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Synthesis of reversed structured triacylglycerols possessing EPA and DHA at their terminal positions

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ABSTRACT

This report describes synthesis of reversed structured triacylglycerols (TAGs) of the LML type, possessing pure EPA or DHA located at the terminal 1,3-positions of the glycerol backbone along with pure even number saturated fatty acids (C6:0–C16:0) occupying the 2-position. These compounds were synthesized by a two-step chemoenzymatic route involving a highly regioselective immobilized *Candida antarctica* lipase to incorporate EPA or DHA activated as acetoxime esters exclusively into the 1,3-positions of glycerol. The saturated fatty acyl groups were subsequently introduced to the remaining 2-position by EDCI coupling agent to accomplish the title compounds highly efficiently. This is the first report on reversed structured TAGs possessing the long-chain n-3 polyunsaturated fatty acids. It is anticipated that these novel compounds and their synthetic methodology will find various important uses such as analytical standards, in screening for bioactivity and in the pharmaceutical area as prodrugs.

1. Introduction

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are by far the most predominant of the long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs) that are characteristic of fish oil and marine fat.[1,2] Numerous beneficial effects on human health and prevention of various diseases are attributed to EPA and DHA. They include cardiovascular diseases, inflammation, autoimmune diseases, rheumatoid arthritis, Alzheimer's disease and related neurogenerative disorders, type-2 diabetes and cancer.[3–9] EPA and DHA are precursors to various bioactive eicosanoid and docosanoid mediators that include prostaglandins, leukotrienes, prostacyclins and thromboxans[5,6] as well as the more recently discovered highly potent anti-inflammatory and pro-resolving protectins, resolvins and maresins.[7–9] Therefore, EPA and DHA may be regarded as prodrugs.[10] Their ethyl esters are in fact available as prescription drugs registered as an adjuvant therapy to treat hypertriglyceridemia, both as a mixture of EPA and DHA[11,12] as well as virtually pure EPA.[13,14]

Triacylglycerols (TAGs) are important constituents of the human and animal diet. They are by far the largest class of nonpolar lipids that occur ubiquitously in fats and oils of plant and animal origin. TAGs are not only a source of energy but also provide essential fatty acids for various biological roles including the n-6 and n-3 PUFAs. They constitute a glycerol skeleton to which three fatty acyl groups are linked as carboxylate esters. The variety and number of different TAG molecular species in fats and oils is countless as a result of the great number of various fatty acids that differ in length, saturation and location of carbon–carbon double bonds in unsaturated and polyunsaturated fatty acids. This is particularly eminent in fish oils that commonly comprise more than 50 different fatty acids.[1,2] The location of fatty acids within the glycerol backbone adds further to the complexity in terms of positional isomers that are also named regioisomers. And, when the two fatty acids occupying the terminal positions differ the TAGs become chiral offering the possibility of TAG enantiomers present.

The physical, sensory, nutritional and biological properties of the TAGs must be largely influenced by the above described varieties. The situation is somewhat simplified, however, by the fact that the fatty acids are not randomly distributed in the TAGs that are known to differ significantly in animals and plants from species to species. Classical examples of such positionally structured TAGs include cocoa butter[15] used in chocolate manufacturing and human milk TAGs.[16] Generally in fish oil TAGs the mid-position is higher enriched with EPA and particularly DHA, whereas in marine mammals including whale oil and seal oil this is the other way around.[17] There are also multiple reports on enantiospecific positioning of fatty acids in animal and plant TAGs.[18–20]

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Structured lipids usually refer to acylglycerols constituting selected fatty acids that are located at predetermined positions of the glycerol backbone.[21] Structured TAGs possessing long-chain polyunsaturated bioactive fatty acids such as EPA and DHA at the 2-position and saturated medium chain (C6:0, C8:0 and C10:0) fatty acids (MCFAs) at the terminal 1,3-positions have gained a continuing interest of scientists as a result of their nutritional value and properties.[22,23] Preparation of such MLM (medium-long-medium) type structured TAGs constituting varying enrichment levels of MCFAs and n-3 PUFAs has been described in numerous reports.[24] There are also number of reports on syntheses of MLM type structured TAGs containing a pure MCFA at the terminal positions and a pure EPA or DHA at the 2-position of the glycerol backbone.[25–27]

All these reports take advantage of lipases that are ideally suited as biocatalysts for structured lipid syntheses.[24] This is based on their high regioselectivity and strong preference to act on the primary alcohol positions of the glycerol moiety in various esterification and transesterification reactions. The mild conditions in terms of temperature under which they act are also of crucial importance to maintain full regiocontrol by hampering disruptive intramolecular acyl-migration side reactions that are associated with partially acylated glycerol and polyol syntheses.[28,29]

We have reported an efficient two-step chemoenzymatic synthesis of MLM type structured TAGs constituting pure EPA and DHA at the 2-position with a pure MCFA at the terminal positions of the glycerol backbone starting from glycerol.[30] That work was extended to a focused library of similarly structured TAGs covering all saturated even carbon number fatty acids from C2–C16:0.[30,31] This has been further extended to similarly structured enantiopure diacylglyceryl ethers (DAGEs) of the 1-*O*-alkyl-*sn*-glycerol type possessing pure saturated even carbon number fatty acids located at the terminal *sn*-3 position of the glycerol backbone with pure EPA or DHA at the *sn*-2 position.[32] A comprehensive well-defined library of such single pure structured lipid compounds will enable their systematic screening for various important chemical and biological properties. And, very recently, the focused library was further expanded to include reversed structured DAGEs, this time possessing the pure EPA and DHA acyl groups at the terminal *sn*-3 position with the saturated fatty acyl groups located at the *sn*-2 position of the glycerol framework.[33,34]

By the work disclosed herein the focused library is further diversified to include oppositely structured TAGs, this time possessing a pure EPA or DHA acyl group at the terminal positions with the saturated fatty acyl group located at the 2-position of the glycerol framework. Fig. 1 illustrates the chemical structures of a normal MLM type structured TAG (constituting EPA and capric acid, C10:0) and a reversed structured TAG of the LML (long-medium-long) type (possessing DHA and caprylic acid, C8:0).

