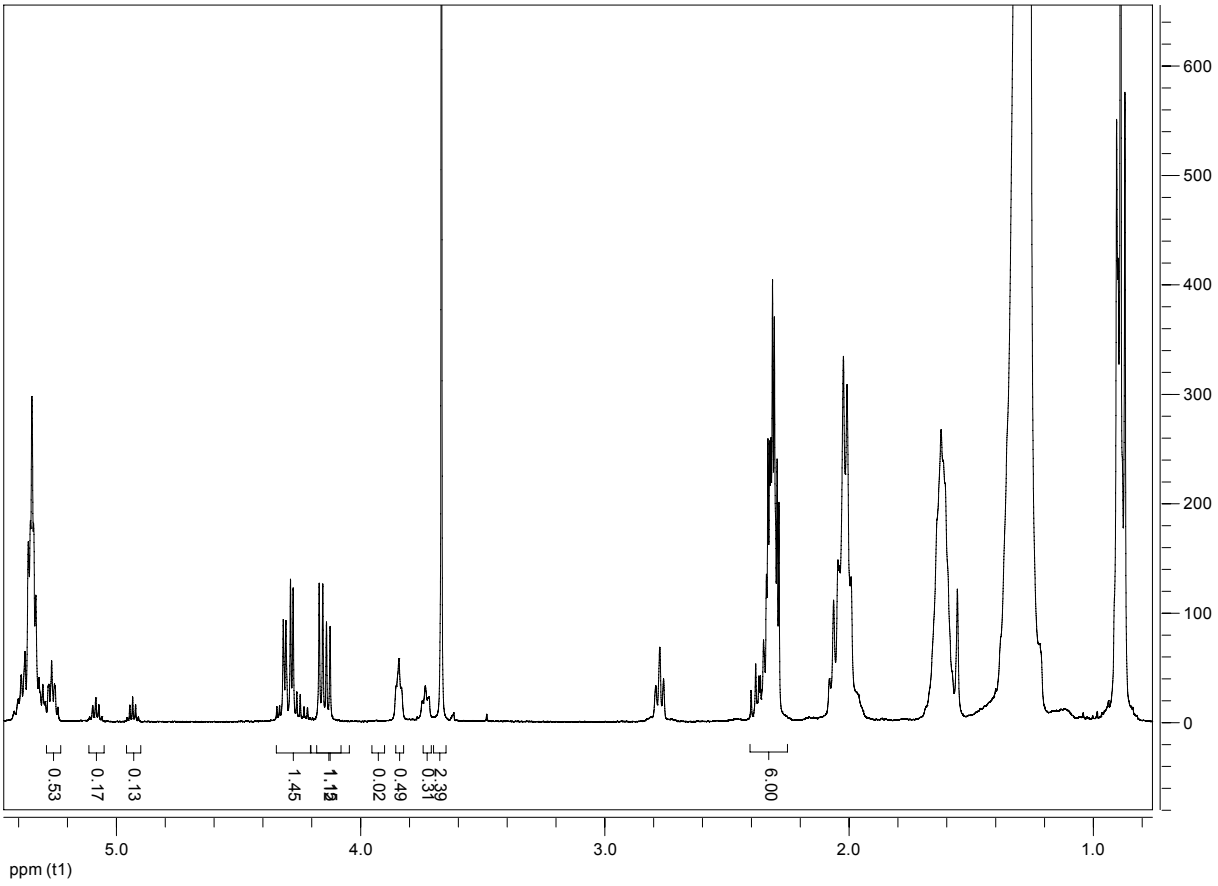

Figure 19. ^{1}H NMR spectrum of the methanolysis after 4 hour reaction. Conversion: 26.3%.

3.4 Summary

Ten immobilized lipases were screened for their ability to catalyse the ethanolysis of palm olein and their selectivity for fatty acids. After initial testing two lipases, A and C, were selected for further experiments as based on the conversion, saturated FA /unsaturated FA ratio obtained in the ethyl esters produced and the composition of the remaining acylglycerols. The next step involving the two selected lipases was to find out whether ethanol concentration or water content of the system played some role in their conversion rate and selectivity. Addition of water did not enhance the selectivity of the lipases or the conversion rate. Removal of water almost made lipase C inactive and reduced the conversion rate of lipase A. Using higher ethanol concentration also decreased the conversion rate of the lipases. Titrations of selected samples from reactions with these two lipases and two more, H and J, showed that the amount of FFA remained between 1.1 and 2.4% when absolute ethanol was used and 2.4-3.3% for ethanol fortis (Table 18). Fatty acid analysis of the acylglycerol fractions of samples from reactions with these four lipases and calculations done from these results, back up the results of the analysis of the ethyl esters. Summarising these results, it is clear, even though the consistency of them was good, that none of the lipases or conditions tested gave satisfactory selectivity at high conversion.

For this project two methods were developed for determining the conversion of ethanolysis by high-resolution ^{1}H NMR. The mol percentage of each of the glyceryl moieties was also determined from ^{1}H NMR to aid further insight into the details of the ethanolysis reaction. The first method is based on choosing between three peaks as a reference peak and then determining the integral of EE. The overall conversion at each time point was determined from the EE peak and the α, β and methyl peaks. This method requires a subjective interpretation of each spectrum which could lead to different results. A more consistent and easily applied method was therefore needed. The second method was thus developed with that in mind. In that method all NMR spectra were integrated in exactly the same way every time by defined areas and always using the same reference peak. The equations given can be put in an Excel spreadsheet to determine the conversion and amount of glyceryl moieties as the integrals are typed in, both saving time and making the analysis easier. The conversion determined with these two methods was very similar but the amount of glycerol estimated to be higher with the second method. Different approaches were used to determine the glycerol in the methods but both employ the fact that each TAG has three fatty acids which can be made into EE; one from the DAGs, two from the MAGs and three from glycerol. When the second method was employed, determining the glycerol in same manner as in the first method, a very similar result was obtained as from the first method. The second method was easily applied for both methanolysis and ethanolysis; however, it might be a little more accurate for methanolysis. The results from these conversion determinations have yet to be confirmed by other quantitative methods, like HPLC, but we are quite confident that our ^{1}H NMR methods gives a good estimate of what is going on in the ethanolysis and methanolysis reactions.

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4 Conclusions

A six step chemoenzymatic process to synthesize enantiopure structured triacylglycerols of the ABC type has been described. A series containing stearic acid at one of the end positions, DHA at the mid position and a saturated fatty acid varying from acetic acid to palmitic acid at the other end position has been synthesized for both enantiomers. All compounds isolated were characterized by traditional organic chemistry methods. Specific rotation of all isolated compounds was also determined. In the synthesis of (*S*)-**3a-b** and (*R*)-**3a-b** a side reaction caused some problems involving formation of 1,3-dioctanoylglycerol but this was resolved using two different solvents in recrystallization of (*S*)-**3a-b** and (*R*)-**3a-b**. Enantiopurity of the compounds synthesized has yet to be confirmed.

Ten lipases were screened for their ability to catalyse the ethanolysis of palm olein at a good conversion rate and with selectivity towards saturated fatty acids. Two methods were developed based on high resolution ^{1}H NMR to determine the conversion of the alcoholysis and monitor the progress of the reactions. None of the lipases or conditions tested gave satisfactory selectivity at high conversion. Both ^{1}H NMR methods are considered to give a good estimate of the conversion and reaction mechanism of both methanolysis and ethanolysis reactions, but the results have yet to be confirmed with HPLC.

5 Materials and methods

5.1 Materials and instruments

Solketals ((S)-(+)-1,2-isopropylideneglycerol and (R)-(+)-2,3-isopropylideneglycerol), both of 98% purity and 99% ee) were obtained from Sigma-Aldrich (Steinheim, Germany). Benzyl bromide (98%), 4-dimethylaminopyridine (DMAP, 99%) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 98+%) were obtained from Acros Organics (Geel, Belgium), anhydrous magnesium sulphate from Merck (Darmstadt, Germany), 10% palladium on carbon catalyst from Merck (Munich, Germany) and sodium hydride (60% dispersion in mineral oil), boron trifluoride methanol solution (14%), vinyl acetate (≥99%) and vinyl stearate from Sigma-Aldrich (Steinheim, Germany) Vinyl esters of butanoic acid (>97%), hexanoic acid (>97%), decanoic acid (>99%), myristic acid (>99%) and palmitic acid(>96%) were obtained from TCI Europe (Zwindrecht, Belgium), vinyl caprylate (>99%) from TCI Japan (Tokyo, Japan) and vinyl laurate from Fluka Chemie (Taufkirchen, Germany). DHA ethyl ester was obtained from Pronova BioPharma ASA (Sandefjord, Norway) and was hydrolysed before use in reactions. Chloroform, dichloromethane, ethyl acetate and methanol were all obtained HPLC grade from Sigma-Aldrich (Steinheim, Germany) along with petroleum ether (boiling range 40-60°C), benzene and chloroform-d (99.8 atom % D). Absolute ethanol was purchased from Riedel-de Haën. Tetrahydrofuran was obtained from Acros Organics (Geel, Belgium) and dried over Na wire in presence of benzophenone under dry nitrogen atmosphere. Silica gel for chromatography (0.060-0.200 mm, 60 Å) was obtained from Acros Organics (Geel, Belgium) and TLC Plates both analytical (250 μm, Glass Backed TLC Extra Hard Layer, 60 Å, indicator: F-254) and preparative (1000 μm, Glass Backed TLC Extra Hard Layer, 60 Å, indicator: F-254) were from Silicycle (Quebec, Canada). The TLC eluent used was 80:20:2 mixture of petroleum ether/diethyl ether/acetic acid. Spots were visualized with UV light and Rodamin 6G (Merck). The immobilized *Candida antarctica* lipase (Novozym 435) was a gift from Novozymes A/S. Palm olein and nine lipases listed in Table 28 were provided by Novozymes A/S. Lipase PS-C "Amano" I was from Amano Enzymes Inc. (Nagoya, Japan). All chemicals and solvents were used without further purification unless otherwise stated.

