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Activation of n-3 polyunsaturated fatty acids as oxime esters: a novel approach for their exclusive incorporation into the primary alcoholic positions of the glycerol moiety by lipase

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ABSTRACT

A novel approach has been developed for activating the highly bioactive long-chain n-3 polyunsaturated fatty acids EPA and DHA as oxime esters and incorporating them exclusively to the end-positions of glycerol and enantiopure 1-*O*-alkylglycerols. The *Candida antarctica* lipase B was observed to display a superb regioselectivity when using the acetoxime esters of EPA and DHA as acyldonors under mild condition to keep acyl-migration side-reaction under complete control. Regiopure 1,3-diacylglycerols, 1-*O*-alkyl-3-acyl-*sn*-glycerols and their antipodes possessing EPA and DHA were afforded in very high purity and yields.

1. Introduction

The long-chain n-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are characteristic of marine oil and biologically active compounds essential to human health. Their cardioprotective and anti-inflammatory properties are now firmly established (Harris et al., 2008; Holub and Holub, 2004; James et al., 2003; Simopoulos, 2002). For instance, Omacor® (Lovaza® in USA), comprised of ethyl esters highly enriched with EPA and DHA, is a prescription drug registered as an adjuvant therapy in the treatment of hypertriglyceridemia and for secondary prevention of post-myocardial infarction (Hoy and Keating, 2009). Furthermore, EPA and DHA are considered to have important effects on cognitive health and brain function (Peet and Stokes, 2005; Ross et al., 2007) and, more recently, they have emerged as anti-carcinogenic nutrients in the treatment of certain types of tumors (Anderson and Ma, 2009; Colomer et al., 2007).

Symmetric 1,3-diacylglycerols (1,3-DAG), constituting saturated medium-chain fatty acids (MCFA) at the end-positions of the glycerol backbone, are important key intermediates in the synthesis of symmetrically structured triacylglycerols (TAG) possessing a bioactive long-chain n-3 PUFA at the 2-position (Christensen and Hoy, 1996; Iwasaki and Yamane, 2000) and for the rational design of acylglycerol prodrugs. Indeed, labile drugs such as L-DOPA and some non-steroidal anti-inflammatory drugs (NSAID) have been incorporated into the 2-position of 1,3-DAG to increase drugs absorption and delivery by the intestinal lymphatic system following oral administration (Charman and Porter, 1996; Lambert, 2000). Although symmetric 1,3-DAG have found various valuable applications, their potential is far from being extensively exploited. Their syntheses 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.

The naturally occurring 1-*O*-alkyl-2,3-diacyl-*sn*-glycerols, generally known as diacylglyceryl ethers (DAGE), are 1-*O*-alkyl-*sn*-glycerol based ether lipids (EL) present in high amounts in the liver oils of various cartilaginous fish species (Deniau et al., 2010; Kayama and Mankura, 1998; Magnusson and Haraldsson, 2011; Mangold and Paltauf, 1983; Wetherbee and Nichols, 2000). The 1-*O*-alkyl-*sn*-glycerols have been claimed to possess numerous biological activities, including antineoplastic (Hallgren, 1983; Pedrono et al., 2004) and antibacterial (Ved et al., 1984) properties, to increase fertility (Cheminade et al., 2002) and to induce immune stimulant and adjuvant effects (Acevedo et al., 2006; Ngwenya and Foster, 1991; Yamamoto and Ngwenya, 1987). More recently, they have been exploited as drug-carriers including alkylglycerol based prodrugs (Gopinath et al., 2002; Hammond et al., 2001).

Symmetric 1,3-DAG comprising n-3 PUFA are compounds of high value for pharmaceutical and medical purposes since they may

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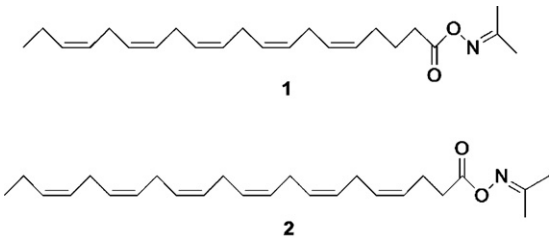


Fig. 1. Acetoxime esters of EPA **1** and DHA **2**.

be esterified with a drug at the 2-position. Such prodrugs acquire the biological properties of n-3 PUFA and the drug in one and the same molecule and might indeed provide improvements to drug's therapeutic value (Chan et al., 2010; Savelieva et al., 2010). Likewise, 1-*O*-alkyl-3-acyl-*sn*-glycerols (3-monoacylglyceryl ethers, 3-MAGE) comprised of an n-3 PUFA at their end-position might be conveniently acylated at their *sn*-2 position to produce prodrugs which combine the benign effects of n-3 PUFA, bioactive alkylglycerols and the pharmaceutical properties of the drug.