2. Results and discussion

2.1. Previous synthesis of normal structured MLM type TAGs

We reported a two-step chemoenzymatic process designed for synthesis of MLM type structured TAGs possessing pure EPA or DHA at the mid-position and pure MCFA at the end-positions.[30] This chemoenzymatic approach is demonstrated in Scheme 1 for caprylic acid and DHA. In the first step glycerol was acylated exclusively at the terminal positions by employing a highly regioselective immobilized *Candida antarctica* lipase (CAL-B from Novozymes) using the saturated fatty acids activated as vinyl esters. The use of vinyl esters secures fast and irreversible reactions under sufficiently mild conditions to eliminate detrimental acyl-migration side reactions.

EPA and DHA were subsequently introduced to the remaining 2-position of the resulting 1,3-diacylglycerol (1,3-DAG) intermediates by aid of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) as a chemical coupling agent in presence of dimethylaminopyridine (DMAP) serving both as a base and catalyst in dichloromethane at rt. All products and intermediates were obtained in excellent yields with excellent chemical and regiopurity.

2.2. Synthesis of reversed structured LML type TAGs

The intended reversed structured TAG synthesis was more of a challenge. For that task an activated form of EPA and DHA was needed for the enzymatic step to proceed at adequate rate under the mild conditions needed to retard the acyl-migration side reaction. In structured TAG synthesis it is essential to avoid such acyl migration reactions that result in loss of regiocontrol when the fatty acids migrate from the intended position to an unwanted position within the glycerol framework.[30,31] The use of vinyl esters was no longer an option since the polyunsaturated fatty acids do not tolerate the chemical conditions required for preparing such derivatives as was discussed in details in a recent report.[33] Moreover, most of the commercially available microbial lipases do not accept EPA or DHA and their derivatives as substrates.

Both obstacles were successfully overcome by the use of EPA and DHA activated as oxime esters on which the immobilized *C. antarctica* lipase (CAL-B) acted at sufficient rate under mild enough condition to eliminate any acyl-migration side reaction. The mild conditions are also greatly beneficial for transformations involving the highly labile n-3 PUFAs.[35] There is no doubt that CAL-B is superior to all currently known lipases in dealing with EPA and DHA as may be witnessed from numerous reports.[27,36] The 1,3-DAG intermediate adducts **1** (EPA) and **2** (DHA) were obtained regiopure and in very high yields (85% for both EPA and DHA). The details have been reported in a recent publication.[33] Fig. 2 shows the chemical structure of the acetoxime ester of DHA.

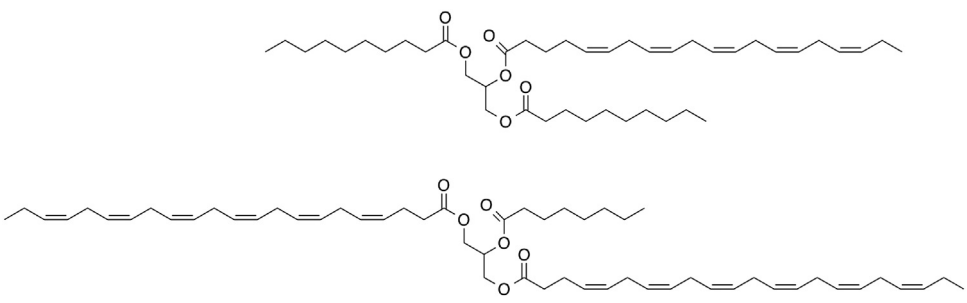
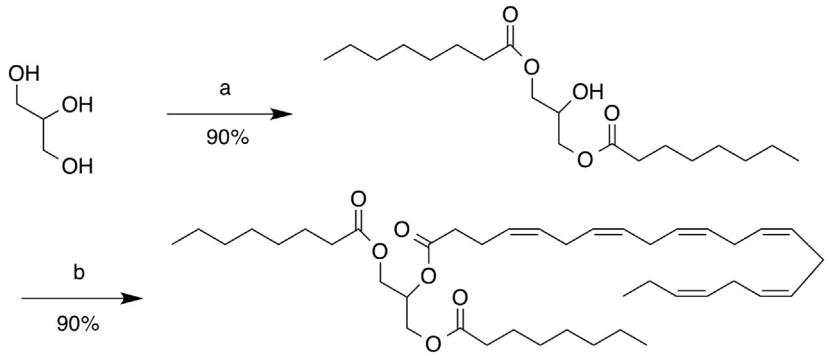


Fig. 1. Chemical structures of a normal structured MLM type TAG possessing EPA at the 2-position and capric acid (C10:0) at the 1,3-positions (top), and a reversed structured LML type TAG possessing DHA at the 1,3-positions and caprylic acid (C8:0) at the 2-position (bottom).



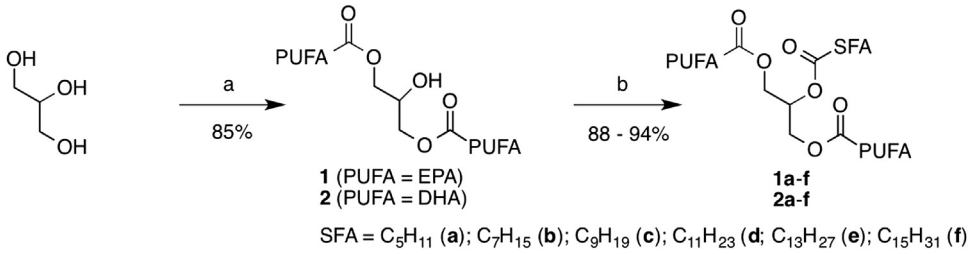
Scheme 1. The two-step chemoenzymatic synthesis of normal structured MLM type TAG shown for caprylic acid (C8:0) and DHA. Reagents and conditions: (a) *C. antarctica* lipase, vinyl octanoate, CH_2Cl_2, rt, 3 h; (b) DHA, EDCI, DMAP, CH_2Cl_2, rt, 12 h.

Fig. 2. The chemical structure of a DHA acetoxime ester.

