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Chemoenzymatic synthesis of structured triacylglycerols by highly regioselective acylation

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Abstract—A highly efficient two-step chemoenzymatic synthesis of structured triacylglycerols comprising a pure n-3 polyunsaturated fatty acid at the mid-position and a pure saturated fatty acid located at the end-positions is described. In the first step an immobilized *Candida antarctica* lipase was observed to display an excellent regioselectivity toward the end-positions of glycerol at 0–4°C using vinyl esters as acylating agents. The n-3 fatty acids were introduced into the remaining mid-position highly efficient and in excellent yields using EDCI coupling agent.
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1. Introduction

The term structured lipids refers to lipids that have a predetermined composition and distribution of fatty acids at the glycerol backbone.[1,2] Structured triacylglycerols (TAG) comprising certain types of fatty acids at the end-positions and different fatty acids at the mid-position of the glycerol moiety have gained increasing attention of scientists as dietary and health supplements. Of particular interest are structured TAG possessing biologically active long-chain polyunsaturated fatty acids (PUFA) located at the mid-position, with medium-chain fatty acids (MCFA) at the end-positions.[3]

The long-chain n-3 PUFA are characteristic of marine fat.[4,5] Their beneficial health effects are well established and have been attributed to *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA), the two most prevalent n-3 PUFA in fish.[6]

Therefore, structured TAG containing EPA or DHA located at the mid-position with MCFA at the end-positions are in high demand.[7] Figure 1 displays such structured TAG comprising EPA and capric acid (left) and DHA and caprylic acid (right).

The synthesis of such positionally labeled structured TAG requires full regioselectivity control and can hardly be undertaken by traditional synthetic organic chemistry methods without multi-step protection–deprotection processes. Based on their high regioselectivity, lipases[8,9] are ideally suited as biocatalysts for the synthesis of structured lipids, by acting preferably or exclusively at the primary positions of the glycerol moiety.[4,7] Another important feature offered by lipases is the mild conditions under which they act, which may become crucial in hampering intramolecular acyl-migration side-reactions. Such processes are well-known to create problems in association with the synthesis of partially acylated diols and polyols and must be avoided to maintain the required regiocontrol.[10,11]

Numerous reports describe the preparation of structured TAG comprising various enrichment levels of these fatty acids, where lipases play a key role as biocatalysts, by various esterification and transesterification reactions, usually as a complicated mixture of compounds.[4,12–14] There are also reports on homogeneous TAG of the type described in Figure 1, but the products have not been fully characterized by conventional organic chemistry methods.[15–17]

A previous communication reports the synthesis of structured TAG comprising pure MCFA (C_8, C_{10} or C_{12}) and EPA or DHA by a two-step chemoenzymatic approach, starting from glycerol.[18] In the first step an immobilized 1,3-regioselective *Rhizomucor miehei* lipase was exploited to prepare the 1,3-diacylglycerol (1,3-DAG) intermediate adducts **1a–c** (Scheme 1) in moderate to good yields (55–70%) by a method of Schneider and coworkers.[19,20] Subsequently, pure EPA or DHA were chemically introduced to the remaining mid-position in excellent yields (90–95%) using a coupling agent (Scheme 2). The main objectives of the work described in this paper were to

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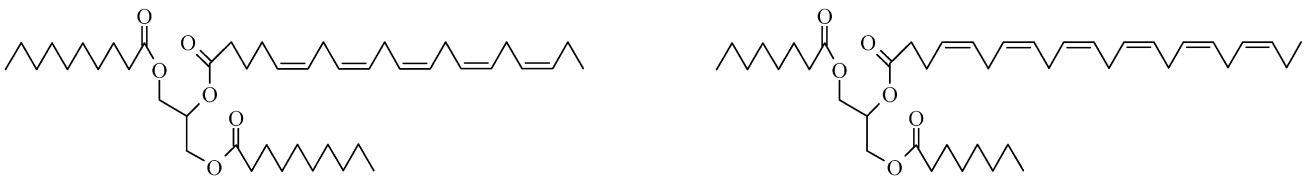


Figure 1.

improve the regioselectivity and thus the yields of the enzymatic step of the synthesis.

The current work describes a dramatic improvement of the enzymatic step and the regiocontrol of the structured TAG synthesis. This is based on a rapid, irreversible transesterification of glycerol using vinyl esters of the MCFA and related fatty acids and an immobilized *Candida antarctica* lipase offering excellent results at 0–4°C (Scheme 1). Under these conditions the lipase displayed a perfect regioselectivity by acting exclusively at the primary alcoholic positions of the glycerol. There were no signs of any acyl-migration taking place and the 1,3-DAG adducts **1a–e** were produced in virtually quantitative yields. Subsequently, pure EPA and DHA were introduced into the mid-position by EDCI coupling agent in excellent yields to accomplish structured TAG of the type described above (Scheme 2).

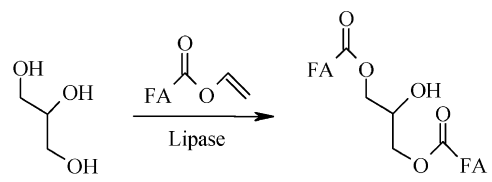
2. Results and discussion

Preparation of structured TAG comprising n-3 PUFA at the mid-position with MCFA at the end-positions is described in numerous reports. These structured TAG usually comprise various enrichment levels of these fatty acids. They are prepared by treating fish oils with 1,3-regioselective lipases under transesterification conditions with MCFA as free acids or ethyl esters.[4,13] Fish oils, such as tuna oil, usually have the mid-position of the TAG more enriched with the n-3 PUFA, especially DHA. The aim of that sort of product is oriented towards nutrition or food supplements. Reactions are frequently conducted without any organic solvent, which favours the food supplement application. Another approach is to use a 1,3-regioselective lipase to hydrolyse[12] or ethanolyse[14] the end-positions of the fish oil TAG. A different lipase is then employed to reesterify the end-positions of the resulting 2-MAG with MCFA.

There are reports describing the preparation of homogeneous structured TAG comprising pure EPA or DHA at the mid-position and a pure MCFA at the end-positions.[15–17] The aim of that type of product is more toward the pharmaceutical area for drug and clinical purposes. The synthesis has been based on a two-enzymatic step process. The first step involves the synthesis of pure trieicosapentaenoin or tridocosahexaenoin by a procedure of Haraldsson et al.[21] The second step involves a subsequent transesterification of the homogeneous TAG with caprylic acid or its ethyl esters into the end-positions by a 1,3-regioselective lipase.[15] From the viewpoint of synthetic organic chemistry, this approach involves various drawbacks. One relates to the use of three equivalents of the pure highly valuable EPA or DHA required and a vast excess of the MCFA or its ethyl esters. Another is related to rather complicated mixtures obtained requiring tedious separation and purification procedures, which lowers the yields considerably. Third, the regiocontrol of these methods is not very high.