Table 28. Lipases used in the ethanolysis.

	Name	Lipase
Lipase A	BE-2007-00011	Lipozyme RM IM
Lipase B	BE-2007-00014	Experimental
Lipase C	BE-2007-00016	Candida A lipase experimental
Lipase D	BE-2007-00017	Experimental
Lipase E	BE-2008-00021	Novozym-435
Lipase F	BE-2008-00025	Lipozymes TL IM
Lipase G	BE-2008-00030	Lipozymes TL experimental
Lipase H	BE-2008-00055	Candida B experimental
Lipase I	BE-2008-00064	Experimental
Lipase J	Amano	Lipase PS-C "Amano" I

High-resolution ^{1}H and ^{13}C NMR spectra were recorded on Avance 400 Bruker NMR spectrometer in deuteriated chloroform as solvent at 400.12 and 100.61 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) and the coupling constants (*J*) in Hertz (Hz). The following abbreviations are used to describe the multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet. 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. The IR spectra were measured on Nicolet Avatar 360 FT-IR spectrophotometer (E.S.P) on a KBr pellet (MAG and DAGs) or as a neat liquid (benzylglycerol and TAGs). Melting points were determined on a Büchi 520 melting point apparatus and are uncorrected. The high-resolution mass spectra (HRMS) were acquired on a Bruker mircOTOF-Q mass spectrometer equipped with an atmospheric pressure chemical ionization chamber. All data analysis was done on Bruker software. Optical activity measurements were performed on Autopol V Automatic Polarimeter from Rudolf Research Analytical using a 40T-2.5-100-0.7 Temp Trol polarimetric cell with 2.5 mm inside diameter, 100 mm optical path length and 0.7 ml volume. Fatty acid analyses were performed on ethyl and methyl esters in a Perkin-Elmer Clarus 400 Gas Chromatograph.

5.2 Synthesis of triacylglycerols

5.2.1 Benzylglycerol

3-*O*-Benzyl-sn-glycerol [(*R*)-1]. NaH (60% mineral oil dispersion; 1.466 g) was added to a three-necked round bottom flask and treated several times with dry petroleum ether using a syringe to rid off the mineral oil prior to addition of freshly dried THF (30 ml). (*S*)-Solketal (3.003 g; 22.72 mmol) was added via syringe of rate appropriate for keeping the heat formation under control. The resulting suspension was stirred for few minutes before the benzyl bromide (3.970 g; 23.21 mmol) was added via syringe. The resulting suspension was refluxed for 4 hours after which all solketal had been consumed. The reaction mixture was allowed to cool to room temperature and1 M HCl (30 ml) added together with ethanol sufficient to make the solution homogeneous. The resulting mixture was refluxed for 30 minutes or until all 1-*O*-benzyl-2,3-isopropylidene-*sn*-glycerol intermediate had reacted.

After cooling to room temperature the reaction mixture was extracted with diethyl ether and the organic phase treated several times with 14% $NaHCO_3$ until pH > 7 and finally dried over $MgSO_4$. The solvent was removed in vacuo on a rotavapor and the residue introduced to a Kugelrohr distillation at 2.8 Torr. First fraction was collected at 50 °C and discarded. The desired product was collected as a colourless liquid (2.685 g; 14.73 mmol) in 65% overall yields. $[\alpha]_D^{20}$ 3.42 (*c* 10, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38-7.30 (m, 5H, Ph-H), 4.56 (s, 2H, Ph-CH_2), 3.92-3.87 (m, 1H, C*H*OH), 3.73-3.67 (m, 1H, C*H*$_2$OH), 3.65-3.61 (m, 1H, C*H*$_2$OH), 3.57 (dd, J = 9.6, 4.4 Hz, 1H, C*H*$_2$OCH$_2$Ph), 3.53 (dd, J = 9.6, 6.4 Hz, 1H, C*H*$_2$OCH$_2$Ph), 2.81 (br d, J = 4.4 Hz, 1H, OH), 2.35 (br t, J = 5.4 Hz, 1H, OH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 137.6, 128.5 (2), 127.9, 127.8 (2), 73.6, 71.8, 70.6, 64.0 ppm. FT-IR (Neat): 3150-3600 (br, OH), 2926 (vs, CH), 2869 (vs, CH) cm^{-1}.

1-*O*-Benzyl-sn-glycerol [(*S*)-1]. The same procedure was follow as described for (*R*)-**1**: NaH (60% mineral oil dispersion; 1.313 g), (*S*)-solketal (2.556 g; 19.34 mmol) benzyl bromide (3.375 g; 19.73 mmol) and dried THF (30 ml). The desired product was collected as a colourless liquid (1.951 g; 10.71 mmol) in 55% overall yields. $[\alpha]_D^{20}$ -3.02 (*c* 6, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38-7.30 (m, 5H, Ph-H), 4.56 (s, 2H, Ph-CH_2), 3.92-3.87 (m, 1H, C*H*OH), 3.73-3.67 (m, 1H, C*H*$_2$OH), 3.65-3.61 (m, 1H, C*H*$_2$OH), 3.57 (dd, J = 9.6, 4.4 Hz, 1H, C*H*$_2$OCH$_2$Ph), 3.53 (dd, J = 9.6, 6.4 Hz, 1H, C*H*$_2$OCH$_2$Ph), 2.81 (br d, J = 4.4 Hz, 1H, OH), 2.35 (br t, J = 5.4 Hz, 1H, OH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 137.6, 128.5 (2), 127.9, 127.8 (2), 73.6, 71.8, 70.6, 64.0 ppm. FT-IR (Neat): 3150-3600 (br, OH), 2926 (vs, CH), 2869 (vs, CH) cm^{-1}.

5.2.2 MAG

1-Octadecanoyl-*sn*-glycerol [(*S*)-2]. 3-*O*-benzyl-*sn*-glycerol [(*R*)-**1**] (1.277 g; 7.01mmol) and vinyl stearate (2.730 g; 8.79 mmol) were dissolved in dichloromethane (6 ml). Immobilized *Candida antarctica* lipase (397 mg) was then added and the resulting suspension stirred at room temperature for 2 ½ hours (monitored by TLC). The reaction was discontinued by separating the lipase by filtration and the solvent removed by rotavapor. The residue was dissolved in petroleum ether (140 ml), Pd-catalyst (323 mg) added and put in a Parr reactor for 2 ½ hours. During the reaction crystals were formed which were dissolved in THF (70 ml) before the Pd-catalyst was filtered away. The solvent was removed and the crystals were recrystallized in petroleum ether which afforded 1-octadecanoyl-*sn*-glycerol [(*S*)-**2**] (2.41 g; 6.72 mmol) as white shining crystals in 96% yield; m.p. 69.7-70.5 °C. $[\alpha]_D^{20}$ 2.43 (*c* 6, THF). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21 (dd, *J* = 11.7, 4.6 Hz, 1H, OCOC*H*$_2$), 4.14 (dd, *J* = 11.7, 6.1 Hz, 1H, OCOC*H*$_2$), 3.97-3.90 (m, 1H, CH$_2$C*H*CH$_2$), 3.73-3.67 (m, 1H, C*H*$_2$OH), 3.63-3.57 (m, 1H, C*H*$_2$OH), 2.44 (d, *J* = 5.1, 1H, CH$_2$O*H*), 2.35 (t, *J* = 7.6 Hz, 2H, CH$_2$COO), 1.99 (t, *J* = 6.1, 1H, CH$_2$O*H*), 1.67-1.59 (m, 2H, C*H*$_2$CH$_2$COO), 1.35-1.20 (m, 28H, CH$_2$), 0.88 (t, *J* = 6.8, 3H, CH$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.3, 70.3, 65.2, 63.3, 34.1, 31.9, 29.7 (5C), 29.6 (2C), 29.5, 29.4, 29.3, 29.2, 29.1, 24.9, 22.7, 14.1 ppm. FT-IR (KBr): 3150-3600 (br, OH), 2918 (vs, CH), 2849 (vs, CH), 1735 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{21}H_{42}O_4$ - OH: 341.3050; found: 341.3042 amu.

3-Octadecanoyl-*sn*-glycerol [(*R*)-2]. The same procedure was follow as described for (*S*)-**2**: 1-*O*-benzyl-*sn*-glycerol [(*S*)-**1**] (1.401 g; 7.69 mmol), vinyl stearate (2.985 g; 9.61 mmol), dichloromethane (6 ml) and immobilized *Candida antarctica* lipase (439 mg). Deprotection: petroleum ether (200 ml), Pd-aktivkol (396 mg) and THF (120 ml).