To obtain the reversed structured TAGs the pure even carbon number saturated fatty acids ranging from C6:0 to C16:0 were introduced by chemical coupling to the 2-position of the resulting 1,3-DAGs **1** and **2** derived from glycerol possessing pure EPA and DHA at their terminal 1,3-positions. In our previous reports this turned out to be easy, in the symmetric 1,3-DAGs possessing the straight-chain saturated acyl groups being readily acylated with EPA and DHA using EDCI as a coupling agent. The question remained as to whether reversing this, with two of the long-chain n-3 PUFAs this time occupying both terminal positions of the glycerol skeleton, would create problems related to steric hindrance in the coupling reaction. The PUFAs possess number of permanent kinks on their hydrocarbon chain as a result from each of the double bonds affecting their shape, rigidity and bulkiness. The two-step overall chemoenzymatic process is illustrated in Scheme 2 for the synthesis of **1a**–**f** (the EPA series) and **2a**–**f** (the DHA series) with **a**, **b**, **c**, **d**, **e** and **f** referring to C6:0, C8:0, C10:0, C12:0, C14:0 and C16:0, respectively.

sterically hindered 2-position of a 1,3-DAG intermediate as in the current case. This relates to the lipase incorporating two equivalents of the PUFAs into the less crowded terminal positions to form a 1,3-DAG intermediate that subsequently underwent an acyl-migration promoted by the acidic conditions offered by the presence of free fatty acids at an elevated temperature (65 °C) to form a racemic 1(3),2-DAG with the final acylation step taking place at the less hindered primary alcohol outer position.[36]

The coupling reaction was successfully conducted in dichloromethane at room temperature under similar conditions as previously described for the MLM type structured TAG synthesis.[30,31] The saturated fatty acids were used in about 10% molar excess with approximately 1.5 molar equivalents of the EDCI coupling agent and 1 molar equivalent of DMAP as based on the starting 1,3-DAG adduct. The reaction was allowed to proceed overnight, although the reaction was possibly already completed at an earlier stage (3–4 h), like in the preparation of the MLM type structured TAGs described before.[31] The reversed LML type



Scheme 2. The two-step chemoenzymatic synthesis of LML type reversed structured TAGs. Reagents and conditions: (a) *C. antarctica* lipase, PUFA as an acetoxime ester, CH_2Cl_2, rt, 3.5 h; (b) SFA, EDCI, DMAP, CH_2Cl_2, rt, 12 h. (For the sake of clarity the use of SFA (saturated fatty acid) and PUFA (polyunsaturated fatty acid) in this scheme refers to the hydrocarbon chains of these molecules being saturated or polyunsaturated.).

Earlier, we had successfully prepared homogeneous TAGs possessing pure EPA or DHA as the sole fatty acid accommodating all three positions of the glycerol moiety by use of lipase, where glycerol was acylated with stoichiometric amount (three molar equivalents) of EPA or DHA as free acids.[36] In that case there were strong evidences that the introduction of the third and final equivalent of the PUFAs was taking place at a terminal 1- or 3-position of a 1(3),2-DAG intermediate rather than the more

structured TAG products were obtained chemically and regioisomerically pure as was confirmed by ^1H and ^{13}C NMR spectroscopy, in very high to excellent yields (88–93% for the EPA series and 89–94% for the DHA series) after purification by flash chromatography on short silica gel column. Table 1 shows the yields obtained for these reversed structured TAG products, the total of 12 such reversed structured TAG derivatives, **1a**–**f** for EPA and **2a**–**f** for DHA.

Table 1
The reversed structured TAG products constituting a pure saturated fatty acid (SFA) at their 2-position and EPA (**1a**–**f**) or DHA (**2a**–**f**) at the 1,3-positions, along with their obtained yields

Compound	SFA	PUFA	Yield
1a	C6:0	EPA	91%
1b	C8:0	EPA	90%
1c	C10:0	EPA	88%
1d	C12:0	EPA	89%
1e	C14:0	EPA	93%
1f	C16:0	EPA	90%
2a	C6:0	DHA	89%
2b	C8:0	DHA	94%
2c	C10:0	DHA	90%
2d	C12:0	DHA	91%
2e	C14:0	DHA	90%
2f	C16:0	DHA	90%

It is hard to see how synthesis of structured TAGs of the current type may be brought about without a lipase. Traditional organic synthesis is much dependent on protection-deprotection based approaches. The use of many of the most practised protective groups such as acetals requires mild acidic conditions for their deprotection under which acyl-migration is unescapable. And, in the current case involving polyunsaturated fatty acids alternative use of benzyl based protective groups can also be ruled out since catalytic hydrogenolysis is no longer an option when that type of acyl groups are present. This underlines the importance of the lipase and use of activated PUFAs in tasks of this type to prepare reversed structured TAGs and other related acylglycerol based lipids.

These compounds are the first reversed structured TAGs of the LML type possessing the long-chain n-3 PUFAs to be reported. There is little doubt that such compounds will become an important complementary to the already existing focused library of well defined chemically and regiopure lipid compounds.[30–34] The reversed structured TAGs may certainly offer unique opportunities in terms of screening for biological activity of EPA and DHA in relation to their location at the end-positions versus the mid-position in TAGs bearing in mind the difference between fish oil and marine mammal TAGs.[17] Ackman pointed out a possible relevance to that in relation to a lower incidence of cardiovascular diseases among Greenland Inuits consuming seal fat rather than much fish in their diet.[37]

The reversed structured TAGs may also find value as important chemical standards and fine chemicals for various purposes. These structured TAGs as well as their preparation methodology may also find important use in the pharmaceutical area as prodrugs offering the possibility of attaching an active drug molecule to the 2-position of the glycerol backbone in a combination with bioactive EPA or DHA already within the same molecule. Interestingly enough, EPA and DHA have been described as prodrugs in relation to their serving as precursors to drug-like bioactive eicosanoids, resolvins and protectins.[10] Finally, the straightforward and simple methodology may also become of use in introducing radiolabelled molecules into the acylglycerol structure.

For rational design of acylglycerol prodrugs labile drugs such as L-Dopa and some non-steroidal anti-inflammatory drugs (NSAIDs) have been incorporated into the 2-position of 1,3-DAGs to increase drugs absorption and delivery by the intestinal lymphatic system following oral administration.[38,39] Up to now their syntheses, however, have been primarily confined to saturated acyl chains due to lack of efficient methods to insert other types of more labile fatty acids into the end-positions of the glycerol backbone. Symmetric 1,3-DAGs comprised of EPA and DHA are compounds of high value for pharmaceutical and medical purposes since they may be esterified with a drug at the 2-position, making use of the methodology described herein. The resulting prodrugs acquire the biological properties of n-3 PUFAs and the pharmaceutical properties of the drug in one and the same molecule and may indeed improve the therapeutic value of the drug.[40,41]

2.3. Regiocontrol by ^{1}H and ^{13}C NMR spectroscopy studies

^{1}H NMR spectroscopy was of great importance to monitor the progress of the reactions and confirm the chemical identity and purity of the intermediates and products. It was also of high utility to evaluate the extent of possible acyl-migration side reaction and therefore the regiopurity and regiocontrol of these processes. This has been extensively discussed and described in our previous reports on the chemoenzymatic synthesis of the MLM type structured TAGs.[30,31] The acyl-migration side reaction is a well-recognized problem in dealing with syntheses that involve partially acylated carbohydrates, polyols and glycerol derivatives.[28–31] This is a spontaneous intramolecular process controlled by thermodynamics and independent of the biocatalyst. It is promoted by various parameters that include pH, presence of acid or base, type of solvent, immobilized enzyme carrier material and temperature, the most crucial parameter in the current studies. As before, there were no signs of acyl-migration taking place neither in the enzymatic part[33] of the current synthesis nor the coupling reaction described herein as was firmly established by the ^{1}H NMR studies.