Yamane and coworkers[16,17] have reported on an interesting modification and improvement of these synthesis. This is based on a highly 1,3-regioselective *Candida antarctica* lipase catalyzed ethanolysis of the TAG intermediates homogeneous with EPA and DHA, and a subsequent lipase promoted esterification of the resulting 2-MAG with ethyl esters of MCFA using the *Rhizomucor miehei* lipase. Although the first lipase apparently displayed high regioselectivity under the ethanolic conditions and there was only minor acyl-migration taking place during the second enzymatic step, there were unwanted regioisomers present in the products. No attempts were made to isolate the desired structured TAG and characterize them by traditional organic chemistry methods.

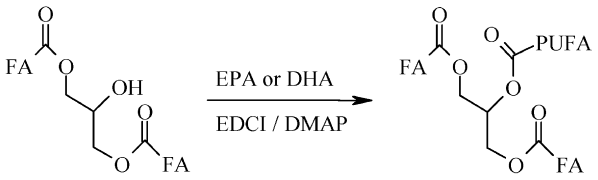
In the lipase step of the current work the lipase acted exclusively at the end-positions and no acyl-migration took place. Toward the end of the reactions virtually quantitative conversion into the desired 1,3-DAG adduct was obtained and there were only traces of 1-MAG present. The structured TAG products remained regioisomerically pure after the n-3 PUFA introduction, unlike in all the above stated reports. It may be argued that the previous methods are advantageous as a consequence of not using any toxic organic solvents or hazardous chemical reagents. That



1a-e

FA = C_7H_{15} (**a**), C_9H_{19} (**b**), $C_{11}H_{23}$ (**c**), $C_{13}H_{27}$ (**d**) or $C_{15}H_{31}$ (**e**)

Scheme 1.



1a-e

2a-e (PUFA = EPA)
3a-e (PUFA = DHA)

FA = C_7H_{15} (**a**), C_9H_{19} (**b**), $C_{11}H_{23}$ (**c**), $C_{13}H_{27}$ (**d**) or $C_{15}H_{31}$ (**e**)

Scheme 2.

argument, however, is not as important when pure adducts and potential drug candidates are at issue and the absolute regioisomeric purity, which is the real aim of these syntheses.

2.1. The lipase reaction

The enzymatic reaction (Scheme 1) was conducted at 0–4°C in dichloromethane or chloroform using 10% dosage of lipase as based on weight of the substrates. Only minimum amount of solvent needed to dissolve the substrates was used, approximately 0.1 mL per g of substrates. The progress of the reaction was monitored by TLC on silica and ^{1}H NMR spectroscopy. During the reaction only 1-monoacylglycerol (1-MAG) intermediate was detected in small quantities with the 1,3-DAG product largely dominating the reaction mixture. After 3–5 h the reaction was completed with only traces of the 1-MAG left and there were no signs of any 1,2-DAG nor TAG present and thereby no indications of any acyl-migration side-reactions nor the lipase acting at the mid-position. However, prolonged reaction time resulted in some 1,2-DAG and TAG formation presumably involving acyl-migration. Also, at higher temperature (20°C) there were clear indications of loss of regioselectivity, implying that the temperature is a crucial factor in terms of controlling the regioselectivity of the reaction. By reason of lower solubility and an immediate crystallization of the 1,3-DAG adduct out of solution upon formation, the reaction involving the longest chain palmitates was an exception and could easily be run at rt overnight without any detrimental effects on the regioselectivity and yield.

To secure yields greater than 90%, 2.5 equiv. of the vinyl esters were needed, corresponding to 1.25 fold stoichiometric amount or 25% excess. This relates to an increased rate of reaction and the hydrolysis side-reaction[20] competing with glycerol and 1-MAG for the vinyl esters to produce free fatty acids. The products were crystallized from petroleum ether or *n*-hexane after storage in a freezer (−18°C). The yields (90–92%, see Table 1) are based on pure material after recrystallization.

Table 1. Preparation of 1,3-diacylglycerols

Compound	FA	Yield (%)
1a	$-C_7H_{15}$	90
1b	$-C_9H_{19}$	92
1c	$-C_{11}H_{23}$	92
1d	$-C_{13}H_{27}$	92
1e	$-C_{15}H_{31}$	90

2.2. The coupling reaction

In the subsequent coupling reaction 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) was used as a chemical coupling agent to introduce EPA and DHA into the mid-position of the 1,3-DAG adducts (Scheme 2). The reaction was conducted at rt in dichloromethane in the presence of 0.3–0.5 mol equiv. of 4-dimethylaminopyridine (DMAP) using an exact stoichiometric amount of EPA or DHA as based on the 1,3-DAG adduct. The reactions were completed in 12–15 h. Pure 2-EPA-TAG (**4a**–**e**) and 2-DHA-TAG (**5a**–**e**) were afforded as colourless oils and slightly yellowish oils, respectively for the EPA and DHA adducts, in yields of 90–95% after chromatography treatment on silica gel (see Table 2).

Table 2. Synthesis of structured triacylglycerols

Compound	FA	PUFA	Yield (%)
2a	$-C_7H_{15}$	EPA	90
2b	$-C_9H_{19}$	EPA	93
2c	$-C_{11}H_{23}$	EPA	92
2d	$-C_{13}H_{27}$	EPA	92
2e	$-C_{15}H_{31}$	EPA	90
3a	$-C_7H_{15}$	DHA	90
3b	$-C_9H_{19}$	DHA	94
3c	$-C_{11}H_{23}$	DHA	95
3d	$-C_{13}H_{27}$	DHA	95
3e	$-C_{15}H_{31}$	DHA	92

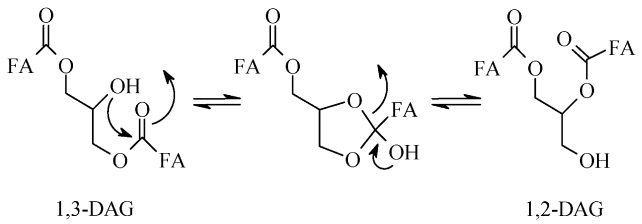
Besides much higher yields, EDCI offers the advantage over other coupling agents such as dicyclohexyl carbodiimide (DCC)[18] to possess a polar amino group and to be water soluble, which is of benefit when working up the reaction and purifying the products. DMAP presumably serves both as a base and a catalyst for the acylation process, and far more than a catalytic amount was required to obtain the optimal yields. No sign of any acyl-migration side-reaction was observed to take place during the coupling reaction.

2.3. Acyl-migration

Acyl-migration is a major problem in regioselective acylation of polyhydroxy compounds such as glycerols and carbohydrates. The acyl-migration process is an enzyme independent intramolecular rearrangement working against regioselectivity. Such processes are speeded up by various factors including temperature, pH, the presence of acid or base, type of solvent, and immobilized enzyme support material.[9,10,24]

Although the acyl-migration is an enzyme independent process it may be induced by lipase. This occurs in association with the presence of low quantities of water in the organic reaction medium essential for lipases to retain their optimal activity and consequent hydrolysis side-reactions.[22,23] Such hydrolysis of a 1,3-DAG acyl group may easily maintain the presence of 1-MAG in the reaction mixture. It is believed that the acyl-migration rate for 1,3-DAG is lower than for 1-MAG, the latter being more prone to acyl-migration.[24] The extent of such hydrolysis side-reaction is related to the optimal water content of the reaction medium which appears to vary considerably among different lipases. The initial water content of the current lipase preparation was 1–2% by weight.