Herein, a novel approach is disclosed to incorporate EPA and DHA exclusively into the end-positions of glycerol and enantiomerically pure 1-*O*-alkylglycerols. Acetoxime esters of EPA **1** and DHA **2** (Fig. 1) prepared from the corresponding free fatty acids and acetoxime, using a chemical coupling agent, played a crucial role by permitting a rapid and highly regioselective transesterification process catalyzed by the *Candida antarctica* lipase B (CAL-B from Novozymes). Reversely structured 1,3-DAG, possessing EPA and DHA at the terminal positions, and enantiopure 1-*O*-alkylglycerols, possessing EPA and DHA at their *sn*-1 or *sn*-3 terminal position, were obtained in very high to excellent yields (85–93%).

This is the first report on n-3 PUFA as activated acyl-donors to be exclusively directed by lipase to the end-positions of the glycerol moiety devoid of acyl-migration side-reactions.

2. Results and discussion

Structured TAG possessing saturated fatty acids at the terminal positions along with a bioactive PUFA such as EPA or DHA located at the 2-position are relatively easy to obtain by a highly efficient two-step chemoenzymatic approach (Halldorsson et al., 2003; Magnusson and Haraldsson, 2010). The key step devoid of protection–deprotection transformations involves a highly regioselective lipase and fatty acids activated as vinyl esters to accomplish regiopure 1,3-DAG. High reactivity and fast reactions were offered at mild temperature to retard acyl-migration side-reaction (Bloomer et al., 1991; Fureby et al., 1996; Kodali et al., 1990) that usually hampers regioselective acylation of glycerols and polyhydroxy compounds. The bioactive n-3 PUFA were then introduced to the 2-position by chemical coupling. Similar approach was used to prepare similarly structured DAGE possessing a MCFA at the *sn*-3 position and n-3 PUFA at the *sn*-2 position (Haraldsson, 2007; Magnusson et al., 2011).

Reversing this to prepare reversely structured 1,3-DAG or TAG and DAGE possessing PUFA at the terminal positions is far more of a challenge for several reasons. One is that as a result of their lability it is rather difficult to activate PUFA as vinyl esters or to obtain them in other activated forms. What makes the situation even worse is the fact that most lipases do not tolerate the highly polyunsaturated fatty acids, including EPA and DHA, very well as substrates so that slow reactions are to be anticipated for such substrates with lipase (Halldorsson et al., 2004a; Haraldsson and Hjaltason, 2006). However, the immobilized *C. antarctica* lipase was observed to acylate both glycerol (Haraldsson et al., 1995) and 1-*O*-alkyl-*sn*-glycerols (Haraldsson and Thorarensen, 1994) with EPA and DHA as free acids rather efficiently under less gentle conditions that indeed promoted acyl-migration.

2.1. Activation of EPA and DHA

Previous reports have demonstrated the superb regioselectivity offered by the immobilized *C. antarctica* lipase toward the less hindered terminal hydroxyl groups of glycerol (Halldorsson et al., 2003; Magnusson and Haraldsson, 2010) and 1-*O*-alkyl-*sn*-glycerols (Haraldsson, 2007; Magnusson et al., 2011) in transesterification processes, when vinyl esters of saturated fatty acids were used as acyl donors. The vinyl esters brought about a fast irreversible reaction by formation of an enol, which rapidly tautomerized to non-nucleophilic volatile acetaldehyde (Degueil-Castaing et al., 1987; Wang et al., 1988). In light of these results, and the fact that the *C. antarctica* lipase has been reported to accommodate rather well the intrinsic n-3 PUFA structures into its catalytic site, a similar incorporation of activated EPA and DHA into the end-positions of glycerol and the glycerol backbone of enantiopure alkylglycerols was envisaged.

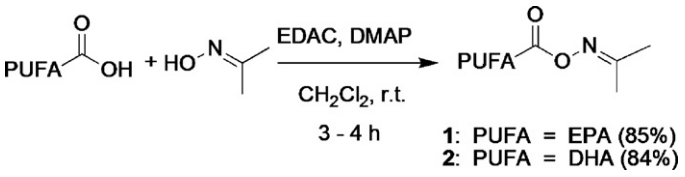
It was evident that such incorporation taking place with high regioselectivity and high conversion rate, required activated esters of EPA and DHA for triggering a fast acylation process. However, there are no reports on preparation of such n-3 PUFA activated esters, to the best of our knowledge. As a first option, the synthesis of vinyl esters of n-3 PUFA was considered.