Despite significantly lower accuracy ^{13}C NMR spectroscopy was also useful as an additional support to the regiocontrol of the syntheses described. This relates to the carbonyl carbons of each of the three categories of fatty acids, the saturated fatty acids, EPA and DHA, each of them displaying a distinct resonance peak that depends upon their location at the terminal 1,3-positions (α) or the 2-position (β) of the TAG glycerol backbone. The carbonyl carbons of all the saturated fatty acids C6:0–C16:0 investigated in the current work were observed to resonate at δ 172.8 ppm when located at the 2-position of the TAGs. The corresponding resonance value for EPA located at the 1,3-positions remained at δ 173.0 ppm and that for DHA at these positions at δ 172.5 ppm. As can be noticed in the experimental section these values were quite consistently obtained for these fatty acids. This compares to δ 173.3 ppm for the saturated fatty acids when located at the 1,3-positions and δ 172.6 ppm for EPA and δ 172.1 ppm for DHA when located at the 2-position as obtained for normal structured MLM type TAGs from previous studies.[30,31] Table 2 lists the characteristic ^{13}C NMR chemical shift values for these carbonyl groups. Some deviations from previously reported data for MLM structured TAGs[30] may be noticed that are most likely related to the current data being derived from a 400 MHz instrument as compared to 250 MHz instrument (as based on protons) in the previous case. These differences affecting EPA when located at the β-position and DHA at the α-position were evident in the second decimal digits.

Table 2
The ^{13}C NMR chemical shift values characteristic for the carbonyl carbons of saturated fatty acids (C6:0–C16:0), EPA and DHA depending on their location at the 1,3-positions (α) or 2-position (β) of the glycerol backbone of structured TAGs

Fatty acid type	δ (ppm), α C=O	δ (ppm), β C=O
C6:0–C16:0	173.3	172.8
EPA	173.0	172.6
DHA	172.5	172.1

3. Conclusion

A novel approach was recently disclosed to incorporate EPA and DHA exclusively into the terminal positions of glycerol and enantiopure 1-*O*-alkylglycerols by use of EPA and DHA activated as acetoxime esters in a highly regioselective transesterification process catalyzed by immobilized *C. antarctica* lipase B.[33] This resulted in reversed structured 1,3-DAGs, possessing EPA and DHA at their end-positions, and enantiopure MAGEs, possessing EPA and DHA at their *sn*-1 or *sn*-3 end-position, in very high to excellent yields (85–93%). A subsequent introduction of saturated fatty acids into their 2-position resulted in novel reversed structured TAGs and DAGEs by use of chemical coupling. This was very recently reported for the reversed structured DAGEs, which were obtained in very high to excellent yields (85–98%),[34] and is disclosed in the current report for the similarly structured TAGs that were obtained in 88–94% yields.

4. Material and methods

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer using $CDCl_3$ as a solvent. 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. In assignments for the ^{1}H NMR spectra FA refers to the saturated fatty acyl part of the molecule. 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 (IR) spectra for all the products as neat liquids were conducted on a Nicolet Avatar 360 FTIR (E.S.P.) Spectrophotometer on a ZnSe plate. The high-resolution mass spectra (HRMS) were acquired on a Bruker micrOTOF-Q mass spectrometer equipped with an atmospheric pressure chemical ionization chamber (APCI) or an E-spray ionization chamber (ESI). All data analysis was done on a Bruker software.

All chemicals and solvents were used without further purification unless otherwise stated. EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) was obtained from Sigma–Aldrich (Steinheim, Germany). Hexanoic acid (98%, zur synthese) and decanoic acid (98%, zur synthese) were obtained from Merck (Germany) and hexadecanoic acid (99%) from Fluka (Switzerland). Octanoic acid (>99.5%), dodecanoic acid (>99.5%), tetradecanoic acid (>99.5%) and 4-dimethylaminopyridine (DMAP, 99%) were obtained from Acros Organics (Geel, Belgium). CH_2Cl_2 was obtained HPLC grade from Sigma–Aldrich (Steinheim, Germany) and dried over CaH_2 under nitrogen atmosphere when needed. Column chromatography was performed on Silica gel 60 (Silicycle, Ontario). Reactions were monitored by TLC on Silica gel 60 F254 (Silicycle, Ontario), with detection by quenching of fluorescence, rhodamine 6G in CH_3OH and/or with phosphomolybdic acid in ethanol.