Scheme 3 illustrates a simplified mechanism of such an intramolecular rearrangement, involving a nucleophilic attack of a free hydroxyl group at an adjacent ester carbonyl group, for the conversion of 1,3-DAG of capric acid into the undesired 1,2-DAG by acyl-migration. This results in formation of a tetrahedral intermediate in where the two vicinal hydroxyl groups are bridged over a five-membered hemi-*ortho* ester type ring. At equilibrium there is approximately, a 60:40 ratio of the neat DAG counterparts

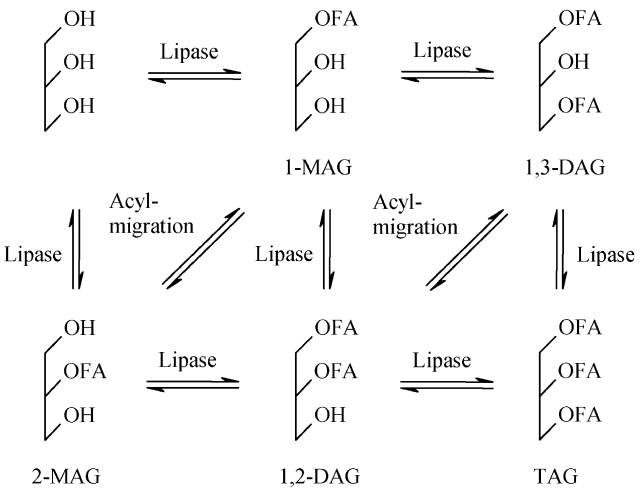


Scheme 3.

in favour of the more stable 1,3-DAG adduct. The corresponding equilibrium composition for the 1-MAG vs 2-MAG is 90:10 favouring the 1-MAG adduct.[24] The equilibrium composition may be slightly shifted when the glycerides are dissolved in a solvent. There are indications from the current work that longer acyl groups were less prone to undergo acyl-migration.

2.4. Regioselectivity control

The good results predominantly relate to the absence of any acyl-migration side-reactions and the enzyme displaying an excellent regioselectivity. This is interrelated to various important factors including the temperature, apparently the most crucial single parameter, fast and efficient reaction, support material of lipase not inducing acyl-migration, type of reaction, acyl donor and reaction conditions. Scheme 4 illustrates the complexity involved and all potential reactions, reaction intermediates and products that may take part in the lipase promoted reaction.



Scheme 4.

The temperature was maintained low enough to keep the acyl-migration and lipase regioselectivity completely under control. At the same time the lipase acted fast enough to eliminate any acyl-migration and also irreversibly to eliminate any equilibrium problems. This was brought about by the use of activated vinyl esters, which offered a fast and irreversible reaction, and were tolerated very well as an acylating substrate by the lipase. The type of reaction and the nature of acyl donor is also very important. This is an irreversible transesterification of vinyl esters and the glycerol adducts. It is irreversible because of the enolic leaving group tautomerizing to a non-nucleophilic acetaldehyde.

With the activated vinyl esters, the lipase acted very fast and efficiently. After 5 h virtually 100% conversion had been obtained into the desired 1,3-DAG adduct with no undesirable side-products being detected apart from only traces of the 1-MAG intermediate as was established by ^{1}H NMR spectroscopy analysis of the crude reaction mixture. Prolonged reaction beyond 5 h under the same conditions at 0°C resulted in a noticeable formation of adducts bearing acyl group at the mid-position, 1,2-DAG and TAG. This shows that the reaction rate and length of time under the reaction conditions can also be crucial for hampering undesired acyl-migration reactions, since the acyl-migration process is clearly time-dependent.

Raising the temperature to rt and 40°C resulted in an immediate loss of the strict 1,3-regiocontrol of the reaction system with clear indications of such products. Whether this is solely related to acyl-migration, the lipase losing its regioselectivity, or both, is not easy to determine. Investigations are underway to sort this out in more depth.

The excessive amount of the vinyl esters relates not only to the time factor but also to a water content control since water is presumably much more prone to react with the highly reactive vinyl esters than the glyceryl esters. Thereby, the water content and the related hydrolysis side-reactions are kept under control by the excessive amount of the vinyl esters. An alternative means of controlling the water content was to use a lipase preparation of lower water content, but that might have lowered the initial reaction rate and the rate of the overall reaction with detrimental effects. However, this lipase is known to act perfectly well under extremely low water conditions.[21]

When the *Candida antarctica* lipase was used under direct esterification conditions with free acids the result was a far lower degree of regioselectivity even at 0°C and a much slower reaction. The same lipase was previously used to synthesize homogeneous TAG of EPA and DHA at much higher temperature, 60–65°C, highly efficient.[21] The conclusion was that acyl-migration was playing a key role in such synthesis induced by the high temperature and protons from the free fatty acids. In the current reaction only a limited number of protons associated with the formation of free fatty acids by hydrolysis side-reactions is present without much apparent effect on the regioselectivity.

Yamane and coworkers have also observed the regioselectivity of lipases to be very much dependent upon type of reaction and conditions.[16] The *Candida antarctica* lipase acted highly regioselective and efficiently in its 1,3-regioselective ethanolysis of homogeneous TAG of EPA or DHA to produce 2-MAG. Interestingly, this lipase did not offer satisfactory results when used to reintroduce MCFA into the free 1,3-positions of the resulting 2-MAG, nor could it be used to incorporate MCFA into the end-positions of the homogeneous TAG either by acidolysis or interesterification reactions. The *Rhizomucor miehei* lipase, on the other hand, proved to be highly useful for that sort of process, but far less useful for the TAG ethanolysis approach.

When the *Candida antarctica* lipase was replaced by the *Rhizomucor miehei* lipase under identical conditions losses

in 1,3-regioselectivity and yields were obtained. A mixture of 2-MAG, 1,2-DAG and TAG was clearly noticeable with the latter lipase, as certainly was the case when using the original method of Schneider and coworkers with the same lipase in ether at rt for both free acids and vinyl esters as acylating agents. Similar results were observed in the previous work of Schneider and coworkers[19,20] and, more recently, Yamane and coworker[25] in terms of regiocontrol of the 1,3-DAG preparation. It is quite clear that the *Rhizomucor* lipase is inferior to the *Candida* lipase to produce 1,3-DAG. When comparing the two lipases involved in our work the fact that they are immobilized on different supports should be kept in mind.

Finally, an interesting question emerges as to which factor is the most important to administer in terms of regioselectivity control of these reactions, the acyl-migration or the lipase adhering to its strict 1,3-regioselectivity. Our present and previous[21] results indicate that the acyl-migration is certain. However, it can be unequivocally ruled out that at a higher temperature the lipase may act directly at the mid-position[26] or is the acyl-migration process entirely responsible for the fatty acyl incorporation into that position? These are crucial questions to answer in order to understand the chemistry involved in more depth and we hope to have the answers soon in our continuing study of these reactions.