The two major methods for generating vinyl esters of carboxylic acids involve addition of the carboxylic acids to acetylene gas in the presence of Hg(II) or Zn(II) salts at high temperature (>100 °C), and transvinylation between the carboxylic acids and vinyl acetate, catalyzed by Hg(II) or Pd(II) salts, usually under reflux (Weissermel and Arpe, 2003). However, the high temperature employed and the plausible coordination of the π-orbitals of double bonds to such transition metals, especially for the transvinylation process, may easily promote *cis*/*trans* isomerization and/or double bond migration, therefore strongly excluding their application on n-3 PUFA. Another disadvantage of some of these methods is the use of toxic mercury salts, that are strongly restricted in organic synthesis and in the field of medicine.

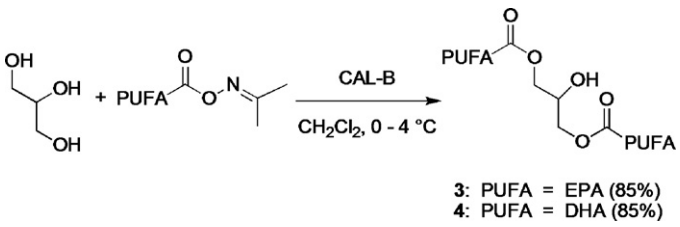
Waldinger and Schneider (1996) have reported on the generation of vinyl esters of oleic acid (C18:1n-9) and linoleic acid (C18:2n-6) by a modified transvinylation method at room temperature, in the presence of $Pd(OAc)_2$ and potassium hydroxide in catalytic amounts. The esters were obtained in high yields, according to the authors, although full characterization data was not provided. This methodology was applied to DHA as free fatty acid under identical reaction conditions. After 48 h reaction time a vinyl ester product was observed in about 20% yield but, not surprisingly, severe detriments on the fine structured methylene-interrupted *cis*-configured double bond framework of the DHA moiety were noticed, as was confirmed by ^{1}H NMR spectroscopy of the final crude mixture.

Other types of activated acyl donors, that have indeed been widely used in lipase catalyzed acylation processes, include isopropenyl esters, anhydrides, cyanomethyl esters, 2-haloethyl esters and oxime esters (Chenevert et al., 2006; Faber, 2004). However, the preparation of isopropenyl esters has the same disadvantages as the closely related aforementioned vinyl esters. Cyanomethyl esters produce toxic cyanomethanol, and anhydrides, although highly reactive, have the main disadvantage of a lack of satisfactory preparation methods, besides an equivalent of the precious acid being abolished during the acylation process.

In view of all this, the generation of 2-haloethyl esters and oxime esters was considered and set out to prepare 2,2,2-trifluoroethyl ester of EPA and acetoxime ester **1** of EPA. They were successfully prepared using the convenient



Scheme 1. Activation of EPA and DHA as acetoxime esters by EDAC coupling.



Scheme 2. Transesterification of EPA and DHA acetoximes with glycerol using *Candida antarctica* lipase B.

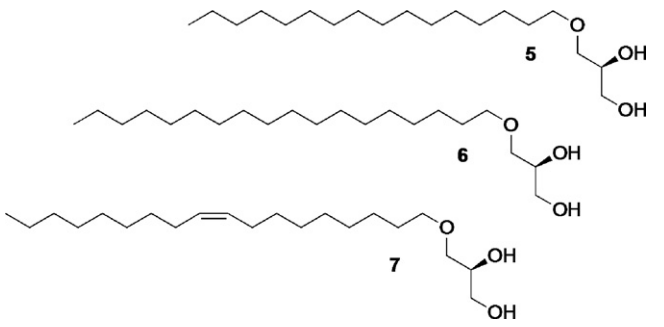


Fig. 2. Chimyl **5**, batyl **6** and selachyl **7** alcohols.

coupling agent 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) in the presence of dimethylaminopyridine (DMAP), under mild reaction conditions known to be very well tolerated by n-3 PUFA (Halldorsson et al., 2003; Magnusson and Haraldsson, 2010), affording the corresponding esters in 85% isolated yields. Other standard procedures for their generation were avoided, since they would have implicated prior generation of acyl chlorides under the corrosive reaction conditions, deleterious to the labile long-chain polyunsaturated framework of these n-3 PUFA (Ghogare and Kumar, 1989; Tsai and Huang, 1999).