*4.1.1. Synthesis of 1,3-dieicosapentaenoyl-2-hexanoylglycerol (**1a**).* To a solution of 1,3-dieicosapentaenoylglycerol **1** (41 mg, 0.062 mmol), DMAP (10 mg, 0.082 mmol) and EDCI (21 mg, 0.109 mmol), in dry CH_2Cl_2 (1 mL) was added hexanoic acid (9 mg, 0.077 mmol) and the resulting solution stirred at rt overnight under nitrogen atmosphere. The reaction mixture was then passed through a short silica column chromatography (CH_2Cl_2) to afford product **1a** (43 mg, 0.057 mmol) as clear oil, yield 91%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.43–5.29 (m, 20H, =C*H*), 5.29–5.24 (m, 1H, CH_2CHCH_2), 4.31 (dd, 2H, J 11.9, J 4.3 Hz, CH_2OCO), 4.15 (dd, 2H, J 11.9, J 6.0 Hz, CH_2OCO), 2.86–2.79 (m, 16H, =CCH_2C=), 2.33 (t, 4H, J 7.6 Hz, CH_2COO in EPA), 2.31 (t, 2H, J 7.5 Hz, CH_2COO in FA), 2.14–2.04 (m, 8H, =CCH_2CH$_3$ and =CCH_2CH$_2$), 1.70 (q (br), 4H, J 7.5 Hz, CH_2CH$_2$COO in EPA), 1.65–1.58 (m, 2H, CH_2CH$_2$COO in FA), 1.35–1.27 (m, 4H, CH_2), 0.97 (t, 6H, J 7.5 Hz, CH_3 in EPA), 0.90 (t, 3H, J 7.0 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0 (2), 172.9, 132.0 (2), 128.9 (2), 128.8 (2), 128.6 (2), 128.3 (2), 128.2 (4), 128.1 (2), 127.9 (2), 127.0 (2), 68.8, 62.2 (2), 34.1, 33.4 (2), 31.2, 26.5 (2), 25.6 (6), 25.5 (2), 24.7 (2), 24.6, 22.3, 20.6 (2), 14.3 (2), 13.9 ppm; FTIR: ν_{max} 3012 (s, CH), 2932 (vs, CH), 2873 (s, CH), 1743 (vs, C=O) cm^{-1}. HRMS m/z calcd for $C_{49}H_{74}O_6$ ($M+NH_4^+$) 776.5824, found 776.5806 amu.

*4.1.2. Synthesis of 1,3-dieicosapentaenoyl-2-octanoylglycerol (**1b**).* The same procedure was followed as for **1a** except using 1,3-dieicosapentaenoylglycerol **1** (40 mg, 0.061 mmol), DMAP (8 mg, 0.065 mmol), EDCI (20 mg, 0.104 mmol) and octanoic acid (9 mg, 0.062 mmol). The product **1b** (43 mg, 0.055 mmol) was afforded as clear oil, yield 90%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.43–5.29 (m, 20H, =C*H*), 5.29–5.24 (m, 1H, CH_2CHCH_2), 4.30 (dd, 2H, J 11.9, J 4.3 Hz, CH_2OCO), 4.15 (dd, 2H, J 11.9, J 6.0 Hz, CH_2OCO), 2.88–2.77 (m, 16H, =CCH_2C=), 2.33 (t, 4H, J 7.6 Hz, CH_2COO in EPA), 2.31 (t, 2H, J 7.5 Hz, CH_2COO in FA), 2.14–2.04 (m, 8H, =CCH_2CH$_3$ and =CCH_2CH$_2$), 1.70 (q (br), 4H, J 7.5 Hz, CH_2CH$_2$COO in EPA), 1.65–1.58 (m, 2H, CH_2CH$_2$COO in FA), 1.35–1.27 (m, 8H, CH_2), 0.97 (t, 6H, J 7.5 Hz, CH_3 in EPA), 0.88 (t, 3H, J 7.0 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0 (2), 172.8, 132.0 (2), 128.9 (2), 128.8 (2), 128.6 (2), 128.3 (2), 128.2 (2), 128.1 (4), 127.8 (2), 127.0 (2), 68.8, 62.2 (2), 34.2, 33.4 (2), 31.6, 29.0, 28.9, 26.5 (2), 25.6 (6), 25.5 (2), 24.9, 24.7 (2), 22.6, 20.6 (2), 14.3 (2), 14.1 ppm; FTIR: ν_{max} 3012 (s, CH), 2930 (vs, CH), 2857 (s, CH), 1740 (vs, C=O) cm^{-1}. HRMS m/z calcd for $C_{51}H_{78}O_6$ ($M+NH_4^+$) 804.6137, found 804.6134 amu.

*4.1.3. Synthesis of 1,3-dieicosapentaenoyl-2-decanoylglycerol (**1c**).* The same procedure was followed as for **1a** except using 1,3-dieicosapentaenoylglycerol **1** (70 mg, 0.106 mmol), DMAP (13 mg, 0.107 mmol), EDCI (30 mg, 0.156 mmol) and decanoic acid (20 mg, 0.116 mmol). The product **1c** (76 mg, 0.093 mmol) was afforded as clear oil, yield 88%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.43–5.29 (m, 20H, =C*H*), 5.29–5.24 (m, 1H, CH_2CHCH_2), 4.30 (dd, 2H, J 11.9, J 4.3 Hz, CH_2OCO), 4.15 (dd, 2H, J 11.9, J 6.0 Hz, CH_2OCO), 2.88–2.77 (m, 16H, =CCH_2C=), 2.35–2.29 (m, 6H, CH_2COO in EPA and FA), 2.14–2.04 (m, 8H, =CCH_2CH$_3$ and =CCH_2CH$_2$), 1.70 (q (br), 4H, J 7.5 Hz, CH_2CH$_2$COO in EPA), 1.65–1.58 (m, 2H, CH_2CH$_2$COO in FA), 1.35–1.27 (m, 12H, CH_2), 0.97 (t, 6H, J 7.5 Hz, CH_3 in EPA), 0.88 (t, 3H, J 7.0 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0 (2), 172.8, 132.0 (2), 128.9 (2), 128.8 (2), 128.6 (2), 128.3 (2), 128.2 (2), 128.1 (4), 127.8 (2), 127.0 (2), 68.8, 62.2 (2), 34.2, 33.4 (2), 31.9, 29.4, 29.3 (2), 29.1, 26.5 (2), 25.6 (6), 25.5 (2), 24.9, 24.7 (2), 22.7, 20.5 (2), 14.3 (2), 14.1 ppm; FTIR: ν_{max} 3012 (s, CH), 2926 (vs, CH), 2855 (s, CH), 1743 (vs, C=O) cm^{-1}. HRMS m/z calcd for $C_{53}H_{82}O_6$ ($M+NH_4^+$) 832.6450, found 832.6451 amu.