Recrystallization from petroleum ether which afforded 3-octadecanoyl-*sn*-glycerol [(*R*)-**2**] (2.632 g; 7.34 mmol) as white shining crystals in 96% yield; m.p. 74.1-75.4 °C. $[\alpha]_D^{20}$ - 2.32 (*c* 6, THF). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.19 (dd, *J* = 11.6, 4.7 Hz, 1H, $OCOCH_2$), 4.14 (dd, *J* = 11.6, 6.1 Hz, 1H, $OCOCH_2$), 3.96-3.90 (m, 1H, CH_2CHCH_2), 3.72-3.67 (m, 1H, CH_2OH), 3.63-3.57 (m, 1H, CH_2OH), 2.60 (d, *J* = 4.7, 1H, CH_2OH), 2.35 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.17 (t, *J* = 5.2, 1H, CH_2OH), 1.67-1.59 (m, 2H, CH_2CH_2COO), 1.37-1.20 (m, 28H, CH_2), 0.88 (t, *J* = 6.8, 3H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.3, 70.3, 65.2, 63.3, 34.1, 31.9, 29.7 (5C), 29.6 (2C), 29.5, 29.4, 29.3, 29.2, 29.1, 24.9, 22.7, 14.1 ppm. FT-IR (KBr): 3150-3600 (br, OH), 2918 (vs, CH), 2849 (vs, CH), 1735 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{21}H_{42}O_4$ - OH: 341.3050; found: 341.3050 amu.

5.2.3 DAG

3-Ethanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-3a]. 1-Octadecanoyl-*sn*-glycerol [(*S*)-**2**] (400 mg; 1.116 mmol) and vinyl acetate (125 mg; 1.452 mmol) were dissolved in dichloromethane (4 ml). Immobilized *Candida antarctica* lipase (56 mg) was then added and the resulting suspension stirred at room temperature for 3 hours (monitored by TLC). The reaction was discontinued by separating the lipase by filtration and the solvent removed by rotavapor. The residue was first recrystallized in methanol for 2 hours at room temperature, the resulting suspension was filtered (residue determined to be 1,3-octadecanoyl-*sn*-glycerol by NMR) and the filtrate was evaporated and crystallized again in petroleum ether which afforded 3-ethanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**3a**] (280 mg; 0.699 mmol) as white crystals in 63% yield; m.p. 41.6-44.0 °C. $[\alpha]_D^{20}$ 0.442 (*c* 1.13, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.43 (s, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 2 H, CH_2COO), 2.10 (s, 3 H, CH_3), 1.66-1.59 (m, 2 H, CH_2CH_2COO), 1.35-1.20 (m, 28 H, CH_2), 0.88 (t, *J* = 6.8 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9, 171.0, 68.3, 65.2, 65.0, 34.1, 31.9, 29.7 (5C), 29.6 (2C), 29.5, 29.4, 29.3, 29.2 ,29.1, 24.9, 22.7, 20.8, 14.1 ppm. IR (KBr): 3300-3600 (br, OH), 2914 (vs, CH), 2849 (vs, CH), 1733 (vs, C=O), 1713 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{23}H_{44}O_5$ - OH: 383.3156; found: 383.3172 amu.

1-Ethanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-3a]. The same procedure was follow as described for (*S*)-**3a**: 3-Octadecanoyl-*sn*-glycerol [(*R*)-**2**] (200 mg; 0.558 mmol), vinyl acetate (64 mg; 0.743 mmol), dichloromethane (4 ml) and Immobilized *Candida antarctica* lipase (29 mg). THF (0.5 ml) were also added to help with the solvation of 1-octadecanoyl-*sn*-glycerol. Crystallization in petroleum ether afforded 1-ethanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**3a**] (135 mg; 0.337 mmol) as white crystals in 60% yield; m.p. 45.6-46.3 °C. $[\alpha]_D^{20}$ -1.039(*c* 0.77, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.42 (s, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 2 H, CH_2COO), 2.10 (s, 3 H, CH_3), 1.66-1.59 (m, 2 H, CH_2CH_2COO), 1.35-1.20 (m, 28 H, CH_2), 0.88 (t, *J* = 6.8 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9, 171.0, 68.3, 65.2, 65.0, 34.1, 31.9, 29.7 (5C), 29.6 (2C), 29.5, 29.4, 29.3, 29.2 ,29.1, 24.9, 22.7, 20.8, 14.1 ppm. IR (KBr): 3300-3600 (br, OH), 2914 (vs, CH), 2849 (vs, CH), 1733 (vs, C=O), 1713 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{23}H_{44}O_5$ - OH: 383.3156; found: 383.3162 amu.

3-Butanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-3b]. The same procedure was follow as described for (*S*)-**3a**: 1-Octadecanoyl-*sn*-glycerol [(*S*)-**2**] (300 mg; 0.837 mmol), vinyl butanoate (125 mg; 1.095 mmol), dichloromethane (3 ml) and immobilized *Candida*

antarctica lipase (45 mg). Crystallization in petroleum ether afforded 3-butanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**3b**] (290 mg; 0.677 mmol) as white crystals in 81% yield; m.p. 50.8-51.7 °C. $[\alpha]_D^{20}$ 0.336 (*c* 1.19, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.42 (s, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 2 H, CH_2COO), 2.34 (t, *J* = 7.4 Hz, 2 H, CH_2COO), 1.72-1.59 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 28 H, CH_2), 0.96 (t, *J* = 7.4 Hz, 3 H, CH_3), 0.88 (t, *J* = 6.8 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9, 173.7, 68.4, 65.0 (2 C), 35.9, 34.1, 31.9, 29.7 (5 C), 29.6 (2 C), 29.5, 29.4, 29.3, 29.2, 29.1, 24.9, 22.7, 18.4, 14.1, 13.6 ppm. IR (KBr): 3300-3600 (br, OH), 2915 (vs, CH), 2849 (vs, CH), 1733 (vs, C=O), 1708 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{25}H_{48}O_5$ - OH: 411.3469; found: 411.3476 amu.

1-Butanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-3b]. The same procedure was follow as described for (*S*)-**3a**: 3-Octadecanoyl-*sn*-glycerol [(*R*)-**2**] (270 mg; 0.753 mmol), vinyl butanoate (112 mg; 0.981 mmol), dichloromethane (5 ml) and immobilized *Candida antarctica* lipase (39 mg). The residue was recrystallized in petroleum ether which gave 290 mg of white crystals. 235 mg of the crystals were recrystallized again in methanol at room temperature for 2 ½ hours giving 15 mg of white crystals which were determined to be 1,3-octadecanoyl-*sn*-glycerol by NMR. The filtrate was evaporated and recrystallized methanol which afforded 1-butanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**3b**] (195 mg; 0.455 mmol) calculated to be in 85% yield; m.p. 54.0-54.7 °C. $[\alpha]_D^{20}$ -1.058 (*c* 1.04, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.44 (s, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 2 H, CH_2COO), 2.34 (t, *J* = 7.4 Hz, 2 H, CH_2COO), 1.71-1.59 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 28 H, CH_2), 0.96 (t, *J* = 7.4 Hz, 3 H, CH_3), 0.88 (t, *J* = 6.8 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9, 173.7, 68.4, 65.0 (2 C), 35.9, 34.1, 31.9, 29.7 (5 C), 29.6 (2 C), 29.5, 29.4, 29.3, 29.2, 29.1, 24.9, 22.7, 18.4, 14.1, 13.6 ppm. IR (KBr): 3300-3600 (br, OH), 2915 (vs, CH), 2849 (vs, CH), 1733 (vs, C=O), 1708 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{25}H_{48}O_5$ - OH: 411.3469; found: 411.3488 amu.

3-Hexanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-3c]. 1-Octadecanoyl-*sn*-glycerol [(*S*)-**2**] (270 mg; 0.753 mmol) and vinyl hexanoate (142 mg; 0.999 mmol) were dissolved in dichloromethane (5 ml). Immobilized *Candida antarctica* lipase (48 mg) was then added and the resulting suspension stirred at room temperature for 3 hours (monitored by TLC). The reaction was discontinued by separating the lipase by filtration and the solvent removed by rotavapor. The residue was recrystallized in petroleum ether which afforded 3-hexanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**3c**] (283 mg; 0.620 mmol) as white crystals in 82% yield; m.p. 57.0-57.5 °C. $[\alpha]_D^{20}$ 0.370 (*c* 1.08, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.42 (d, *J* = 3.4 Hz, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 1.68-1.59 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 32 H, CH_2), 0.90 (t, *J* = 6.9 Hz, 3 H, CH_3), 0.88 (t, *J* = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9 (2 C), 68.4, 65.0 (2 C), 34.1, 34.0, 31.9, 31.3, 29.7 (5 C), 29.6 (2C), 29.5, 29.4, 29.3, 29.2, 29.1, 24.9, 24.6, 22.7, 22.3, 14.1, 13.9 ppm. IR (KBr): 3300-3600 (br, OH), 2915 (vs, CH), 2849 (vs, CH), 1732 (vs, C=O), 1708 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{27}H_{52}O_5$ - OH: 439.3782; found: 439.3793 amu.