The reaction rates for the two types of activated esters of EPA, the 2,2,2-trifluoroethyl ester and oxime ester **1**, were compared in a transesterification reaction with chimyl alcohol (*S*)-**5** (*vide supra*), catalyzed by the immobilized *C. antarctica* lipase. It became clearly evident that the oxime ester reacted faster than the corresponding 2-haloethyl ester, an important fact, since a longer reaction time was anticipated to increase the probabilities of acyl-migration to take place with concomitant loss of regiocontrol. Having established the suitable acylating agent for the n-3 PUFA, the generation of the analogous acetoxime ester of DHA **2** by the same methodology was proceeded with to obtain **2** in high yield (84%). The activation process for EPA and DHA is illustrated in Scheme 1.

Oxime esters of this type have certainly been prepared for various carboxylic acids and used in enzymatic reactions involving lipase (Chenevert et al., 2006; Faber, 2004), but this is the first report of such derivatives of the highly labile EPA and DHA. Previous examples include a recent report of a lipase-catalyzed acylation of secondary alcohols with oxime esters of sterically hindered carboxylic acids (Storz et al., 2010) and regioselective acylation of nucleoside alcohols with acetoxime esters (Moris and Gotor, 1994). Moreover, acetoxime esters of fatty acids including palmitic acid have indeed been reported in regioselective lipase-catalyzed esterification of 1-*O*-alkylglycerols (Aranda and Blanco, 1992). In that case the acyl-migration levels, although low (<2%), were far too high to be acceptable in the current work.

2.2. The 1,3-DAG preparation

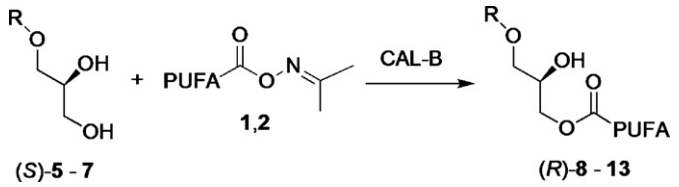
The transesterification reaction involving glycerol was performed under conditions very similar to those previously described for structured TAG synthesis using the immobilized *C. antarctica* lipase (Halldorsson et al., 2003; Magnusson and Haraldsson, 2010). Glycerol was reacted with 2.6 equiv. of the acetoxime ester (30% excess based on mole) in dry dichloromethane at 0–4 °C using 15% dose of the lipase, as based on weight of substrates, in the presence of molecular sieves (4 Å). This compares to 2.5 equiv. of the activated ester and 10% lipase dose in the previous case involving the MCFA vinyl esters, where no molecular sieves were needed (Halldorsson et al., 2003; Magnusson and Haraldsson, 2010). The lipase incorporated the acyl moieties of EPA and DHA highly regioselectively into the outer positions of glycerol. The acetoxime esters of EPA and DHA were very well tolerated as acylating substrates by the lipase and a fast reaction was brought about. The reaction is illustrated in Scheme 2.

The use of acetoxime esters was anticipated to render irreversibility to the transesterification reaction system by co-production of low nucleophilic acetoxime leaving group. Although oxime esters, including the acetoxime ester, do ensure a more or less irreversible system, they do not provide complete irreversibility as may be witnessed by reports on oximolysis reactions involving lipases (Ghogare and Kumar, 1989; Salunkhe and Nair, 2000). In this regard, the presence of molecular sieves in the current report clearly improved the catalytic process, presumably by removing to some extent the released acetoxime, thereby shifting the reaction toward completion. The excessive amount of the activated esters was needed to offset the adverse side-reactions, *i.e.* the reverse oximolysis reaction and the rapid hydrolysis of the acetoxime esters. Both processes are catalyzed by the lipase, the latter caused by the essential water (1–2% by weight) accommodated in the lipase for retaining its integrity (Dordick, 1989; Koskinen and Klibanov, 1996). The reaction temperature was maintained low at 0–4 °C to keep the acyl-migration process under control to prevent formation of undesired regioisomers.

As in previous cases, high-resolution ^{1}H NMR spectroscopy was of great use to monitor the progress of the reaction as it proceeded, and revealed that the 1,3-DAG product predominated, with only minor amounts (<1%) of the 1-monoacylglycerol (1-MAG) intermediate being noticed, throughout as the reaction proceeded. The reaction was completed after 4 h with no signs of the 1-MAG intermediate or other acylglycerol adducts being present, such as 1,2-diacylglycerol (1,2-DAG) or TAG, therefore, implying that no acyl-migration had taken place during the biocatalysis process. Finally, flash chromatography on 4% boric acid impregnated flash silica gel (Christie, 1982) to hamper known acyl-migration, induced by untreated silica gel, was employed, affording the desired 1,3-DAG **3** and **4**, respectively for EPA and DHA, chemically and regioisomerically pure as was established by high-resolution ^{1}H and ^{13}C NMR, in very high yields (85%). GC analysis of the EPA and DHA starting material and the corresponding products **3** and **4** revealed that the chemical purity of both EPA and DHA was preserved and that no degradation products were formed during these processes.