2.5. NMR analysis

High-field ^{1}H and ^{13}C NMR spectroscopy was crucial in monitoring the regioselectivity control in these reactions and to evaluate the purity of the compounds. Previous work revealed that all individual acylglycerol adducts potentially involved in these reactions, 1- and 2-MAG, 1,3- and 1,2-DAG, as well as TAG (see Scheme 4), display quite characteristic ^{1}H NMR spectra in the glyceryl moiety proton region (δ 5.30–3.60 ppm).[21] This is regardless of the type of acyl groups present. Knowing this makes it quite straightforward to recognize all the individual acylglycerol constituents present in the product mixture and to quantify them with reasonable or good accuracy. Thus, the progress of the reaction as it proceeded as well as its regiocontrol could be monitored. In a mixture comprising all constituents there is certainly some interference between these peaks, but this is normally not the case under the optimal reaction conditions described in this work.

^{13}C NMR spectroscopy played an important complementary role in monitoring the regiocontrol of these reactions, not only with respect to the enzymatic reaction but also the coupling reaction. This relates to the fact that the carbonyl group carbon of a certain fatty acid displays two distinct resonance peaks depending on whether the fatty acid is located at the end-positions (α) or the mid-position (β) of the glyceryl backbone.[18,21,27] In structured TAG of the type described in the current work saturated fatty acids including MCFA all resonate at an identical chemical shift value, δ 173.2 ppm, when located at an end-position, but at δ 172.9 ppm when located at the mid-position (see Table 3).

In addition, the EPA and DHA carbonyl group carbons resonate at different chemical shift values from MCFA and from each other. This is a result of the influence of the location of the carbon–carbon double bonds in varying proximity to the carboxyl groups of these fatty acids. As an example, the EPA carbonyl group carbon resonates at δ 172.8 ppm when located at the α-position, but δ 172.5 ppm when located at the β-position of TAG. The DHA carbonyl group carbon, on the other hand, resonates at δ 172.6 ppm when located at the α-position, but δ 172.1 ppm when located at the β-position of TAG. This is summarized in Table 3. This variation has been utilized by chemists to determine the EPA and DHA content at the mid-position vs. the end-positions in fish oil TAG.[28,29] This has also enabled the evaluation of the structured TAG products described in this report in terms of regioisomeric purity. It should, however, be borne in mind that the accuracy of the ^{13}C NMR spectroscopy is not very high, especially when weak carbonyl signals are involved. Still it is a good complement to other methods such as ^{1}H NMR spectroscopy and chromatography. In all the products described in this report, the 1,3-DAG and the structured TAG included, there were no signs of any contamination of any regioisomers to be observed either by ^{1}H or ^{13}C NMR spectroscopy.

Table 3. ^{13}C NMR chemical shift values of carbonyl group carbons

Fatty acid	$\alpha C{=}O$ (δ ppm)	$\beta C{=}O$ (δ ppm)
MCFA	173.2	172.9
EPA	172.8	172.5
DHA	172.6	172.1

3. Conclusion

Structured triacylglycerols constituting pure saturated fatty acids at the end-positions and pure EPA or DHA at the mid-position of the glycerol moiety were synthesized highly efficient and with excellent regiopurity by a chemoenzymatic approach. In the first step, an immobilized *Candida antarctica* lipase acted exclusively at the end-positions of glycerol and regioisomerically pure 1,3-diacylglycerols were accomplished in excellent yields. Pure EPA and DHA were introduced to the remaining mid-position by chemical coupling using EDCI to afford the structured triacylglycerols in excellent yield.

4. Experimental

4.1. General

^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded on a Bruker AC 250 NMR spectrometer in deuterated chloroform as a solvent. 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. Infrared spectra were conducted on a Nicolet Avatar 360 FT-IR (E.S.P.) Spectrophotometer on a KBr pellet. The high resolution mass spectra (HRMS) were aquired on a Micromass Q-Tof II mass spectrometer equipped with an Z-spray atmospheric pressure ionization chamber. The mass spectrometry parameters were as described by Mu et al.[30] All data analysis was done on Micromass MassLynx software.

Elemental analyses were performed at Department of Chemistry, H. C. Ørsted Institute, University of Copenhagen in Denmark. Melting points were determined on a Büchi 520 melting point apparatus and are uncorrected.

The immobilized *Rhizomucor miehei* (Lipozyme RM IM) and *Candida antarctica* (Novozym 435) lipases were supplied as a gift from Novozyme A/S (Bagsvaerd, Denmark). Glycerol (99%) was purchased from Sigma Chemicals (St. Louis, Missouri), vinyl caprylate (>99%), vinyl capriate (>99%), vinyl myristate (>99%) and vinyl palmitate (>96%) were purchased from Tokyo Kasei Kogyo Company (Tokyo, Japan), and vinyl laurate (>99%) from Fluka Chemie (Taufkirchen, Germany). They were all used without further purification. EPA (98%) and DHA (≥95%) were obtained as ethyl esters from Norsk Hydro Research Centre (Porsgrunn, Norway) and were hydrolysed to their corresponding free acids.[27] Solvents (*n*-hexane, pet. ether, boiling range 40–65°C, diethyl ether, chloroform, dichloromethane) were obtained from Acros Organics (Geel, Belgium) and were of analytical grade and used without further purification. Silica gel (Silica gel 60) and analytical TLC plates (DC Alufolien Kieselgel 60 F_{254}) were obtained from Merck (Darmstadt, Germany).

4.1.1. 1,3-Dioctanoylglycerol (1a). To a mixture of glycerol (4.01 g, 43.5 mmol) and vinyl caprylate (18.53 g, 108.8 mmol) in dichloromethane (2.0 mL) in a 100 mL round-bottomed flask at 0–4°C (ice-bath) was added immobilized *Candida antarctica* lipase (1.9 g). The resulting mixture was stirred on a magnetic stirrer hot-plate at 0–4°C for 3 h under nitrogen atmosphere. Then, additional lipase (0.4 g) was added to the reaction mixture which was stirred for additional 2 h at the same temperature. The lipase was separated off by filtration and the solvent removed in vacuo on a rotary evaporator to afford a white crystalline product. The crystals were dissolved in pet. ether (160 mL) and the solution placed in a freezer (−18°C) and allowed to stand overnight. The product was afforded as white crystals (13.43 g, 90% yield). Mp=24.3–25.0°C. ^{1}H NMR δ 4.18–4.05 (m, 5H, CH_2CHCH_2), 2.87 (br s, 1H, OH), 2.32 (t, J=7.5 Hz, 4H, CH_2COO), 1.60 (m, 4H, CH_2CH_2COO), 1.31–1.21 (m, 16H, CH_2) and 0.85 (t, J=6.7 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 173.9 (2), 68.2, 64.9 (2), 34.0 (2), 31.6 (2), 29.0 (2), 28.8 (2), 24.8 (2), 22.5 (2) and 14.0 (2) ppm. IR ν_{max} 3300–3600 (br, O–H), 2926 (vs, C–H), 2856 (vs, C–H), 1739 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{19}H_{36}O_5$+H m/z 345.2641; found 345.2621 amu. Elemental analysis. Found: C, 66.11; H, 10.43. $C_{19}H_{36}O_5$ requires C, 66.24; H, 10.53%.