1-Hexanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-3c]. The same procedure was follow as described for (*S*)-**3c**: 3-Octadecanoyl-*sn*-glycerol [(*R*)-**2**] (260 mg; 0.725 mmol), vinyl hexanoate (135 mg; 0.949 mmol), dichloromethane (5 ml) and immobilized *Candida antarctica* lipase (44 mg). 1-hexanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**3c**] (303 mg; 0.663 mmol) was isolated as white crystals in 92% yield; m.p. 57.3-58.2 °C. $[\alpha]_D^{20}$ -0.392 (*c*

1.02, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.44 (s, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 1.67-1.59 (m, 4 H, C*H*$_2$CH$_2$COO), 1.35-1.20 (m, 32 H, CH_2), 0.90 (t, *J* = 6.9 Hz, 3 H, CH_3), 0.88 (t, *J* = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9 (2 C), 68.4, 65.0 (2 C), 34.1, 34.0, 31.9, 31.3, 29.7 (5 C), 29.6 (2C), 29.5, 29.4, 29.3, 29.2, 29.1, 24.9, 24.6, 22.7, 22.3, 14.1, 13.9 ppm. IR (KBr): 3300-3600 (br, OH), 2915 (vs, CH), 2849 (vs, CH), 1732 (vs, C=O), 1708 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{27}H_{52}O_5$ - OH: 439.3782; found: 439.3785 amu.

1-Octadecanoyl-3-octanoyl-*sn*-glycerol [(*S*)-3d]. The same procedure was follow as described for (*S*)-**3c**: 1-Octadecanoyl-*sn*-glycerol [(*S*)-**2**] (269 mg; 0.750 mmol), vinyl caprylate (164 mg; 0.963 mmol), dichloromethane (5 ml) and immobilized *Candida antarctica* lipase (46 mg). 1-octadecanoyl-3-octanoyl-*sn*-glycerol [(*S*)-**3d**] (290 mg; 0.598 mmol) was isolated as white crystals in 80% yield; m.p. 59.1-59.8 °C. $[\alpha]_D^{20}$ 0.278(*c* 1.08, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.43 (d, *J* = 4.8 Hz, 1 H, OH), 2.35 (t, *J* = 7.5 Hz, 4 H, CH_2COO), 1.67-1.59 (m, 4 H, C*H*$_2$CH$_2$COO), 1.35-1.20 (m, 36 H, CH_2), 0.88 (t, *J* = 6.8 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9 (2 C), 68.4, 65.0 (2 C), 34.1 (2 C), 31.9, 31.6, 29.7 (5 C), 29.6 (2 C), 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 28.9, 24.9 (2 C), 22.7, 22.6, 14.1, 14.0 ppm. IR (KBr): 3300-3600 (br, OH), 2915 (vs, CH), 2849 (vs, CH), 1732 (vs, C=O), 1708 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{29}H_{56}O_5$ - OH: 467.4095; found: 467.4099 amu.

3-Octadecanoyl-1-octanoyl-*sn*-glycerol [(*R*)-3d]. The same procedure was follow as described for (*S*)-**3c**: 3-octadecanoyl-*sn*-glycerol [(*R*)-**2**] (251 mg; 0.700 mmol), vinyl caprylate (165 mg; 0.969 mmol), dichloromethane (4 ml) and immobilized *Candida antarctica* lipase (45 mg). 3-octadecanoyl-1-octanoyl-*sn*-glycerol [(*R*)-**3d**] (282 mg; 0.582 mmol) was isolated as white crystals in 83% yield; m.p. 60.7-62.0 °C. $[\alpha]_D^{20}$ -0.098 (*c* 1.02, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.44 (d, *J* = 4.3 Hz, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 1.66-1.59 (m, 4 H, C*H*$_2$CH$_2$COO), 1.35-1.20 (m, 36 H, CH_2), 0.88 (t, *J* = 6.8 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9 (2 C), 68.4, 65.0 (2 C), 34.1 (2 C), 31.9, 31.6, 29.7 (5 C), 29.6 (2 C), 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 28.9, 24.9 (2 C), 22.7, 22.6, 14.1, 14.0 ppm. IR (KBr): 3300-3600 (br, OH), 2915 (vs, CH), 2849 (vs, CH), 1732 (vs, C=O), 1708 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{29}H_{56}O_5$ - OH: 467.4095; found: 467.4098 amu.

3-Decanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-3e]. The same procedure was follow as described for (*S*)-**3c**: 1-Octadecanoyl-*sn*-glycerol [(*S*)-**2**] (120 mg; 0.335 mmol), vinyl decanoate (90 mg; 0.454 mmol), dichloromethane (3 ml), THF (1 ml) and immobilized *Candida antarctica* lipase (20 mg). 3-decanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**3e**] (133 mg; 0.259 mmol) was isolated as white crystals in 78% yield; m.p. 60.7-62.0 °C. $[\alpha]_D^{20}$ 0.400(*c* 1.00, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.42 (d, *J* = 4.9 Hz, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 1.66-1.59 (m, 4 H, C*H*$_2$CH$_2$COO), 1.35-1.20 (m, 40 H, CH_2), 0.88 (t, *J* = 6.8 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9 (2 C), 68.4, 65.0 (2 C), 34.1 (2 C), 31.9, 31.8, 29.7 (5 C), 29.6 (3 C), 29.4 (3 C), 29.2 (3 C), 29.1 (2 C), 24.9 (2 C), 22.7 (2 C), 14.1 (2 C) ppm. IR (KBr): 3300-3600 (br, OH), 2915 (vs, CH), 2849 (vs, CH), 1732 (vs, C=O), 1708 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{31}H_{60}O_5$ - OH: 495.4408; found: 495.4412 amu.

1-Decanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-3e]. The same procedure was follow as described for (*S*)-**3c**: 3-Octadecanoyl-*sn*-glycerol [(*R*)-**2**] (239 mg; 0.667 mmol), vinyl

decanoate (169 mg; 0.852 mmol), dichloromethane (5 ml) and immobilized *Candida antarctica* lipase (49 mg). 1-decanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**3e**] (299 mg; 0.583 mmol) was isolated as white crystals in 87% yield; m.p. 63.6-64.4 °C. $[\alpha]_D^{20}$ -0.396 (*c* 1.01, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.42 (d, *J* = 4.7 Hz, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 1.66-1.59 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 40 H, CH_2), 0.88 (t, *J* = 6.8 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9 (2 C), 68.4, 65.0 (2 C), 34.1 (2 C), 31.9, 31.8, 29.7 (5 C), 29.6 (3 C), 29.4 (3 C), 29.2 (3 C), 29.1 (2 C), 24.9 (2 C), 22.7 (2 C), 14.1 (2 C) ppm. IR (KBr): 3300-3600 (br, OH), 2915 (vs, CH), 2849 (vs, CH), 1732 (vs, C=O), 1708 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{31}H_{60}O_5$ - OH: 495.4408; found: 495.4390 amu.

3-Dodecanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-3f]. The same procedure was follow as described for (*S*)-**3c**: 1-Octadecanoyl-*sn*-glycerol [(*S*)-**2**] (270 mg; 0.753 mmol), vinyl laurate (217 mg; 0.959 mmol), chloroform (8 ml) and immobilized *Candida antarctica* lipase (51 mg). 3-dodecanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**3f**] (318 mg; 0.588 mmol) was isolated as white crystals in 78% yield; m.p. 59.7-60.2 °C. $[\alpha]_D^{20}$ -0.583(*c* 1.03, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.42 (d, *J* = 4.6 Hz, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 1.66-1.59 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 44 H, CH_2), 0.88 (t, *J* = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9 (2 C), 68.4, 65.0 (2 C), 34.1 (2 C), 31.9 (2 C), 29.7 (5 C), 29.6 (2 C), 29.5 (3 C), 29.4 (2 C), 29.3 (2 C), 29.2 (2 C), 29.1 (2 C), 24.9 (2 C), 22.7 (2 C), 14.1 (2 C) ppm. IR (KBr): 3300-3600 (br, OH), 2915 (vs, CH), 2849 (vs, CH), 1732 (vs, C=O), 1708 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{33}H_{64}O_5$ - OH: 523.4721; found: 523.4713 amu.

1-Dodecanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-3f]. The same procedure was follow as described for (*S*)-**3c**: 3-Octadecanoyl-*sn*-glycerol [(*R*)-**2**] (230 mg; 0.641 mmol), vinyl laurate (184 mg; 0.813 mmol), dichloromethane (5 ml) and immobilized *Candida antarctica* lipase (43 mg). 1-dodecanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**3f**] (292 mg; 0.540 mmol) was isolated as white crystals in 84% yield; m.p. 65.2-66.0 °C. $[\alpha]_D^{20}$ -0.610 (*c* 0.82, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.43 (d, *J* = 4.8 Hz, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 1.66-1.59 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 44 H, CH_2), 0.88 (t, *J* = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9 (2 C), 68.4, 65.0 (2 C), 34.1 (2 C), 31.9 (2 C), 29.7 (5 C), 29.6 (2 C), 29.5 (3 C), 29.4 (2 C), 29.3 (2 C), 29.2 (2 C), 29.1 (2 C), 24.9 (2 C), 22.7 (2 C), 14.1 (2 C) ppm. IR (KBr): 3300-3600 (br, OH), 2915 (vs, CH), 2849 (vs, CH), 1732 (vs, C=O), 1708 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{33}H_{64}O_5$ - OH: 523.4721; found: 523.4717 amu.