2.3. Acylation of 1-O-alkylglycerols with EPA and DHA

The most prevalent 1-*O*-alkyl-*sn*-glycerols present in shark liver oil are chimyl **5**, batyl **6** and selachyl **7** alcohols (Fig. 2), named after



Scheme 3. Acylation of 1-*O*-alkyl-*sn*-glycerols with EPA and DHA using *Candida antarctica* lipase B.

the fish species they were first isolated from, *i.e.* chimeras, rays and sharks, respectively (Magnusson and Haraldsson, 2011; Mangold and Paltauf, 1983).

The above described methodology involving the PUFA acetoximes **1** and **2** was applied on the alkylglycerols (*S*)-**5**, (*S*)-**6** and (*S*)-**7** and their antipodes (*R*)-**5**, (*R*)-**6** and (*R*)-**7**, that were prepared by a procedure of Magnusson and Haraldsson (Halldorsson et al., 2004b; Magnusson et al., 2011). In accordance with the previously reported synthesis of structured DAGE possessing MCFA at the *sn*-3 position and EPA or DHA at the *sn*-2 position (Magnusson et al., 2011) the reactions involving the solid chimyl and batyl alcohols were carried out at room temperature to speed up the reaction without affecting the regiocontrol. In these reactions higher excessive amounts of the acetoxime esters (60% as based on mole) were needed to complete the reaction as compared to the glycerol case described above. The reaction is shown in Scheme 3 for the *S*-enantiomers (for further structural details of the compounds involved see Table 1).

A complete incorporation of both EPA and DHA into the end-positions of the alkylglycerols to obtain the corresponding 3-MAGE was accomplished after 3 h and 30 min, and only traces of the unreacted alkylglycerols were noticed as was confirmed by ^{1}H NMR spectroscopy. The corresponding products (*R*)-**8**, (*R*)-**9**, (*R*)-**10**, (*R*)-**11** and (*S*)-**8**, (*S*)-**9**, (*S*)-**10**, (*S*)-**11** were obtained by means of crystallization from methanol at −40 °C in excellent yields (88–93%). For full details see Table 1 for the (*S*)-adducts and Table 2 for the (*R*)-adducts. There were no indications of the lipase displaying enantiopreference to one of the 1-*O*-alkylglycerol enantiomers over the other in these reactions. This is in agreement with a previous report on very low or no enantioselection of several lipases including the *C. antarctica* and *Rhizomucor miehei* (*vide infra*) lipases in their acylation of the primary alcohol group in racemic 1-*O*-alkylglycerols **5**, **6** and **7** (Halldorsson et al., 2004b).

When dealing with the monounsaturated liquid selachyl alcohol (*S*)-**7** and its antipode (*R*)-**7**, some modifications of the applied methodology described above for the solid chimyl and batyl adducts were necessary to secure high isolated yields. Because the products were not easily crystallizable, even at low temperature such as −40 °C, chromatography became the most viable alternative for their purification. The use of chromatography, on the other hand, became tedious and time-consuming and resulted in lower yields due to relatively high amounts of unreacted acetoxime esters left in the reaction mixture.

To avoid the use of the high excessive quantities of acetoxime esters, a more irreversible system was devised by conducting the reactions involving the selachyl alcohol without solvent under vacuum (0.01 Torr) and maintaining the reaction temperature between 25 and 30 °C. In this manner the co-produced acetoxime leaving group (b.p. 135 °C at 1 atm) was continuously removed and collected into a liquid-nitrogen cooled trap as the reaction proceeded. As a result, only 20% excess (as based on mole) of the acetoxime ester was sufficient to complete the reaction in only 1 h and 30 min. The use of that 'high' temperature (25–30 °C) warrants a special comment in that ether lipids of the DAGE type have been observed to be less prone to undergo acyl-migration under transesterification conditions with lipase as compared to TAG (Haraldsson and Thorarensen, 1994). The products (*R*)-**12**, (*R*)-**13**, (*S*)-**12** and (*S*)-**13** were obtained after flash chromatography on 4% boric acid impregnated flash silica gel in excellent yields (88–92%). Alternatively, these adducts this time allowed purification by crystallization from methanol at −40 °C.