4.1.2. 1,3-Didecanoylglycerol (1b). The same procedure was followed as for **1a** above using glycerol (1.00 g, 10.9 mmol) and vinyl capriate (5.40 g, 27.3 mmol) in dichloromethane (0.5 mL) in a 25 mL round-bottomed flask with the lipase added in two portions (470 mg and 125 mg after 3 h). The product was afforded as white crystals after recrystallization from pet. ether (4.00 g, 92% yield). Mp=40.9–41.2°C. ^{1}H NMR δ 4.20–4.06 (m, 5H, CH_2CHCH_2), 2.53 (br s, 1H, OH), 2.33 (t, J=7.5 Hz, 4H, CH_2COO), 1.68–1.55 (m, 4H, CH_2CH_2COO), 1.41–1.18 (m, 24H, CH_2) and 0.86 (t, J=6.6 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 173.9 (2), 68.3, 65.0 (2), 34.1 (2), 31.8 (2), 29.4 (2), 29.2 (2), 29.1 (4), 24.8 (2), 22.6 (2) and 14.1 (2) ppm. IR ν_{max} 3300–3600 (br, O–H), 2914 (vs, C–H), 2849 (vs, C–H), 1732 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{23}H_{44}O_5$+NH_4 m/z 418.3533; found 418.3522 amu. Elemental analysis. Found: C, 69.05; H, 11.23. $C_{23}H_{44}O_5$ requires C, 68.96; H, 11.07%.

4.1.3. 1,3-Didodecanoylglycerol (1c). The same procedure was followed as for **1a** above using glycerol (0.50 g, 5.43 mmol) and vinyl laurate (3.07 g, 13.6 mmol) in dichloromethane (0.5 mL) in a 10 mL round-bottomed flask with the lipase added in two portions (240 mg and 65 mg after 3 h). The product was afforded as white crystals after recrystallization from pet. ether (2.21 g, 92% yield). Mp=54.3–54.7°C. ^{1}H NMR δ 4.20–4.04 (m, 5H, CH_2CHCH_2), 2.54 (br s, 1H, OH), 2.34 (t, J=7.5 Hz, 4H, CH_2COO), 1.68–1.56 (m, 4H, CH_2CH_2COO), 1.39–1.16 (m, 32H, CH_2) and 0.87 (t, J=6.5 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 173.9 (2), 68.3, 65.0 (2), 34.1 (2), 31.9 (2), 29.6 (4), 29.4 (2), 29.3 (2), 29.2 (2) 29.1 (2), 24.8 (2), 22.6 (2) and 14.1 (2) ppm. IR ν_{max} 3300–3600 (br, O–H), 2918 (vs, C–H), 2856 (vs, C–H), 1739 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{27}H_{52}O_5$+NH_4 m/z 474.4159; found 474.4159 amu. Elemental analysis. Found: C, 71.09; H, 11.62. $C_{27}H_{52}O_5$ requires C, 71.00; H, 11.48%.

4.1.4. 1,3-Ditetradecanoylglycerol (1d). The same procedure was followed as for **1a** above using glycerol (250 mg, 2.71 mmol) and vinyl myristate (1.72 g, 13.6 mmol) in dichloromethane (0.2 mL) in a 5 mL round-bottomed flask with the lipase added in two portions (130 mg and 30 mg after 3 h). The product was afforded as white crystals after recrystallization from pet. ether (1.28 g, 92% yield). Mp=66.5–67.0°C. ^{1}H NMR δ 4.21–4.04 (m, 5H, CH_2CHCH_2), 2.64 (br s, 1H, OH), 2.34 (t, J=7.5 Hz, 4H, CH_2COO), 1.68–1.55 (m, 4H, CH_2CH_2COO), 1.40–1.16 (m, 40H, CH_2) and 0.87 (t, J=6.5 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 174.0 (2), 68.3, 65.0 (2), 34.1 (2), 31.9 (2), 29.7 (4), 29.6 (4), 29.4 (2), 29.3 (2), 29.2 (2) 29.1 (2), 24.9 (2), 22.7 (2) and 14.1 (2) ppm. IR ν_{max} 3300–3600 (br, O–H), 2914 (vs, C–H), 2849 (vs, C–H), 1732 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{31}H_{60}O_5$+NH_4 m/z 530.4785; found 530.4772 amu. Elemental analysis. Found: C, 72.76; H, 11.85. $C_{31}H_{60}O_5$ requires C, 72.60; H, 11.79%.

4.1.5. 1,3-Dihexadecanoylglycerol (1e). The same procedure was followed as for **1a** above using glycerol (250 mg, 2.71 mmol) and vinyl stearate (1.92 g, 6.79 mmol) in dichloromethane (1.0 mL) in a 5 mL round-bottomed flask with the lipase added in one lot (150 mg). The reaction was allowed to proceed overnight at rt. The product was afforded as white crystals after recrystallization from pet. ether (1.40 g, 90% yield). Mp=73.0–73.4°C. ^{1}H NMR δ 4.21–4.03 (m, 5H, CH_2CHCH_2), 2.50 (br s, 1H, OH), 2.34 (t, J=7.5 Hz, 4H, CH_2COO), 1.68–1.53 (m, 4H, CH_2CH_2COO), 1.40–1.15 (m, 48H, CH_2) and 0.87 (t, J=6.5 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 173.9 (2), 68.3, 65.0 (2), 34.1 (2), 31.9 (2), 29.7 (8), 29.6 (4), 29.4 (2), 29.3 (2), 29.2 (2) 29.1 (2), 24.9 (2), 22.7 (2) and 14.1 (2) ppm. IR ν_{max} 3300–3600 (br, O–H), 2915 (vs, C–H), 2849 (vs, C–H), 1733 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{35}H_{68}O_5$+NH_4 m/z 586.5411; found 586.5389 amu. Elemental analysis. Found: C, 73.90; H, 12.19. $C_{35}H_{68}O_5$ requires C, 73.89; H, 12.05%.

4.1.6. 2-Eicosapentaenoyl-1,3-dioctanoylglycerol (2a). To a solution of 1,3-dioctanoylglycerol **1a** (350 mg, 1.02 mmol) and EPA as a free acid (307 mg, 1.02 mmol) in dichloromethane (2.0 mL) was added DMAP (49 mg, 0.40 mmol) and EDCI (234 mg, 1.22 mmol). The resulting solution was stirred on a magnetic stirrer hot-plate at rt for 15 h. The reaction was disconnected by passing the reaction mixture in ether/dichloromethane (10:90) through a short column packed with silica gel. Solvent removal in vacuo afforded the pure product as a yellowish oil (576 mg, 90% yield). ^{1}H NMR δ 5.41–5.33 (m, 10H, =CH), 5.33–5.24 (m, 1H, CH_2CHCH_2), 4.29 (dd, J=11.9, 4.3 Hz, 2H, CH_2CHCH_2), 4.13 (dd, J=11.9, 5.9 Hz, 2H, CH_2CHCH_2), 2.86–2.77 (m, 8H, $=CCH_2C=$), 2.33 (t, J=7.3 Hz, 2H, CH_2COO in EPA), 2.30 (t, J=7.6 Hz, 4H, CH_2COO), 2.15–2.02 (m, 4H, $=CHCH_2CH_2$ and $CH_3CH_2CH=$), 1.75–1.63 (m, 2H, CH_2CH_2COO in EPA), 1.66–1.54 (m, 4H, CH_2CH_2COO in octanoic acid), 1.40–1.16 (m, 16H, CH_2), 0.97 (t, J=7.5 Hz, 3H, CH_3 in EPA) and 0.87 (t, J=6.8 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 173.2 (2, αC=O), 172.6 (1, βC=O, EPA), 132.0 (1), 128.9 (1), 128.7 (1), 128.5 (1), 128.2 (1), 128.1 (2), 128.0 (1), 127.8 (1), 127.0 (1), 68.9 (1), 62.0 (2), 34.0 (2), 33.5 (1), 31.6 (2), 29.0 (2), 28.9 (2), 26.4 (1), 25.6 (3), 25.5 (1), 24.8 (2), 24.7 (1), 22.6 (2), 20.5 (1), 14.2 (1) and 14.0 (2) ppm. IR ν_{max} 3013 (s, C–H), 2927 (vs, C–H), 2856 (vs, C–H), 1740 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{39}H_{64}O_6+NH_4$ m/z 646.5047; found 646.5037 amu.