1-Octadecanoyl-3-tetradecanoyl-*sn*-glycerol [(*S*)-3g]. The same procedure was follow as described for (*S*)-**3c**: 1-Octadecanoyl-*sn*-glycerol [(*S*)-**2**] (250 mg; 0.697 mmol), vinyl myristate (245 mg; 0.963 mmol), dichloromethane (8 ml), THF (2 ml) and immobilized *Candida antarctica* lipase (62 mg). 1-octadecanoyl-3-tetradecanoyl-*sn*-glycerol [(*S*)-**3g**] (350 mg; 0.615 mmol) was isolated as white crystals in 88% yield; m.p. 63.6-64.5 °C. $[\alpha]_D^{20}$ 0.234(*c* 1.28, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.41 (d, *J* = 4.7 Hz, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 1.66-1.59 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 48 H, CH_2), 0.88 (t, *J* = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9 (2 C), 68.4, 65.0 (2 C), 34.1 (2 C), 31.9 (2 C), 29.7 (7 C), 29.6 (3 C), 29.5 (2 C), 29.4 (2 C), 29.3 (2 C), 29.2 (2 C), 29.1 (2 C), 24.9 (2 C), 22.7 (2 C), 14.1 (2 C) ppm. IR (KBr): 3300-3600 (br, OH), 2915 (vs, CH), 2849 (vs, CH), 1732

(vs, C=O), 1708 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{35}H_{68}O_5$ - OH: 551.5034; found: 551.5026 amu.

3-Octadecanoyl-1-tetradecanoyl-*sn*-glycerol [(*R*)-3g]. The same procedure was follow as described for (*S*)-**3c**: 3-Octadecanoyl-*sn*-glycerol [(*R*)-**2**] (220 mg; 0.614 mmol), vinyl myristate (198 mg; 0.778 mmol), dichloromethane (2 ml), THF (2 ml) and immobilized *Candida antarctica* lipase (47 mg). 3-octadecanoyl-1-tetradecanoyl-*sn*-glycerol [(*R*)-**3g**] (314 mg; 0.552 mmol) was isolated as white crystals in 90% yield; m.p. 68.2-69.6 °C. $[\alpha]_D^{20}$ 0.000 (*c* 1.25, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.44 (d, *J* = 4.8 Hz, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 1.66-1.59 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 48 H, CH_2), 0.88 (t, *J* = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9 (2 C), 68.4, 65.0 (2 C), 34.1 (2 C), 31.9 (2 C), 29.7 (7 C), 29.6 (3 C), 29.5 (2 C), 29.4 (2 C), 29.3 (2 C), 29.2 (2 C), 29.1 (2 C), 24.9 (2 C), 22.7 (2 C), 14.1 (2 C) ppm. IR (KBr): 3300-3600 (br, OH), 2915 (vs, CH), 2849 (vs, CH), 1732 (vs, C=O), 1708 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{35}H_{68}O_5$ - OH: 551.5034; found: 551.5012 amu.

3-Hexadecanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-3h]. The same procedure was follow as described for (*S*)-**3c**: 1-octadecanoyl-*sn*-glycerol [(*S*)-**2**] (252 mg; 0.703 mmol), vinyl palmitate (246 mg; 0.871 mmol), dichloromethane (7 ml), THF (2 ml) and immobilized *Candida antarctica* lipase (51 mg). 3-hexadecanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**3h**] (383 mg; 0.642 mmol) was isolated as white crystals in 91% yield; m.p. 68.3-69.4 °C. $[\alpha]_D^{20}$ 0.291(*c* 1.03, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.43 (d, *J* = 4.8 Hz, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 1.66-1.59 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 52 H, CH_2), 0.88 (t, *J* = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9 (2 C), 68.4, 65.0 (2 C), 34.1 (2 C), 31.9 (2 C), 29.7 (12 C), 29.6 (2 C), 29.5 (2 C), 29.4 (2 C), 29.2 (2 C), 29.1 (2 C), 24.9 (2 C), 22.7 (2 C), 14.1 (2 C) ppm. IR (KBr): 3300-3600 (br, OH), 2914 (vs, CH), 2849 (vs, CH), 1734 (vs, C=O), 1713 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{37}H_{72}O_5$ - OH: 579.5347; found: 579.5328 amu.

1-Hexadecanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-3h]. The same procedure was follow as described for (*S*)-**3c**: 3-Octadecanoyl-*sn*-glycerol [(*R*)-**2**] (220 mg; 0.614 mmol), vinyl palmitate (218 mg; 0.772 mmol), dichloromethane (5 ml), THF (1 ml) and immobilized *Candida antarctica* lipase (46 mg). 1-hexadecanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**3h**] (346 mg; 0.580 mmol) was isolated as white crystals in 95% yield; m.p. 70.4-72.2 °C. $[\alpha]_D^{20}$ 0.079 (*c* 1.27, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.21-4.06 (m, 5 H, CH_2CHCH_2), 2.42 (d, *J* = 4.7 Hz, 1 H, OH), 2.35 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 1.66-1.59 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 52 H, CH_2), 0.88 (t, *J* = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ =173.9 (2 C), 68.4, 65.0 (2 C), 34.1 (2 C), 31.9 (2 C), 29.7 (12 C), 29.6 (2 C), 29.5 (2 C), 29.4 (2 C), 29.2 (2 C), 29.1 (2 C), 24.9 (2 C), 22.7 (2 C), 14.1 (2 C) ppm. IR (KBr): 3300-3600 (br, OH), 2914 (vs, CH), 2849 (vs, CH), 1734 (vs, C=O), 1713 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{37}H_{72}O_5$ - OH: 579.5347; found: 579.5334 amu.

5.2.4 TAG

2-Docosahexaenoyl-3-ethanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-4a]. 3-Ethanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**3a**] (120 mg; 0.300 mmol), DHA as free acid (109 mg; 0.332 mmol), EDCI (73 mg; 0.381 mmol) and DMAP (15 mg; 0.123 mmol) were dissolved in dichloromethane (3 ml). The resulting solution was stirred at room temperature over night (monitored by TLC). The reaction was discontinued by passing the reaction mixture through a short column pack with silica by use of CH_2Cl_2. Solvent removal in vacuo afforded the pure 2-docosahexaenoyl-3-ethanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**4a**] as yellowish liquid (135 mg; 0.190 mmol) in 63% yield. $[\alpha]_D^{20}$ -1.414 (*c* 9.90, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.29 (dd, *J* = 11.9, 4.0 Hz, 1 H, CH_2CHCH_2), 4.28 (dd, *J* = 11.9, 3.9 Hz, 1 H, CH_2CHCH_2), 4.15 (dd, *J* = 11.9, 5.8 Hz, 1 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 6.0 Hz, 1 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, $=CCH_2C=$), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.6 Hz, 2 H, CH_2COO), 2.11-2.04 (m, 2 H, $=CCH_2CH_3$), 2.07 (s, 3 H, CH_3), 1.64-1.57 (m, 2 H, CH_2CH_2COO), 1.35-1.20 (m, 28 H, CH_2), 0.97 (t, *J* = 7.5, 3 H, CH_3 in DHA), 0.88 (t, *J* = 6.8 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3, 172.2, 170.5, 132.0, 129.5, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.0, 62.3, 62.0, 34.0, 31.9, 29.7 (7 C), 29.6, 29.5, 29.4, 29.3, 29.1, 25.6 (5 C), 25.5, 24.9, 22.7, 22.6, 20.7, 20.6, 14.3, 14.1 ppm. IR (neat): 3013 (s, CH), 2923 (vs, CH), 2853 (vs, CH), 1744 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{45}H_{74}O_6 + NH_4$: 728.5824; found: 728.5819.

2-Docosahexaenoyl-1-ethanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-4a]. The same procedure was follow as described for (*S*)-**4a**: 1-Ethanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**3a**] (106 mg; 0.265 mmol), DHA-FFA (100 mg; 0.304 mmol), EDCI (64 mg; 0.334 mmol), DMAP (13 mg; 0.106 mmol) and dichloromethane (3 ml). 2-docosahexaenoyl-1-ethanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**4a**] was isolated as yellowish liquid (132 mg; 0.186 mmol) in 70% yield. $[\alpha]_D^{20}$ +1.486 (*c* 10.03, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.29 (dd, *J* = 11.9, 4.2 Hz, 1 H, CH_2CHCH_2), 4.28 (dd, *J* = 11.9, 4.2 Hz, 1 H, CH_2CHCH_2), 4.15 (dd, *J* = 11.9, 5.8 Hz, 1 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 6.0 Hz, 1 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, $=CCH_2C=$), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.6 Hz, 2 H, CH_2COO), 2.11-2.04 (m, 2 H, $=CCH_2CH_3$), 2.07 (s, 3 H, CH_3), 1.64-1.57 (m, 2 H, CH_2CH_2COO), 1.35-1.20 (m, 28 H, CH_2), 0.97 (t, *J* = 7.5, 3 H, CH_3 in DHA), 0.88 (t, *J* = 6.9 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3, 172.2, 170.5, 132.0, 129.5, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.0, 62.3, 62.0, 34.0, 31.9, 29.7 (7 C), 29.6, 29.5, 29.4, 29.3, 29.1, 25.6 (5 C), 25.5, 24.9, 22.7, 22.6, 20.7, 20.6, 14.3, 14.1 ppm. IR (neat): 3013 (s, CH), 2923 (vs, CH), 2853 (vs, CH), 1744 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{45}H_{74}O_6 + NH_4$: 728.5824; found: 728.5819.