The enantiopure 1-*O*-alkyl-*sn*-glycerol adducts monoacylated with EPA, (*R*)-**8**, (*R*)-**10** and (*R*)-**12**, and DHA, (*R*)-**9**, (*R*)-**11** and (*R*)-**13**, and their corresponding antipodes, (*S*)-**8**, (*S*)-**10** and (*S*)-**12**, and (*S*)-**9**, (*S*)-**11** and (*S*)-**13**, respectively, were afforded chemically and regioisomerically pure as was established by high-resolution ^{1}H NMR spectroscopy. GC analysis of the chimyl 3-MAGE adducts (*R*)-**8** and (*R*)-**9** revealed that no deteriorations to the chemical purity of EPA and DHA had occurred during these transformations. Tables 1 and 2 list all the 3-MAGE adducts along with their isolated yields and specific rotation values, respectively, for the (*R*)- and (*S*)-adducts.

Table 1
3-MAGE adducts (*R*)-**8**–**13** obtained from acylation of enantiopure 1-*O*-alkylglycerols (*S*)-**5**–**7** with EPA and DHA oxime esters promoted by the *Candida antarctica* lipase in accordance with Scheme 3.

Entry	R	PUFA	Yield (%)	$[\alpha]_D$
(*R*)-**8**	—$C_{16}H_{33}$	EPA	90	−3.4
(*R*)-**9**	—$C_{16}H_{33}$	DHA	91	−2.5
(*R*)-**10**	—$C_{18}H_{37}$	EPA	93	−2.8
(*R*)-**11**	—$C_{18}H_{37}$	DHA	89	−2.3
(*R*)-**12**	—$C_{18}H_{35}$	EPA	89	−2.3
(*R*)-**13**	—$C_{18}H_{35}$	DHA	92	−2.7

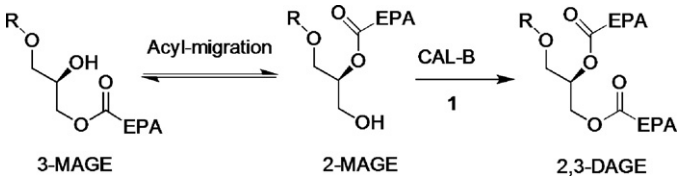
Table 2
3-MAGE adducts (*S*)-**8**–**13** obtained from acylation of enantiopure 1-*O*-alkylglycerols (*R*)-**5**–**7** with EPA and DHA oxime esters promoted by the *Candida antarctica* lipase in accordance with Scheme 3.

Entry	R	PUFA	Yield (%)	$[\alpha]_D$
(*S*)-**8**	—$C_{16}H_{33}$	EPA	90	+3.1
(*S*)-**9**	—$C_{16}H_{33}$	DHA	91	+2.5
(*S*)-**10**	—$C_{18}H_{37}$	EPA	90	+2.9
(*S*)-**11**	—$C_{18}H_{37}$	DHA	90	+2.1
(*S*)-**12**	—$C_{18}H_{35}$	EPA	88	+2.7
(*S*)-**13**	—$C_{18}H_{35}$	DHA	90	+2.4

2.4. Regiocontrol and regioisomeric purity

The successful results accomplished in the lipase reactions relate first and foremost to the high regioselectivity of the *C. antarctica* lipase, strongly preferring the primary alcohol end-position over the secondary mid-position of the glycerol moiety, but also to the absence of acyl-migration side-reactions. Acyl-migration is a thermodynamic intramolecular process, involving an acyl group being transferred to an adjacent hydroxyl group. This takes place through a cyclic five-membered hemiortho ester type intermediate, resulting in migration of the acyl group (Haraldsson et al., 1995). Besides temperature, acyl-migration is influenced by various other parameters such as reaction rate, solvent, type of reaction, type of acyl chain, pH, lipase support and water activity (Compton et al., 2007; Laszlo et al., 2008).

In the reactions described in this report the acyl-migration process was kept under complete control under the mild reaction conditions by an interplay between the fast acting lipase, activated PUFA esters and low temperature, the key parameter in suppressing the acyl-migration process (Halldorsson et al., 2003; Magnusson and Haraldsson, 2010). Such migration of a PUFA located at an end-position in 1,3-DAG to the mid-position of the glycerol backbone, would result in a 1,2-DAG regioisomer, which subsequently may be readily converted into undesired TAG upon



Scheme 4. Acyl-migration of 3-MAGE to form 2-MAGE and a subsequent acylation with EPA by *Candida antarctica* lipase B.

acylation of the resulting primary hydroxyl group in the presence of the lipase and the acyl-donor. Likewise, there is also a possibility of the 1-MAG to undergo acyl-migration to be converted into their 2-monoacylglycerol (2-MAG) regioisomers. Similarly, 3-MAGE may be converted into their 2-MAGE regioisomers through acyl-migration, and in turn converted into their corresponding unwanted 2,3-DAGE during the enzymatic catalysis. This is illustrated in Scheme 4 for the EPA oxime ester **1**.