*4.1.4. Synthesis of 1,3-dieicosapentaenoyl-2-dodecanoylglycerol (**1d**).* The same procedure was followed as for **1a** except using 1,3-dieicosapentaenoylglycerol **1** (43 mg, 0.065 mmol), DMAP (8 mg, 0.065 mmol), EDCI (21 mg, 0.110 mmol) and dodecanoic acid (15 mg, 0.075 mmol). The product **1d** (49 mg, 0.058 mmol) was afforded as clear oil, yield 89%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.43–5.29 (m, 20H, =C*H*), 5.29–5.24 (m, 1H, CH_2CHCH_2), 4.30 (dd, 2H, J 11.9, J 4.3 Hz, CH_2OCO), 4.15 (dd, 2H, J 11.9, J 6.0 Hz, CH_2OCO), 2.88–2.77 (m, 16H, =CCH_2C=), 2.35–2.29 (m, 6H, CH_2COO in EPA and FA), 2.14–2.04 (m, 8H, =CCH_2CH$_3$ and =CCH_2CH$_2$), 1.70 (q (br), 4H, J 7.5 Hz, CH_2CH$_2$COO in EPA), 1.65–1.58 (m, 2H, CH_2CH$_2$COO in FA), 1.35–1.27 (m, 16H, CH_2), 0.97 (t, 6H, J 7.5 Hz, CH_3 in EPA), 0.88 (t, 3H, J 6.8 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0 (2), 172.8, 132.0 (2), 128.9 (2), 128.8 (2),

128.6 (2), 128.3 (2), 128.2 (2), 128.1 (4), 127.9 (2), 127.0 (2), 68.8, 62.2 (2), 34.2, 33.4 (2), 31.9, 29.6 (2), 29.5, 29.3 (2), 29.1, 26.5 (2), 25.6 (6), 25.5 (2), 24.9, 24.7 (2), 22.7, 20.5 (2), 14.3 (2), 14.1 ppm; FTIR: ν_{max} 3012 (s, CH), 2925 (vs, CH), 2854 (s, CH), 1743 (vs, C=O) cm^{-1}. HRMS *m/z* calcd for $C_{55}H_{86}O_6$ ($M+NH_4^+$) 860.6763, found 860.6802 amu.

*4.1.5. Synthesis of 1,3-dieicosapentaenoyl-2-tetradecanoylglycerol (**1e**).* The same procedure was followed as for **1a** except using 1,3-dieicosapentaenoylglycerol **1** (73 mg, 0.110 mmol), DMAP (10 mg, 0.082 mmol), EDCI (32 mg, 0.167 mmol) and tetradecanoic acid (25 mg, 0.109 mmol). The product **1e** (89 mg, 0.102 mmol) was afforded as clear oil, yield 93%. ^1H NMR (400 MHz, $CDCl_3$): δ 5.43–5.29 (m, 20H, =C*H*), 5.29–5.24 (m, 1H, CH_2CHCH_2), 4.30 (dd, 2H, J 11.9, J 4.3 Hz, CH_2OCO), 4.15 (dd, 2H, J 11.9, J 6.0 Hz, CH_2OCO), 2.88–2.77 (m, 16H, $=CCH_2C=$), 2.35–2.29 (m, 6H, CH_2COO in EPA and FA), 2.14–2.04 (m, 8H, $=CCH_2CH_3$ and $=CCH_2CH_2$), 1.70 (q (br), 4H, J 7.5 Hz, CH_2CH_2COO in EPA), 1.65–1.57 (m, 2H, CH_2CH_2COO in FA), 1.35–1.27 (m, 20H, CH_2), 0.97 (t, 6H, J 7.5 Hz, CH_3 in EPA), 0.88 (t, 3H, J 6.8 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0 (2), 172.8, 132.0 (2), 128.9 (2), 128.8 (2), 128.6 (2), 128.3 (2), 128.2 (2), 128.1 (4), 127.8 (2), 127.0 (2), 68.8, 62.2 (2), 34.2, 33.4 (2), 31.9, 29.7, 29.6 (3), 29.5, 29.3 (2), 29.1, 26.5 (2), 25.6 (6), 25.5 (2), 24.9, 24.7 (2), 22.7, 20.5 (2), 14.3 (2), 14.1 ppm; FTIR: ν_{max} 3012 (s, CH), 2924 (vs, CH), 2854 (s, CH), 1743 (vs, C=O) cm^{-1}. HRMS *m/z* calcd for $C_{57}H_{90}O_6$ ($M+NH_4^+$) 888.7076, found 888.7104 amu.

*4.1.6. Synthesis of 1,3-dieicosapentaenoyl-2-hexadecanoylglycerol (**1f**).* The same procedure was followed as for **1a** except using 1,3-dieicosapentaenoylglycerol **1** (70 mg, 0.106 mmol), DMAP (9 mg, 0.074 mmol), EDCI (29 mg, 0.151 mmol) and hexadecanoic acid (27 mg, 0.105 mmol). The product **1f** (86 mg, 0.095 mmol) was afforded as clear oil, yield 90%. ^1H NMR (400 MHz, $CDCl_3$): δ 5.43–5.29 (m, 20H, =C*H*), 5.29–5.24 (m, 1H, CH_2CHCH_2), 4.30 (dd, 2H, J 11.9, J 4.3 Hz, CH_2OCO), 4.15 (dd, 2H, J 11.9, J 6.0 Hz, CH_2OCO), 2.88–2.77 (m, 16H, $=CCH_2C=$), 2.35–2.29 (m, 6H, CH_2COO in EPA and FA), 2.14–2.04 (m, 8H, $=CCH_2CH_3$ and $=CCH_2CH_2$), 1.70 (q (br), 4H, J 7.5 Hz, CH_2CH_2COO in EPA), 1.65–1.57 (m, 2H, CH_2CH_2COO in FA), 1.34–1.20 (m, 24H, CH_2), 0.97 (t, 6H, J 7.5 Hz, CH_3 in EPA), 0.88 (t, 3H, J 6.8 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0 (2), 172.8, 132.0 (2), 128.9 (2), 128.8 (2), 128.6 (2), 128.3 (2), 128.2 (2), 128.1 (4), 127.8 (2), 127.0 (2), 68.8, 62.2 (2), 34.2, 33.4 (2), 31.9, 29.7 (5), 29.6, 29.5, 29.4, 29.3, 29.1, 26.5 (2), 25.6 (6), 25.5 (2), 24.9, 24.7 (2), 22.7, 20.6 (2), 14.3 (2), 14.1 ppm; FTIR: ν_{max} 3012 (s, CH), 2924 (vs, CH), 2853 (s, CH), 1743 (vs, C=O) cm^{-1}. HRMS *m/z* calcd for $C_{59}H_{94}O_6$ ($M+NH_4^+$) 916.7389, found 916.7363 amu.