3-Butanoyl-2-docosahexaenoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-4b]. The same procedure was follow as described for (*S*)-**4a**: 3-Butanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**3b**] (130 mg; 0.303 mmol), DHA-FFA (115 mg; 0.350 mmol), EDCI (77 mg; 0.402 mmol), DMAP (17 mg; 0.139 mmol) and dichloromethane (3 ml). 3-butanoyl-2-docosahexaenoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**4b**] was isolated as yellowish liquid (171 mg; 0.231 mmol) in 76% yield. $[\alpha]_D^{20}$ -0.800 (*c* 10.12, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.29 (dd, *J* = 11.9, 4.3 Hz, 1 H, CH_2CHCH_2), 4.28 (dd, *J* = 11.9, 4.4 Hz, 1 H, CH_2CHCH_2), 4.15 (dd, *J* =

11.9, 5.9 Hz, 1 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 5.9 Hz, 1 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, =CCH_2C=), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.6 Hz, 2 H, CH_2COO), 2.30 (t, *J* = 7.4 Hz, 2 H, CH_2COO), 2.11-2.04 (m, 2 H, =CCH_2CH_3), 1.69-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 28 H, CH_2), 0.97 (t, *J* = 7.5, 3 H, CH_3 in DHA), 0.95 (t, *J* = 7.4 Hz, 3 H, CH_3), 0.88 (t, *J* = 6.9 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3, 173.1, 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 35.9, 34.0, 31.9, 29.7 (7 C), 29.6, 29.5, 29.4, 29.3, 29.1, 25.6 (5 C), 25.5, 24.9, 22.7, 22.6, 20.6, 18.3, 14.3, 14.1, 13.6 ppm. IR (neat): 3013 (s, CH), 2923 (vs, CH), 2853 (vs, CH), 1743 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{47}H_{78}O_6 + NH_4$: 756.6137; found: 756.6128.

1-Butanoyl-2-docosahexaenoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-4b]. The same procedure was follow as described for (*S*)-**4a**: 1-Butanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**3b**] (150 mg; 0.350 mmol), DHA-FFA (128 mg; 0.390 mmol), EDCI (87 mg; 0.454 mmol), DMAP (17 mg; 0.139 mmol) and dichloromethane (3 ml). 1-butanoyl-2-docosahexaenoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**4b**] was isolated as yellowish liquid (214 mg; 0.290 mmol) in 83% yield. $[\alpha]_D^{20}$ +0.835 (*c* 10.18, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2C*H*CH_2), 4.29 (dd, *J* = 11.9, 4.3 Hz, 1 H, CH_2CHCH_2), 4.28 (dd, *J* = 11.9, 4.4 Hz, 1 H, CH_2CHCH_2), 4.15 (dd, *J* = 11.9, 5.9 Hz, 1 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 5.9 Hz, 1 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, =CCH_2C=), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.6 Hz, 2 H, CH_2COO), 2.30 (t, *J* = 7.4 Hz, 2 H, CH_2COO), 2.11-2.04 (m, 2 H, =CCH_2CH_3), 1.69-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 28 H, CH_2), 0.97 (t, *J* = 7.5, 3 H, CH_3 in DHA), 0.95 (t, *J* = 7.4 Hz, 3 H, CH_3), 0.88 (t, *J* = 6.9 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3, 173.1, 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 35.9, 34.0, 31.9, 29.7 (7 C), 29.6, 29.5, 29.4, 29.3, 29.1, 25.6 (5 C), 25.5, 24.9, 22.7, 22.6, 20.6, 18.3, 14.3, 14.1, 13.6 ppm. IR (neat): 3013 (s, CH), 2923 (vs, CH), 2853 (vs, CH), 1743 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{47}H_{78}O_6 + NH_4$: 756.6137; found: 756.6128.

2-Docosahexaenoyl-3-hexanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-4c]. The same procedure was follow as described for (*S*)-**4a**: 3-Hexanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**3c**] (120 mg; 0.263 mmol), DHA-FFA (97 mg; 0.295 mmol), EDCI (62 mg; 0.323 mmol), DMAP (14 mg; 0.115 mmol) and dichloromethane (2.5 ml). 2-docosahexaenoyl-3-hexanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**4c**] was isolated as yellowish liquid (183 mg; 0.239 mmol) in 91% yield. $[\alpha]_D^{20}$ -0.487 (*c* 8.01, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2C*H*CH_2), 4.29 (dd, *J* = 11.9, 4.3 Hz, 1 H, CH_2CHCH_2), 4.28 (dd, *J* = 11.9, 4.4 Hz, 1 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 5.9 Hz, 2 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, =CCH_2C=), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.9 Hz, 4 H, CH_2COO), 2.11-2.04 (m, 2 H, =CCH_2CH_3), 1.65-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 32 H, CH_2), 0.97 (t, *J* = 7.5, 3 H, CH_3 in DHA), 0.89 (t, *J* = 6.9 Hz, 3 H, CH_3), 0.88 (t, *J* = 7.0 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (2 C), 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 34.0 (2 C), 31.9, 31.2, 29.7 (7 C), 29.6, 29.5, 29.4, 29.3, 29.1, 25.6 (5 C), 25.5, 24.9, 24.5, 22.7, 22.6, 22.3, 20.6, 14.3, 14.1, 13.9 ppm. IR (neat): 3013 (s, CH), 2923 (vs, CH), 2853 (vs, CH), 1743 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{49}H_{82}O_6 + NH_4$: 784.6456; found: 784.6429 amu.

2-Docosahexaenoyl-1-hexanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-4c]. The same procedure was follow as described for (*S*)-**4a**: 1-Hexanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**3c**] (120 mg; 0.263 mmol), DHA-FFA (93 mg; 0.283 mmol), EDCI (62 mg; 0.323 mmol), DMAP (13 mg; 0.106 mmol) and dichloromethane (2.5 ml). 2-docosahexaenoyl-1-hexanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**4c**] was isolated as yellowish liquid (172 mg; 0.224 mmol) in 85% yield. $[\alpha]_D^{20}$ +0.453 (*c* 10.15, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.29 (dd, *J* = 11.9, 4.3 Hz, 1 H, CH_2CHCH_2), 4.28 (dd, *J* = 11.9, 4.4 Hz, 1 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 5.9 Hz, 2 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, $=CCH_2C=$), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.9 Hz, 4 H, CH_2COO), 2.11-2.04 (m, 2 H, $=CCH_2CH_3$), 1.65-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 32 H, CH_2), 0.97 (t, *J* = 7.5, 3 H, CH_3 in DHA), 0.89 (t, *J* = 6.9 Hz, 3 H, CH_3), 0.88 (t, *J* = 7.0 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (2 C), 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 34.0 (2 C), 31.9, 31.2, 29.7 (7 C), 29.6, 29.5, 29.4, 29.3, 29.1, 25.6 (5 C), 25.5, 24.9, 24.5, 22.7, 22.6, 22.3, 20.6, 14.3, 14.1, 13.9 ppm. IR (neat): 3013 (s, CH), 2923 (vs, CH), 2853 (vs, CH), 1743 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{49}H_{82}O_6 + NH_4$: 784.6456; found: 784.6429 amu.

2-Docosahexaenoyl-1-octadecanoyl-3-octanoyl-*sn*-glycerol [(*S*)-4d]. The same procedure was follow as described for (*S*)-**4a**: 1-Octadecanoyl-3-octanoyl-*sn*-glycerol [(*S*)-**3d**] (120 mg; 0.248 mmol), DHA-FFA (91 mg; 0.277 mmol), EDCI (58 mg; 0.303 mmol), DMAP (13 mg; 0.106 mmol) and dichloromethane (2.5 ml). 2-docosahexaenoyl-1-octadecanoyl-3-octanoyl-*sn*-glycerol [(*S*)-**4d**] was isolated as yellowish liquid (172 mg; 0.216 mmol) in 87% yield. $[\alpha]_D^{20}$ -0.220 (*c* 9.08, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.29 (dd, *J* = 11.9, 4.4 Hz, 2 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 5.9 Hz, 2 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, $=CCH_2C=$), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 2.11-2.04 (m, 2 H, $=CCH_2CH_3$), 1.64-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 36 H, CH_2), 0.97 (t, *J* = 7.5, 3 H, CH_3 in DHA), 0.88 (t, *J* = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (2 C), 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 34.0 (2 C), 31.9, 31.6, 29.7 (7 C), 29.6, 29.5, 29.4, 29.3, 29.1, 29.0, 28.9, 25.6 (5 C), 25.5, 24.9 (2 C), 22.7, 22.6, 22.5, 20.6, 14.3, 14.1, 14.0 ppm. IR (neat): 3013 (s, CH), 2923 (vs, CH), 2853 (vs, CH), 1743 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{51}H_{86}O_6 + NH_4$: 812.6763; found: 812.6759 amu.