4.1.7. 2-Eicosapentaenoyl-1,3-didecanoylglycerol (2b). The procedure was identical to that for preparing **2a** using 1,3-didecanoylglycerol **1b** (350 mg, 0.874 mmol), EPA (264 mg, 0.874 mmol), DMAP (43 mg, 0.35 mmol) and EDCI (201 mg, 1.05 mmol) in dichloromethane (2.0 mL). The product was afforded as a yellowish oil (556 mg, 93% yield). ^{1}H NMR δ 5.41–5.33 (m, 10H, =CH), 5.33–5.24 (m, 1H, $-CH_2CHCH_2$), 4.29 (dd, J=11.9, 4.3 Hz, 2H, CH_2CHCH_2), 4.13 (dd, J=11.9, 5.9 Hz, 2H, CH_2CHCH_2), 2.88–2.75 (m, 8H, $=CCH_2C=$), 2.33 (t, J=7.3 Hz, 2H, CH_2COO in EPA), 2.30 (t, J=7.6 Hz, 4H, CH_2COO), 2.15–2.00 (m, 4H, $=CHCH_2CH_2$ and $CH_3CH_2CH=$), 1.75–1.63 (m, 2H, CH_2CH_2COO in EPA), 1.66–1.53 (m, 4H, CH_2CH_2COO in decanoic acid), 1.40–1.17 (m, 24H, CH_2), 0.96 (t, J=7.5 Hz, 3H, CH_3 in EPA) and 0.87 (t, J=6.8 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 173.2 (2, αC=O), 172.6 (1, βC=O, EPA), 132.0 (1), 128.9 (1), 128.7 (1), 128.5 (1), 128.2 (1), 128.1 (2), 128.0 (1), 127.8 (1), 126.9 (1), 68.9 (1), 62.0 (2), 34.0 (2), 33.5 (1), 31.8 (2), 29.4 (2), 29.2 (4), 29.0 (2), 26.4 (1), 25.6 (3), 25.5 (1), 24.8 (2), 24.7 (1), 22.6 (2), 20.5 (1), 14.2 (1) and 14.0 (2) ppm. IR ν_{max} 3013 (s, C–H), 2924 (vs, C–H), 2854 (vs, C–H), 1740 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{43}H_{72}O_6+NH_4$ m/z 702.5673; found 702.5656 amu.

4.1.8. 2-Eicosapentaenoyl-1,3-didodecanoylglycerol (2c). The procedure was identical to that for preparing **2a** using 1,3-didodecanoylglycerol **3c** (350 mg, 0.766 mmol), EPA (232 mg, 0.766 mmol), DMAP (38 mg, 0.31 mmol) and EDCI (176 mg, 0.92 mmol) in dichloromethane (2.0 mL). The product was afforded as a yellowish oil (523 mg, 92% yield). ^{1}H NMR δ 5.41–5.33 (m, 10H, =CH), 5.33–5.23 (m, 1H, CH_2CHCH_2), 4.29 (dd, J=11.9, 4.3 Hz, 2H, CH_2CHCH_2), 4.13 (dd, J=11.9, 5.9 Hz, 2H, CH_2CHCH_2), 2.88–2.75 (m, 8H, $=CCH_2C=$), 2.33 (t, J=7.4 Hz, 2H, CH_2COO in EPA), 2.30 (t, J=7.6 Hz, 4H, CH_2COO), 2.15–2.00 (m, 4H, $=CHCH_2CH_2$ and $CH_3CH_2CH=$), 1.75–1.63 (m, 2H, CH_2CH_2COO in EPA), 1.66–1.53 (m, 4H, CH_2CH_2COO in dodecanoic acid), 1.40–1.16 (m, 32H, CH_2), 0.96 (t, J=7.5 Hz, 3H, CH_3 in EPA) and 0.87 (t, J=6.8 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 173.2 (2, αC=O), 172.6 (1, βC=O, EPA), 132.0 (1), 128.9 (1), 128.7 (1), 128.5 (1), 128.2 (1), 128.1 (2), 128.0 (1), 127.8 (1), 126.9 (1), 68.9 (1), 62.0 (2), 34.0 (2), 33.5 (1), 31.9 (2), 29.6 (4), 29.4 (2), 29.3 (2), 29.2 (2), 29.1 (2), 26.4 (1), 25.6 (3), 25.5 (1), 24.8 (2), 24.7 (1), 22.6 (2), 20.5 (1), 14.2 (1) and 14.0 (2) ppm. IR ν_{max} 3013 (s, C–H), 2923 (vs, C–H), 2854 (vs, C–H), 1743 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{47}H_{80}O_6+NH_4$ m/z 758.6299; found 758.6311 amu.

4.1.9. 2-Eicosapentaenoyl-1,3-ditetradecanoylglycerol (2d). The procedure was identical to that for preparing **2a** using 1,3-ditetradecanoylglycerol **1d** (350 mg, 0.683 mmol), EPA (206 mg, 0.683 mmol), DMAP (33 mg, 0.27 mmol) and EDCI (156 mg, 0.82 mmol) in dichloromethane (2.0 mL). The product was afforded as a yellowish oil (501 mg, 92% yield). ^{1}H NMR δ 5.41–5.33 (m, 10H, =CH), 5.33–5.24 (m, 1H, CH_2CHCH_2), 4.29 (dd, J=11.9, 4.3 Hz, 2H, CH_2CHCH_2), 4.14 (dd, J=11.9, 5.9 Hz, 2H, CH_2CHCH_2), 2.88–2.75 (m, 8H, $=CCH_2C=$), 2.33 (t, J=7.4 Hz, 2H, CH_2COO in EPA), 2.30 (t, J=7.6 Hz, 4H, CH_2COO), 2.15–2.00 (m, 4H, $=CHCH_2CH_2$ and $CH_3CH_2CH=$), 1.75–1.63 (m, 2H, CH_2CH_2COO in EPA), 1.66–1.53 (m, 4H, CH_2CH_2COO in tetradecanoic acid), 1.40–1.16 (m, 40H, CH_2), 0.97 (t, J=7.5 Hz, 3H, CH_3 in EPA) and 0.87 (t, J=6.8 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 173.3 (2, αC=O), 172.6 (1, βC=O, EPA), 132.0 (1), 128.9 (1), 128.7 (1), 128.5 (1), 128.2 (2), 128.1 (1), 128.0 (1), 127.8 (1), 127.0 (1), 68.9 (1), 62.0 (2), 34.0 (2), 33.5 (1), 31.9 (2), 29.7 (2), 29.6 (6), 29.5 (2), 29.4 (2), 29.3 (2), 29.1 (2), 26.4 (1), 25.6 (3), 25.5 (1), 24.8 (2), 24.7 (1), 22.7 (2), 20.5 (1), 14.3 (1) and 14.1 (2) ppm. IR ν_{max} 3013 (s, C–H), 2923 (vs, C–H), 2853 (vs, C–H), 1743 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{51}H_{88}O_6+NH_4$ m/z 814.6925; found 814.6897 amu.