*4.1.7. Synthesis of 1,3-didocosahexaenoyl-2-hexanoylglycerol (**2a**).* The same procedure was followed as for **1a** except using 1,3-didocosahexaenoylglycerol **2** (70 mg, 0.098 mmol), DMAP (10 mg, 0.082 mmol), EDCI (28 mg, 0.146 mmol) and hexanoic acid (14 mg, 0.121 mmol). The product **2a** (71 mg, 0.092 mmol) was afforded as light yellow oil, yield 89%. ^1H NMR (400 MHz, $CDCl_3$): δ 5.44–5.29 (m, 24H, =C*H*), 5.29–5.24 (m, 1H, CH_2CHCH_2), 4.31 (dd, 2H, J 11.9, J 4.3 Hz, CH_2OCO), 4.16 (dd, 2H, J 11.9, J 6.0 Hz, CH_2OCO), 2.88–2.80 (m, 20H, $=CCH_2C=$), 2.41–2.37 (m, 8H, CH_2CH_2COO in DHA), 2.32 (t, 2H, J 7.5 Hz, CH_2COO in FA), 2.11–2.04 (m, 4H, $=CCH_2CH_3$), 1.66–1.58 (m, 2H, CH_2CH_2COO in FA), 1.38–1.26 (m, 4H, CH_2), 0.97 (t, 6H, J 7.5 Hz, CH_3 in DHA), 0.89 (t, 3H, J 7.0 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 172.5 (2), 132.0 (2), 129.5 (2), 128.5 (2), 128.3 (4), 128.2 (2), 128.1 (4), 128.0 (2), 127.9 (2), 127.6 (2), 127.0 (2), 68.8, 62.2 (2), 34.1, 33.9 (2), 31.2, 25.6 (8), 25.5 (2), 24.5, 22.6 (2), 22.3, 20.5 (2), 14.3 (2), 13.9 ppm; FTIR: ν_{max} 3013 (s, CH), 2932 (vs, CH), 2873 (s, CH), 1742 (vs, C=O) cm^{-1}. HRMS *m/z* calcd for $C_{53}H_{78}O_6$ ($M+NH_4^+$) 828.6137, found 828.6142 amu.

*4.1.8. Synthesis of 1,3-didocosahexaenoyl-2-octanoylglycerol (**2b**).* The same procedure was followed as for **1a** except using 1,3-didocosahexaenoylglycerol **2** (41 mg, 0.058 mmol), DMAP (10 mg, 0.082 mmol), EDCI (22 mg, 0.115 mmol) and octanoic acid (9 mg, 0.062 mmol). The product **2b** (45 mg, 0.054 mmol) was afforded as light yellow oil, yield 94%. ^1H NMR (400 MHz, $CDCl_3$): δ 5.44–5.29 (m, 24H, =C*H*), 5.29–5.24 (m, 1H, CH_2CHCH_2), 4.31 (dd, 2H, J 11.9, J 4.3 Hz, CH_2OCO), 4.16 (dd, 2H, J 11.9, J 6.0 Hz, CH_2OCO), 2.88–2.78 (m, 20H, $=CCH_2C=$), 2.39–2.37 (m, 8H, CH_2CH_2COO in DHA), 2.32 (t, 2H, J 7.5 Hz, CH_2COO in FA), 2.11–2.03 (m, 4H, $=CCH_2CH_3$), 1.65–1.57 (m, 2H, CH_2CH_2COO in FA), 1.34–1.23 (m, 8H, CH_2), 0.97 (t, 6H, J 7.5 Hz, CH_3 in DHA), 0.88 (t, 3H, J 6.9 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 172.5 (2), 132.0 (2), 129.5 (2), 128.5 (2), 128.3 (4), 128.2 (2), 128.1 (4), 128.0 (2), 127.9 (2), 127.6 (2), 127.0 (2), 68.8, 62.2 (2), 34.2, 33.9 (2), 31.6, 29.0, 28.9, 25.6 (8), 25.5 (2), 24.9, 22.6 (3), 20.5 (2), 14.3 (2), 14.0 ppm; FTIR: ν_{max} 3013 (s, CH), 2929 (vs, CH), 2857 (s, CH), 1743 (vs, C=O) cm^{-1}. HRMS *m/z* calcd for $C_{55}H_{82}O_6$ ($M+NH_4^+$) 856.6450, found 856.6461 amu.

*4.1.9. Synthesis of 1,3-didocosahexaenoyl-2-decanoylglycerol (**2c**).* The same procedure was followed as for **1a** except using 1,3-didocosahexaenoylglycerol **2** (42 mg, 0.059 mmol), DMAP (11 mg, 0.090 mmol), EDCI (15 mg, 0.078 mmol) and decanoic acid (13 mg, 0.075 mmol). The product **2c** (46 mg, 0.053 mmol) was afforded as light yellow oil, yield 90%. ^1H NMR (400 MHz, $CDCl_3$): δ 5.44–5.29 (m, 24H, =C*H*), 5.29–5.24 (m, 1H, CH_2CHCH_2), 4.31 (dd, 2H, J 11.9, J 4.3 Hz, CH_2OCO), 4.16 (dd, 2H, J 11.9, J 6.0 Hz, CH_2OCO), 2.88–2.78 (m, 20H, $=CCH_2C=$), 2.39–2.37 (m, 8H, CH_2CH_2COO in DHA), 2.31 (t, 2H, J 7.5 Hz, CH_2COO in FA), 2.11–2.04 (m, 4H, $=CCH_2CH_3$), 1.65–1.57 (m, 2H, CH_2CH_2COO in FA), 1.34–1.21 (m, 12H, CH_2), 0.97 (t, 6H, J 7.5 Hz, CH_3 in DHA), 0.88 (t, 3H, J 6.9 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 172.5 (2), 132.0 (2), 129.5 (2), 128.5 (2), 128.3 (4), 128.2 (2), 128.1 (4), 128.0 (2), 127.8 (2), 127.6 (2), 127.0 (2), 68.8, 62.2 (2), 34.2, 33.9 (2), 31.8, 29.4, 29.3 (2), 29.1, 25.6 (8), 25.5 (2), 24.9, 22.6 (3), 20.5 (2), 14.3 (2), 14.1 ppm; FTIR: ν_{max} 3013 (s, CH), 2926 (vs, CH), 2855 (s, CH), 1743 (vs, C=O) cm^{-1}. HRMS *m/z* calcd for $C_{57}H_{86}O_6$ ($M+NH_4^+$) 884.6763, found 884.6752 amu.