Accordingly, it may be inferred that the absence of 2-MAG, 1,2-DAG and TAG, and the ether linked 2-MAGE and DAGE, in the enzymatic reactions, is a strong indication that no losses of regiocontrol owing to acyl-migration took place during the enzymatic catalysis. Obviously, similar arguments support the firm regiocontrol of the lipase and the lipase not acting directly on the mid-position of the glycerol moiety.

The combination of the mild reaction conditions applied and the lipase acting fast and efficiently kept the acyl-migration suppressed. Previous reports have shown such reaction conditions to be crucial to maintain a full regiocontrol in lipase catalyzed transesterification processes (Halldorsson et al., 2003; Magnusson and Haraldsson, 2010). In the current case, there were indications that the acetoxime esters did not provide complete irreversibility. To outweigh that disadvantage, several parameters needed to be adjusted, including the use of higher excessive amounts of the acylating agent, use of molecular sieves and a total removal of the acetoxime leaving group by evaporation under vacuum conditions.

When fine-tuning the reaction conditions to maximize the reaction rate of the transesterification process, while keeping the acyl-migration under control, other solvents were screened. For instance, when dichloromethane was replaced with tetrahydrofuran or toluene the acyl-migration side-product 2-MAGE was clearly noticeable. When the *C. antarctica* lipase was replaced with an immobilized *R. miehei* lipase (Lipozyme RM IM from Novozyme) under identical conditions, the conversion rate of chimyl alcohol (*S*)-**5** to (*R*)-**8** with the EPA oxime ester dropped to only 10% after 5 h. This implies that the acetoxime ester of EPA is far worse tolerated as an acylating substrate by that lipase as compared to the *C. antarctica* lipase. It should, however, be added that the *C. antarctica* lipase has been observed to display much higher tolerance for EPA and DHA than the *R. miehei* lipase that also discriminates strongly against DHA (Halldorsson et al., 2004a; Haraldsson and Hjaltason, 2006).

High-resolution ^{1}H NMR spectroscopy served as a pivotal tool for monitoring in details the progress of the reactions as they proceeded and evaluating their regioselectivity and regiocontrol in a similar manner as was described in the previous structured TAG (Halldorsson et al., 2003; Haraldsson et al., 1995; Magnusson and Haraldsson, 2010) and DAGE (Magnusson et al., 2011) syntheses. In the previous studies the limit of quantification for possible acyl-migration products as detected by 400 MHz ^{1}H NMR spectroscopy was determined on freshly prepared and accurately weighed standards. The results indicate that the levels of such acyl-migration products can be accurately quantified down to 0.25 mol % for practical sample concentration levels with the limit of detection well below that (Magnusson et al., 2011; Magnusson and Haraldsson, 2010). It is quite reasonable to estimate the degree of detection in the current report to remain close to that in the previous studies. Therefore, regiopurity levels of the minimum of 99.9% can be confidently asserted for both 1,3-DAG and 3-MAGE in the current work.

3. Conclusion

A convenient enzymatic approach offering high efficiency has been developed for inserting for the first time the bioactive long-chain n-3 polyunsaturated fatty acids EPA and DHA exclusively into the end-positions of glycerol and enantiomerically pure 1-*O*-alkylglycerols. This methodology has enabled generation of novel and valuable positionally labeled acylglycerol adducts such as 1,3-DAG and the monoacylated ether linked 1-*O*-alkyl-3-acyl-*sn*-glycerols of chimyl, batyl and selachyl alcohols, and their corresponding antipodes, constituting EPA or DHA. The symmetric 1,3-DAG possessing EPA and DHA may offer high potential in the design and development of lipid prodrugs. Along with their various types of 'reversely' structured TAG products they may find numerous applications as fine chemicals for analytical, therapeutic and pharmaceutical purposes. Likewise, the 1-*O*-alkyl-3-acylglycerols possessing EPA and DHA are interesting compounds whose properties remain to be explored. They can serve as scaffolds for drugs and may therefore become important intermediates in the development of prodrugs.

4. Experimental

4.1. General

^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded on a Bruker Avance 400 spectrometer in deuterated chloroform as a solvent at 400.12 and 100.61 MHz, respectively. Chemical shifts (δ) are quoted in parts per million (ppm) and the coupling constants (J) in Hertz (Hz). The following abbreviations are used to describe the multiplicity: s, singlet; d, doublet; t, triplet; qr, quartet; qn, quintet; 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 ^{13}C assignments stated for the carbons belonging to the glyceryl backbone are based on conformation from previous work for related compounds (Magnusson et al., 2011). All Infrared spectra were conducted on a FT-IR (E.S.P.) Spectrophotometer on a ZnSe plate and on KBr pellets for neat liquid and solid compounds respectively. The optical activities were measured on an Autopol V. The high-resolution mass spectra (HRMS) were acquired on a micrOTOF-Q mass spectrometer equipped with an atmospheric pressure chemical ionization chamber (APCI). Fatty acid analyses were performed on methyl esters employing a Perkin Elmer Clarus 400 gas chromatograph using a 30-m capillary column, DB-225 30 N 0.25 mm (J&W Scientific, Folsom, CA) with hydrogen as a carrier gas according to our previously described procedure (Haraldsson and Almarsson, 1991).