4.1.10. 2-Eicosapentaenoyl-1,3-dihexadecanoylglycerol (2e). The procedure was identical to that for preparing **2a** using 1,3-dihexadecanoylglycerol **1e** (400 mg, 0.703 mmol), EPA (213 mg, 0.703 mmol), DMAP (34 mg, 0.28 mmol) and EDCI (162 mg, 0.84 mmol) in dichloromethane (2.0 mL). The product was afforded as a yellowish oil (540 mg, 90% yield). ^{1}H NMR δ 5.41–5.33 (m, 10H, =CH), 5.33–5.23 (m, 1H, CH_2CHCH_2), 4.29 (dd, J=11.9, 4.3 Hz, 2H, CH_2CHCH_2), 4.14 (dd, J=11.9, 5.9 Hz, 2H, CH_2CHCH_2), 2.88–2.75 (m, 8H, $=CCH_2C=$), 2.33 (t, J=7.4 Hz, 2H, CH_2COO in EPA), 2.30 (t, J=7.6 Hz, 4H, CH_2COO), 2.16–2.01 (m, 4H, $=CHCH_2CH_2$ and $CH_3CH_2CH=$), 1.75–1.63 (m, 2H, CH_2CH_2COO in EPA), 1.66–1.53 (m, 4H, CH_2CH_2COO in hexadecanoic acid), 1.40–1.17 (m, 48H, CH_2), 0.97 (t, J=7.5 Hz, 3H, CH_3 in EPA) and 0.88 (t, J=6.8 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 173.3 (2, αC=O), 172.6 (1, βC=O, EPA), 132.0 (1), 128.9 (1), 128.8 (1), 128.6 (1), 1283 (1), 128.2 (1), 128.1 (1), 128.0 (1), 127.8 (1), 127.0 (1), 69.0 (1), 62.0 (2), 34.0 (2), 33.6 (1), 31.9 (2), 29.7 (10), 29.6 (2), 29.5 (2), 29.4 (2), 29.3 (2), 29.1 (2), 26.5 (1), 25.6 (3), 25.5 (1), 24.8 (2), 24.7 (1), 22.7 (2),

20.5 (1), 14.3 (1) and 14.1 (2) ppm. IR ν_{max} 3013 (s, C–H), 2922 (vs, C–H), 2853 (vs, C–H), 1743 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{55}H_{96}O_6+NH_4$ *m/z* 870.7551; found 870.7532 amu.

4.1.11. 2-Docosahexaenoyl-1,3-dioctanoylglycerol (3a). The procedure was identical to the one for preparing the corresponding 2-EPA-TAG **2a** adduct using 1,3-dioctanoylglycerol **1a** (350 mg, 1.02 mmol), DHA (334 mg, 1.02 mmol), DMAP (50 mg, 0.41 mmol) and EDCI (234 mg, 1.22 mmol) in dichloromethane (2.0 mL). The product was afforded as a yellowish oil (599 mg, 90% yield). ^{1}H NMR δ 5.42–5.30 (m, 12H, =CH), 5.29–5.24 (m, 1H, CH_2CHCH_2), 4.29 (dd, *J*=11.9, 4.3 Hz, 2H, CH_2CHCH_2), 4.14 (dd, *J*=11.9, 5.9 Hz, 2H, CH_2CHCH_2), 2.88–2.75 (m, 10H, $=CCH_2C=$), 2.39–2.35 (m, 4H, CH_2CH_2COO in DHA), 2.30 (t, *J*=7.5 Hz, 4H, CH_2COO), 2.12–2.01 (m, 2H, $CH_3CH_2CH=$), 1.66–1.54 (m, 4H, CH_2CH_2COO in octanoic acid), 1.35–1.18 (m, 16H, CH_2), 0.96 ppm (t, *J*=7.5 Hz, 3H, CH_3) and 0.87 (t, *J*=6.8 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 173.2 (2, αC=O), 172.1 (1, βC=O), 132.0 (1), 129.4 (1), 128.5 (1), 128.3 (1), 128.2 (2), 128.0 (2), 127.9 (1), 127.8 (1), 127.6 (1), 127.0 (1), 69.0 (1), 62.0 (2), 34.0 (3), 31.6 (2), 29.0 (2), 28.9 (2), 25.6 (3), 25.5 (1), 24.8 (3), 22.6 (3), 20.5 (1), 14.2 (1) and 14.0 (2) ppm. IR ν_{max} 3013 (s, C–H), 2927 (vs, C–H), 2856 (vs, C–H), 1740 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{41}H_{66}O_6+NH_4$ *m/z* 672.5203; found 672.5179 amu.

4.1.12. 2-Docosahexaenoyl-1,3-didecanoylglycerol (3b). The procedure was identical to that for preparing **2a** using 1,3-didecanoylglycerol **1b** (350 mg, 0.874 mmol), DHA (287 mg, 0.874 mmol), DMAP (42 mg, 0.34 mmol) and EDCI (201 mg, 1.05 mmol) in dichloromethane (2.0 mL). The product was afforded as a yellowish oil (585 mg, 94% yield). ^{1}H NMR δ 5.43–5.30 (m, 12H, =CH), 5.29–5.22 (m, 1H, CH_2CHCH_2), 4.29 (dd, *J*=11.9, 4.3 Hz, 2H, CH_2CHCH_2), 4.14 (dd, *J*=11.9, 5.9 Hz, 2H, CH_2CHCH_2), 2.88–2.75 (m, 10H, $=CCH_2C=$), 2.39–2.35 (m, 4H, CH_2CH_2COO in DHA), 2.30 (t, *J*=7.5 Hz, 4H, CH_2COO), 2.12–2.01 (m, 2H, $CH_3CH_2CH=$), 1.66–1.54 (m, 4H, CH_2CH_2COO in decanoic acid), 1.38–1.18 (m, 24H, CH_2), 0.96 ppm (t, *J*=7.5 Hz, 3H, CH_3) and 0.87 (t, *J*=6.8 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 173.2 (2, αC=O), 172.1 (1, βC=O), 132.0 (1), 129.4 (1), 128.5 (1), 128.3 (1), 128.2 (2), 128.0 (2), 127.9 (1), 127.8 (1), 127.6 (1), 127.0 (1), 69.0 (1), 62.0 (2), 34.0 (3), 31.8 (2), 29.4 (2), 29.2 (4), 29.1 (2), 25.6 (3), 25.5 (1), 24.8 (3), 22.6 (3), 20.5 (1), 14.2 (1) and 14.0 (2) ppm. IR ν_{max} 3013 (s, C–H), 2924 (vs, C–H), 2854 (vs, C–H), 1743 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{45}H_{74}O_6+NH_4$ *m/z* 728.5829; found 728.5817 amu.