*4.1.10. Synthesis of 1,3-didocosahexaenoyl-2-dodecanoylglycerol (**2d**).* The same procedure was followed as for **1a** except using 1,3-didocosahexaenoylglycerol **2** (70 mg, 0.098 mmol), DMAP (10 mg, 0.082 mmol), EDCI (28 mg, 0.146 mmol) and dodecanoic acid (20 mg, 0.100 mmol). The product **2d** (80 mg, 0.089 mmol) was afforded as light yellow oil, yield 91%. ^1H NMR (400 MHz, $CDCl_3$): δ 5.44–5.30 (m, 24H, =C*H*), 5.29–5.24 (m, 1H, CH_2CHCH_2), 4.31 (dd, 2H, J 11.9, J 4.3 Hz, CH_2OCO), 4.16 (dd, 2H, J 11.9, J 6.0 Hz, CH_2OCO), 2.88–2.78 (m, 20H, $=CCH_2C=$), 2.41–2.37 (m, 8H, CH_2CH_2COO in DHA), 2.31 (t, 2H, J 7.5 Hz, CH_2COO in FA), 2.11–2.03 (m, 4H, $=CCH_2CH_3$), 1.65–1.57 (m, 2H, CH_2CH_2COO in FA), 1.34–1.24 (m, 16H, CH_2), 0.97 (t, 6H, J 7.5 Hz, CH_3 in DHA), 0.88 (t, 3H, J 6.8 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 172.5 (2), 132.0 (2), 129.5 (2), 128.6 (2), 128.3 (4), 128.2 (2), 128.1 (4), 128.0 (2), 127.9 (2), 127.6 (2), 127.0 (2), 68.8, 62.3 (2), 34.2, 33.9 (2), 31.9, 29.6 (2), 29.5, 29.3 (2), 29.1, 25.6 (8), 25.5 (2), 24.9, 22.7, 22.6 (2), 20.6 (2), 14.3 (2), 14.1 ppm; FTIR: ν_{max} 3013 (s, CH), 2925 (vs, CH), 2854 (s, CH), 1743 (vs, C=O) cm^{-1}. HRMS *m/z* calcd for $C_{59}H_{90}O_6$ ($M+NH_4^+$) 912.7076, found 912.7062 amu.

*4.1.11. Synthesis of 1,3-didocosahexaenoyl-2-tetradecanoylglycerol (**2e**).* The same procedure was followed as for **1a** except using 1,3-didocosahexaenoylglycerol **2** (41 mg, 0.058 mmol), DMAP

(10 mg, 0.082 mmol), EDCI (15 mg, 0.078 mmol) and tetradecanoic acid (14 mg, 0.061 mmol). The product **2e** (48 mg, 0.052 mmol) was afforded as light yellow oil, yield 90%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.44–5.30 (m, 24H, =C*H*), 5.29–5.24 (m, 1H, CH_2CHCH_2), 4.31 (dd, 2H, J 11.9, J 4.3 Hz, CH_2OCO), 4.16 (dd, 2H, J 11.9, J 6.0 Hz, CH_2OCO), 2.88–2.77 (m, 20H, =CCH_2C=), 2.39–2.37 (m, 8H, CH_2CH_2COO in DHA), 2.31 (t, 2H, J 7.5 Hz, CH_2COO in FA), 2.11–2.04 (m, 4H, =CCH_2CH_3), 1.65–1.57 (m, 2H, CH_2CH_2COO in FA), 1.33–1.23 (m, 20H, CH_2), 0.97 (t, 6H, J 7.5 Hz, CH_3 in DHA), 0.88 (t, 3H, J 6.8 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 172.5 (2), 132.0 (2), 129.5 (2), 128.6 (2), 128.3 (4), 128.2 (2), 128.1 (4), 128.0 (2), 127.9 (2), 127.6 (2), 127.0 (2), 68.8, 62.3 (2), 34.2, 33.9 (2), 31.9, 29.7 (2), 29.6 (2), 29.5, 29.3 (2), 29.1, 25.6 (8), 25.5 (2), 24.9, 22.7, 22.6 (2), 20.6 (2), 14.3 (2), 14.1 ppm; FTIR: ν_{max} 3013 (s, CH), 2924 (vs, CH), 2854 (s, CH), 1743 (vs, C=O) cm^{-1}. HRMS *m*/*z* calcd for $C_{61}H_{94}O_6$ ($M+NH_4^+$) 940.7389, found 940.7402 amu.

*4.1.12. Synthesis of 1,3-didocosahexaenoyl-2-hexadecanoylglycerol (**2f**).* The same procedure was followed as for **1a** except using 1,3-didocosahexaenoylglycerol **2** (42 mg, 0.059 mmol), DMAP (10 mg, 0.082 mmol), EDCI (16 mg, 0.083 mmol) and hexadecanoic acid (15 mg, 0.059 mmol). The product **2f** (50 mg, 0.053 mmol) was afforded as light yellow oil, yield 90%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.44–5.30 (m, 24H, =C*H*), 5.29–5.24 (m, 1H, CH_2CHCH_2), 4.31 (dd, 2H, J 11.9, J 4.3 Hz, CH_2OCO), 4.16 (dd, 2H, J 11.9, J 6.0 Hz, CH_2OCO), 2.88–2.80 (m, 20H, =CCH_2C=), 2.41–2.37 (m, 8H, CH_2CH_2COO in DHA), 2.31 (t, 2H, J 7.5 Hz, CH_2COO in FA), 2.11–2.04 (m, 4H, =CCH_2CH_3), 1.65–1.57 (m, 2H, CH_2CH_2COO in FA), 1.33–1.22 (m, 24H, CH_2), 0.97 (t, 6H, J 7.5 Hz, CH_3 in DHA), 0.88 (t, 3H, J 6.8 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 172.5 (2), 132.0 (2), 129.5 (2), 128.6 (2), 128.3 (4), 128.2 (2), 128.1 (4), 128.0 (2), 127.9 (2), 127.6 (2), 127.0 (2), 68.8, 62.3 (2), 34.2, 33.9 (2), 31.9, 29.7 (5), 29.6, 29.5, 29.3 (2), 29.1, 25.6 (8), 25.5 (2), 24.9, 22.7, 22.6 (2), 20.6 (2), 14.3 (2), 14.1 ppm; FTIR: ν_{max} 3013 (s, CH), 2924 (vs, CH), 2853 (s, CH), 1743 (vs, C=O) cm^{-1}. HRMS *m*/*z* calcd for $C_{63}H_{98}O_6$ ($M+NH_4^+$) 968.7702, found 968.7727 amu.

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