2-Docosahexaenoyl-3-octadecanoyl-1-octanoyl-*sn*-glycerol [(*R*)-4d]. The same procedure was follow as described for (*S*)-**4a**: 3-Octadecanoyl-1-octanoyl-*sn*-glycerol [(*R*)-**3d**] (110 mg; 0.227 mmol), DHA-FFA (90 mg; 0.274 mmol), EDCI (55 mg; 0.287 mmol), DMAP (11 mg; 0.090 mmol) and dichloromethane (3 ml). 2-docosahexaenoyl-3-octadecanoyl-1-octanoyl-*sn*-glycerol [(*R*)-**4d**] was isolated as yellowish liquid (168 mg; 0.211 mmol) in 93% yield. $[\alpha]_D^{20}$ +0.154 (*c* 10.36, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.29 (dd, *J* = 11.9, 4.4 Hz, 2 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 5.9 Hz, 2 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, $=CCH_2C=$), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 2.11-2.04 (m, 2 H, $=CCH_2CH_3$), 1.64-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 36 H, CH_2), 0.97 (t, *J* = 7.5, 3 H, CH_3 in DHA), 0.88 (t, *J* = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (2 C), 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 34.0 (2 C), 31.9, 31.6, 29.7 (7 C), 29.6, 29.5, 29.4, 29.3, 29.1, 29.0, 28.9, 25.6 (5 C), 25.5, 24.9 (2 C), 22.7, 22.6, 22.5, 20.6, 14.3, 14.1, 14.0 ppm. IR (neat): 3013 (s, CH), 2923 (vs, CH), 2853 (vs,

CH), 1743 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{51}H_{86}O_6 + NH_4$: 812.6763; found: 812.6759 amu.

3-Decanoyl-2-docosahexaenoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-4e]. The same procedure was follow as described for (*S*)-**4a**: 3-decanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**3e**] (110 mg; 0.215 mmol), DHA-FFA (87 mg; 0.265 mmol), EDCI (52 mg; 0.271 mmol), DMAP (19 mg; 0.156 mmol) and dichloromethane (2 ml). 3-decanoyl-2-docosahexaenoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**4e**] was isolated as yellowish liquid (166 mg; 0.202 mmol) in 94% yield. $[\alpha]_D^{20}$ -0.120 (*c* 10.02, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.29 (dd, *J* = 11.9, 4.4 Hz, 2 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 5.9 Hz, 2 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, $=CCH_2C=$), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 2.11-2.04 (m, 2 H, $=CCH_2CH_3$), 1.64-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 40 H, CH_2), 0.97 (t, *J* = 7.5, 3 H, CH_3 in DHA), 0.88 (t, *J* = 6.8 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (2 C), 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 34.0 (2 C), 31.9, 31.8, 29.7 (7 C), 29.6, 29.5, 29.4, 29.3, 29.2 (3 C), 29.1 (2 C), 25.6 (5 C), 25.5, 24.9 (2 C), 22.7 (2 C), 22.6, 20.6, 14.3, 14.1 (2 C) ppm. IR (neat): 3013 (s, CH), 2923 (vs, CH), 2853 (vs, CH), 1743 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{53}H_{90}O_6 + NH_4$: 840.7076; found: 840.7094 amu.

1-Decanoyl-2-docosahexaenoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-4e]. The same procedure was follow as described for (*S*)-**4a**: 1-Decanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**3e**] (110 mg; 0.215 mmol), DHA-FFA (83 mg; 0.253 mmol), EDCI (85 mg; 0.443 mmol), DMAP (15 mg; 0.123 mmol) and dichloromethane (2 ml). 1-decanoyl-2-docosahexaenoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**4e**] was isolated as yellowish liquid (169 mg; 0.205 mmol) in 95% yield. $[\alpha]_D^{20}$ +0.083 (*c* 6.05, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.29 (dd, *J* = 11.9, 4.4 Hz, 2 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 5.9 Hz, 2 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, $=CCH_2C=$), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 2.11-2.04 (m, 2 H, $=CCH_2CH_3$), 1.64-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 40 H, CH_2), 0.97 (t, *J* = 7.5, 3 H, CH_3 in DHA), 0.88 (t, *J* = 6.8 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (2 C), 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 34.0 (2 C), 31.9, 31.8, 29.7 (7 C), 29.6, 29.5, 29.4, 29.3, 29.2 (3 C), 29.1 (2 C), 25.6 (5 C), 25.5, 24.9 (2 C), 22.7 (2 C), 22.6, 20.6, 14.3, 14.1 (2 C) ppm. IR (neat): 3013 (s, CH), 2923 (vs, CH), 2853 (vs, CH), 1743 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{53}H_{90}O_6 + NH_4$: 840.7076; found: 840.7094 amu.

2-Docosahexaenoyl-3-dodecanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-4f]. The same procedure was follow as described for (*S*)-**4a**: 3-Dodecanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**3f**] (110 mg; 0.203 mmol), DHA-FFA (68 mg; 0.207 mmol), EDCI (47 mg; 0.245 mmol), DMAP (10 mg; 0.082 mmol) and dichloromethane (2.5 ml). 2-docosahexaenoyl-3-dodecanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**4f**] was isolated as yellowish liquid (154 mg; 0.181 mmol) in 89% yield. $[\alpha]_D^{20}$ -0.030 (*c* 10.01, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.29 (dd, *J* = 11.9, 4.4 Hz, 2 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 5.9 Hz, 2 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, $=CCH_2C=$), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 2.11-2.04 (m, 2 H, $=CCH_2CH_3$), 1.64-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20

(m, 44 H, CH_2), 0.97 (t, J = 7.5, 3 H, CH_3 in DHA), 0.88 (t, J = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (2 C), 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 34.0 (2 C), 31.9 (2 C), 29.7 (7 C), 29.6 (3 C), 29.5 (2 C), 29.4, 29.3, 29.2 (2 C), 29.1 (2 C), 25.6 (5 C), 25.5, 24.9 (2 C), 22.7 (2 C), 22.6, 20.6, 14.3, 14.1 (2 C) ppm. IR (neat): 3013 (s, CH), 2922 (vs, CH), 2853 (vs, CH), 1744 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{55}H_{94}O_6$ + NH_4: 868.7389; found: 868.7374 amu.

2-Docosahexaenoyl-1-dodecanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-4f]. The same procedure was follow as described for (*S*)-**4a**: 1-Dodecanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**3f**] (111 mg; 0.205 mmol), DHA-FFA (76 mg; 0.231 mmol), EDCI (47 mg; 0.245 mmol), DMAP (10 mg; 0.082 mmol) and dichloromethane (3 ml). 2-docosahexaenoyl-1-dodecanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**4f**] was isolated as yellowish liquid (164 mg; 0.193 mmol) in 94% yield. $[\alpha]_D^{20}$ +0.061 (*c* 3.30, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.29 (dd, J = 11.9, 4.4 Hz, 2 H, CH_2CHCH_2), 4.14 (dd, J = 11.9, 5.9 Hz, 2 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, $=CCH_2C=$), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, J = 7.6 Hz, 4 H, CH_2COO), 2.11-2.04 (m, 2 H, $=CCH_2CH_3$), 1.64-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 44 H, CH_2), 0.97 (t, J = 7.5, 3 H, CH_3 in DHA), 0.88 (t, J = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (2 C), 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 34.0 (2 C), 31.9 (2 C), 29.7 (7 C), 29.6 (3 C), 29.5 (2 C), 29.4, 29.3, 29.2 (2 C), 29.1 (2 C), 25.6 (5 C), 25.5, 24.9 (2 C), 22.7 (2 C), 22.6, 20.6, 14.3, 14.1 (2 C) ppm. IR (neat): 3013 (s, CH), 2922 (vs, CH), 2853 (vs, CH), 1744 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{55}H_{94}O_6$ + NH_4: 868.7389; found: 868.7374 amu.

2-Docosahexaenoyl-1-octadecanoyl-3-tetradecanoyl-*sn*-glycerol [(*S*)-4g]. The same procedure was follow as described for (*S*)-**4a**: 1-Octadecanoyl-3-tetradecanoyl-*sn*-glycerol [(*S*)-**3g**] (140 mg; 0.246 mmol), DHA-FFA (89 mg; 0.271 mmol), EDCI (75 mg; 0.391 mmol), DMAP (13 mg; 0.106 mmol) and dichloromethane (3 ml). 2-docosahexaenoyl-1-octadecanoyl-3-tetradecanoyl-*sn*-glycerol [(*S*)-**4g**] was isolated as yellowish liquid (207 mg; 0.235 mmol) in 96% yield. $[\alpha]_D^{20}$ -0.020 (*c* 10.02, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.28 (dd, J = 11.9, 4.4 Hz, 2 H, CH_2CHCH_2), 4.14 (dd, J = 11.9, 5.9 Hz, 2 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, $=CCH_2C=$), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, J = 7.6 Hz, 4 H, CH_2COO), 2.11-2.04 (m, 2 H, $=CCH_2CH_3$), 1.64-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 48 H, CH_2), 0.97 (t, J = 7.5, 3 H, CH_3 in DHA), 0.88 (t, J = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (2 C), 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 34.0 (2 C), 31.9 (2 C), 29.7 (10 C), 29.6 (2 C), 29.5 (2 C), 29.4 (2 C), 29.3 (2 C), 29.1 (2 C), 25.6 (5 C), 25.5, 24.9 (2 C), 22.7 (2 C), 22.6, 20.6, 14.3, 14.1 (2 C) ppm. IR (neat): 3013 (s, CH), 2922 (vs, CH), 2853 (vs, CH), 1743 (vs, C=O), cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{57}H_{98}O_6$ + NH_4: 896.7702; found: 896.7678 amu.