4.1.13. 2-Docosahexaenoyl-1,3-didodecanoylglycerol (3c). The procedure was identical to that for preparing **2a** using 1,3-dioctanoylglycerol **1c** (350 mg, 0.766 mmol), DHA (252 mg, 0.766 mmol), DMAP (37 mg, 0.31 mmol) and EDCI (176 mg, 0.92 mmol) in dichloromethane (2.0 mL). The product was afforded as a yellowish oil (560 mg, 95% yield). ^{1}H NMR δ 5.43–5.30 (m, 12H, =CH), 5.29–5.22 (m, 1H, CH_2CHCH_2), 4.29 (dd, *J*=11.9, 4.3 Hz, 2H, CH_2CHCH_2), 4.13 (dd, *J*=11.9, 5.9 Hz, 2H, CH_2CHCH_2), 2.88–2.75 (m, 10H, $=CCH_2C=$), 2.39–2.35 (m, 4H, CH_2CH_2COO in DHA), 2.30 (t, *J*=7.5 Hz, 4H, CH_2COO), 2.12–2.01 (m, 2H, $CH_3CH_2CH=$), 1.66–1.54 (m, 4H, CH_2CH_2COO in dodecanoic acid), 1.35–1.18 (m, 32H, CH_2), 0.96 ppm (t, *J*=7.5 Hz, 3H, CH_3) and 0.87 (t, *J*=6.8 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 173.2 (2, αC=O), 172.1 (1, βC=O), 132.0 (1), 129.4 (1), 128.5 (1), 128.3 (1), 128.2 (2), 128.0 (2), 127.9 (1), 127.8 (1), 127.6 (1), 127.0 (1), 69.0 (1), 62.0 (2), 34.0 (3), 31.9 (2), 29.6 (4), 29.4 (2), 29.3 (2), 29.2 (2), 29.1 (2), 25.6 (3), 25.5 (1), 24.8 (3), 22.6 (3), 20.5 (1), 14.2 (1) and 14.0 (2) ppm. IR ν_{max} 3013 (s, C–H), 2927 (vs, C–H), 2856 (vs, C–H), 1740 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{49}H_{82}O_6+NH_4$ *m/z* 784.6455; found 784.6461 amu.

4.1.14. 2-Docosahexaenoyl-1,3-ditetradecanoylglycerol (3d). The procedure was identical to that for preparing **2a** using 1,3-ditetradecanoylglycerol **1d** (350 mg, 0.683 mmol), DHA (224 mg, 0.683 mmol), DMAP (34 mg, 0.28 mmol) and EDCI (157 mg, 0.82 mmol) in dichloromethane (2.0 mL). The product was afforded as a yellowish oil (532 mg, 95% yield). ^{1}H NMR δ 5.41–5.32 (m, 12H, =CH), 5.32–5.22 (m, 1H, CH_2CHCH_2), 4.29 (dd, *J*=11.9, 4.4 Hz, 2H, CH_2CHCH_2), 4.13 (dd, *J*=11.9, 5.9 Hz, 2H, CH_2CHCH_2), 2.88–2.76 (m, 10H, $=CCH_2C=$), 2.39–2.35 (m, 4H, CH_2CH_2COO in DHA), 2.30 (t, *J*=7.5 Hz, 4H, CH_2COO), 2.12–2.01 (m, 2H, $CH_3CH_2CH=$), 1.65–1.54 (m, 4H, CH_2CH_2COO in tetradecanoic acid), 1.35–1.18 (m, 40H, CH_2), 0.96 ppm (t, *J*=7.5 Hz, 3H, CH_3) and 0.87 (t, *J*=6.8 Hz, 6H, CH_3) ppm. ^{13}C NMR δ 173.3 (2, αC=O), 172.1 (1, βC=O), 132.0 (1), 129.4 (1), 128.5 (1), 128.3 (1), 128.2 (2), 128.0 (2), 127.9 (1), 127.8 (1), 127.6 (1), 127.0 (1), 69.0 (1), 62.0 (2), 34.0 (3), 31.9 (2), 29.7 (2), 29.6 (6), 29.5 (2), 29.3 (2), 29.2 (2), 29.1 (2), 25.6 (3), 25.5 (1), 24.8 (3), 22.7 (2), 22.6 (1), 20.5 (1), 14.3 (1) and 14.1 (2) ppm. IR ν_{max} 3013 (s, C–H), 2922 (vs, C–H), 2853 (vs, C–H), 1743 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{53}H_{90}O_6+NH_4$ *m/z* 840.7081; found 840.7065 amu.

4.1.15. 2-Docosahexaenoyl-1,3-dihexadecanoylglycerol (3e). The procedure was identical to that for preparing **2a** using 1,3-dihexadecanoylglycerol **1e** (400 mg, 0.703 mmol), DHA (231 mg, 0.703 mmol), DMAP (34 mg, 0.28 mmol) and EDCI (162 mg, 0.84 mmol) in dichloromethane (2.0 mL). The product was afforded as a yellowish oil (570 mg, 92% yield). ^{1}H NMR δ 5.44–5.30 (m, 12H, =CH), 5.29–5.22 (m, 1H, CH_2CHCH_2), 4.29 (dd, *J*=11.9, 4.4 Hz, 2H, CH_2CHCH_2), 4.14 (dd, *J*=11.9, 5.8 Hz, 2H, CH_2CHCH_2), 2.88–2.75 (m, 10H, $=CCH_2C=$), 2.40–2.35 (m, 4H, CH_2CH_2COO in DHA), 2.31 (t, *J*=7.5 Hz, 4H, CH_2COO), 2.13–2.01 (m, 2H, $CH_3CH_2CH=$), 1.66–1.54 (m, 4H, CH_2CH_2COO in hexadecanoic acid), 1.35–1.18 (m, 48H, CH_2), 0.97 ppm (t, *J*=7.5 Hz, 3H, CH_3) and 0.88 (t, *J*=6.8 Hz, 6H, CH_2) ppm. ^{13}C NMR δ 173.3 (2, αC=O), 172.1 (1, βC=O), 132.0 (1), 129.4 (1), 128.5 (1), 128.3 (1), 128.2 (2), 128.0 (2), 127.9 (1), 127.8 (1), 127.6 (1), 127.0 (1), 69.1 (1), 62.0 (2), 34.0 (3), 31.9 (2), 29.7 (10), 29.6 (2), 29.5 (2), 29.4 (2), 29.3 (2), 29.1 (2), 25.6 (3), 25.5 (1), 24.8 (3), 22.7 (2), 22.6 (1), 20.5 (1), 14.3 (1) and 14.1 (2) ppm. IR ν_{max} 3014 (s, C–H), 2922 (vs, C–H), 2853 (vs, C–H), 1743 (vs, C=O) cm^{-1}. HRMS (API): calcd for $C_{57}H_{98}O_6+NH_4$ *m/z* 896.7707; found 896.7679 amu.

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