2-Docosahexaenoyl-3-octadecanoyl-1-tetradecanoyl-*sn*-glycerol [(*R*)-4g]. The same procedure was follow as described for (*S*)-**4a**: 3-Octadecanoyl-1-tetradecanoyl-*sn*-glycerol [(*R*)-**3g**] (110 mg; 0.193 mmol), DHA-FFA (69 mg; 0.210 mmol), EDCI (46 mg; 0.240 mmol), DMAP (11 mg; 0.090 mmol) and dichloromethane (2 ml). 2-docosahexaenoyl-3-octadecanoyl-1-tetradecanoyl-*sn*-glycerol [(*R*)-**4g**] was isolated as yellowish liquid (165

mg; 0.188 mmol) in 97% yield. $[\alpha]_D^{20}$ +0.049 (*c* 8.17, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.28 (dd, *J* = 11.9, 4.4 Hz, 2 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 5.9 Hz, 2 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, $=CCH_2C=$), 2.42-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 2.11-2.04 (m, 2 H, $=CCH_2CH_3$), 1.64-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 48 H, CH_2), 0.97 (t, *J* = 7.5, 3 H, CH_3 in DHA), 0.88 (t, *J* = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (2 C), 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 34.0 (2 C), 31.9 (2 C), 29.7 (10 C), 29.6 (2 C), 29.5 (2 C), 29.4 (2 C), 29.3 (2 C), 29.1 (2 C), 25.6 (5 C), 25.5, 24.9 (2 C), 22.7 (2 C), 22.6, 20.6, 14.3, 14.1 (2 C) ppm. IR (neat): 3013 (s, CH), 2922 (vs, CH), 2853 (vs, CH), 1743 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{57}H_{98}O_6 + NH_4$: 896.7702; found: 896.7678 amu.

2-Docosahexaenoyl-3-hexadecanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-4h]. The same procedure was follow as described for (*S*)-**4a**: 3-Hexadecanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**3h**] (120 mg; 0.201 mmol), DHA-FFA (77 mg; 0.234 mmol), EDCI (48 mg; 0.250 mmol), DMAP (10 mg; 0.082 mmol) and dichloromethane (3 ml). 2-docosahexaenoyl-3-hexadecanoyl-1-octadecanoyl-*sn*-glycerol [(*S*)-**4h**] was isolated as yellowish liquid (145 mg; 0.160 mmol) in 80% yield. $[\alpha]_D^{20}$ 0.00 (*c* 10.16, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.28 (dd, *J* = 11.9, 4.4 Hz, 2 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 5.9 Hz, 2 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, $=CCH_2C=$), 2.41-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 2.11-2.04 (m, 2 H, $=CCH_2CH_3$), 1.64-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 52 H, CH_2), 0.97 (t, *J* = 7.5, 3 H, CH_3 in DHA), 0.88 (t, *J* = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (2 C), 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 34.0 (2 C), 31.9 (2 C), 29.7 (12 C), 29.6 (2 C), 29.5 (2 C), 29.4 (2 C), 29.3 (2 C), 29.1 (2 C), 25.6 (5 C), 25.5, 24.9 (2 C), 22.7 (2 C), 22.6, 20.6, 14.3, 14.1 (2 C) ppm. IR (neat): 3013 (s, CH), 2913 (vs, CH), 2849 (vs, CH), 1728 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{59}H_{102}O_6 + NH_4$: 924.8015; found: 924.7984 amu.

2-Docosahexaenoyl-1-hexadecanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-4h]. The same procedure was follow as described for (*S*)-**4a**: 1-Hexadecanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**3h**] (111 mg; 0.186 mmol), DHA-FFA (72 mg; 0.219 mmol), EDCI (43 mg; 0.224 mmol), DMAP (10 mg; 0.082 mmol) and dichloromethane (2 ml). 2-docosahexaenoyl-1-hexadecanoyl-3-octadecanoyl-*sn*-glycerol [(*R*)-**4h**] was isolated as yellowish liquid (163 mg; 0.180 mmol) in 97% yield. $[\alpha]_D^{20}$ +0.010 (*c* 10.31, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44-5.30 (m, 12 H, =CH), 5.29-5.24 (m, 1 H, CH_2CHCH_2), 4.28 (dd, *J* = 11.9, 4.4 Hz, 2 H, CH_2CHCH_2), 4.14 (dd, *J* = 11.9, 5.9 Hz, 2 H, CH_2CHCH_2), 2.88-2.80 (m, 10 H, $=CCH_2C=$), 2.41-2.37 (m, 4 H, CH_2CH_2COO in DHA), 2.31 (t, *J* = 7.6 Hz, 4 H, CH_2COO), 2.11-2.04 (m, 2 H, $=CCH_2CH_3$), 1.64-1.57 (m, 4 H, CH_2CH_2COO), 1.35-1.20 (m, 52 H, CH_2), 0.97 (t, *J* = 7.5, 3 H, CH_3 in DHA), 0.88 (t, *J* = 6.9 Hz, 6 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (2 C), 172.1, 132.0, 129.4, 128.6, 128.3, 128.2 (2 C), 128.1 (2 C), 128.0, 127.9, 127.6, 127.0, 69.1, 62.0 (2 C), 34.0 (2 C), 31.9 (2 C), 29.7 (12 C), 29.6 (2 C), 29.5 (2 C), 29.4 (2 C), 29.3 (2 C), 29.1 (2 C), 25.6 (5 C), 25.5, 24.9 (2 C), 22.7 (2 C), 22.6, 20.6, 14.3, 14.1 (2 C) ppm. IR (neat): 3013 (s, CH), 2913 (vs, CH), 2849 (vs, CH), 1728 (vs, C=O) cm^{-1}. HRMS (APCI): *m/z* calcd for $C_{59}H_{102}O_6 + NH_4$: 924.8015; found: 924.7984 amu.

5.3 Alcoholysis of palm olein

Ethanolysis experiment 1 (Table 5): 2 g of palm olein, 221 mg of absolute ethanol (2 equivalents based on mol TAG) and 200 mg of lipase (10% based on weight of palm olein) were mixed together and gently agitated at 35°C under nitrogen. After an appropriate time samples were collected and the reaction discontinued by separating the lipase by filtration.

Ethanolysis experiment 2 (Table 6): Same as in experiment 1, except using 295 µl of 95% ethanol instead of absolute ethanol.

Ethanolysis experiment 3 (Table 7): Same as in experiment 1, except using 20% lipase dosage (400 mg) and 295 µl of 95% ethanol instead of absolute ethanol.

Ethanolysis experiment 4 (Table 8 and Table 9): Same as in experiment 1, except using 5 g of oil, 20% lipase dosage (1000 mg) and 702 µl of absolute ethanol.

Ethanolysis experiment 5 (Table 10 and Table 11): Same as in experiment 1, except using 5 g of oil, 20% lipase dosage (1000 mg) and 1404 µl of absolute ethanol (4 molar equivalents based on mol TAG).

Ethanolysis experiment 6 (Table 12 and Table 13): Same as in experiment 1, except using 5 g of oil, 20% lipase dosage (1000 mg) and 1404 µl of absolute ethanol (4 molar equivalents based on mol TAG). Prior to the reaction the lipase had been incubated three times with excess oil.

Ethanolysis experiment 7 (Table 14 and Table 15): Same as in experiment 1, except using 1 g of oil, 20% lipase dosage (200 mg) and 140 µl of absolute ethanol. Prior to the reaction the lipase had been mixed with oil and the resulting suspension dried under vacuum. That oil was then replaced with new dried oil before reactions.

Ethanolysis experiment 8 (Table 16 and Table 17): Same as in experiment 1, except using 1 g of oil, 20% lipase dosage (200 mg) and 140 µl of absolute ethanol. Prior to the reaction the lipase had been dried separately under vacuum. The oil used in the reactions had also been dried under vacuum.

Methanolysis experiment (Table 20): 2 g of palm olein, 195 µl of methanol (2 equivalents based on mol TAG) and 200 mg of lipase A (10% based on weight of palm olein) were mixed together and gently agitated at 35°C under nitrogen. After 1, 2, 4, 8 and 24 hours samples were collected and the reaction discontinued by separating the lipase by filtration.

Titration: Final samples obtained after 24 hours in experiments 1 and 2 from the four lipases, A, C, H and J, were dissolved in 25 mL of 2-propanol and titrated with 0,010M NaOH. Phenolphthalein was used as an indicator. 25 mL of 2-propanol were first titrated to get the background.

Analysis of fatty acid composition of the acylglycerol fractions: Samples obtained after 4 hours in experiment 1 from the four lipases, A, C, H and J, were separated by preparative thin-layer chromatography using 80:20:2 mixture of petroleum ether/diethyl ether/acetic acid as an eluent. Rhodamine was used to visualise the bands, which were then scraped off

to methylate the acylglycerol fractions. Methylation was performed by dissolving 20-30 mg of sample in methanolic solution of sodium hydroxide (1.5-2 ml) at 70°C, 1.5-2 ml of boron trifluoride in methanol solution were added and the mixture maintained at 70°C for about half an hour. Water and petroleum ether were added and the methyl esters extracted into petroleum ether. The organic phase was subsequently analysed by GLC.

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