The immobilized *C. antarctica* lipase (Novozym 435; CAL-B) and the immobilized *R. miehei* lipase (Lipozyme RM IM) were supplied as a gift from Novozyme A/S (Bagsvaerd, Denmark). All chemicals and solvents were used without further purification unless otherwise stated. (*S*)-Solketal, ((+)-1,2-*O*-isopropylidene-*sn*-glycerol; 98%, 99% ee) and commercially grade EDAC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) were obtained from Sigma–Aldrich (Steinheim, Germany) and 4-dimethylaminopyridine (DMAP, 99%) and acetone oxime (98%) from Acros Organics (Geel, Belgium). EPA (98%) and DHA (≥95%) were obtained as ethyl esters from Pronova Biocare (Sandefjord, Norway) and were hydrolyzed to their corresponding free acids (Haraldsson et al., 2000). Dichloromethane (dried over CaH_2),

ethyl acetate and benzene were all obtained HPLC grade from Sigma–Aldrich (Steinheim, Germany). Petroleum ether, boiling range 40–60 °C, was obtained as p.a. from Riedel-de Haën (Seelze, Germany). Silica gel (230–400 mesh, 60 Å) and preparative TLC plates (250 μm, F-254) were obtained from Silicycle (Quebec, Canada).

4.2. Eicosapentaenoic acid acetoxime ester **1**

To a solution of eicosapentaenoic acid (EPA) (1.0 g, 3.31 mmol), DMAP (81 mg, 0.66 mmol) and EDAC (761 mg, 3.97 mmol) in dichloromethane (10 ml), acetoxime (242 mg, 3.31 mmol) was added and the resulting solution stirred for 3–4 h. The reaction solution was passed through a short silica gel column with ethyl acetate/petroleum ether (60:40) as an eluent, affording the product **1** as a yellowish oil after evaporation of the solvents (1.0 g, 85% yield). IR (ZnSe) 3012 (=C—H *cis*), 1760 (C=O), 1652 (C=C) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{23}H_{35}NO_2$ + H: 358.2741; found 358.2752 amu. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44–5.28 (m, 10 H, =CH), 2.88–2.77 (m, 8 H, =CCH_2C=), 2.42 (t, J = 7.5 Hz, 2 H, CH_2COO), 2.18–2.13 (m, 2 H, =CCH_2CH$_2$), 2.11–2.04 (m, 2 H, =CCH_2CH$_3$), 2.04 (s, 3 H, N=C(CH_3)$_2$), 1.98 (s, 3 H, N=C(CH_3)$_2$), 1.78 (qn, J = 7.5 Hz, 2 H, CH_2CH$_2$COO), 0.97 (t, J = 7.5 Hz, 3 H, CH$_2$CH_3) ppm; ^{13}C NMR ($CDCl_3$) δ 170.9 (*C*=O), 163.6 (*C*=N), 132.0, 129.0, 128.8, 128.5, 128.24, 128.17, 128.13, 128.06, 127.8, 127.0, 32.4, 26.56, 25.61 (3), 25.5, 24.8, 22.0, 20.5, 16.9, 14.2 ppm.

4.3. Docosahexaenoic acid acetoxime ester **2**

A procedure identical to that described above for the EPA acetoxime ester **1** was followed using docosahexaenoic acid (DHA) (2.0 g, 6.09 mmol), DMAP (149 mg, 1.22 mmol), EDAC (1.4 g, 7.31 mmol), dichloromethane (15 ml) and acetoxime (467 mg, 6.39 mmol). The product **2** was afforded as a yellowish oil after evaporation of the solvents (1.96 g, 84% yield). IR (ZnSe) 3012 (=C—H *cis*), 1760 (C=O), 1651 (C=C) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{25}H_{37}NO_2$ + H: 384.2897; found 384.2902 amu. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46–5.27 (m, 12 H, =CH), 2.88–2.78 (m, 10 H, =CCH_2C=), 2.49–2.44 (m, 4 H, CH_2CH$_2$COO), 2.11–2.04 (m, 2 H, =CCH_2CH$_3$), 2.04 (s, 3 H, N=C(CH_3)$_2$), 1.99 (s, 3 H, N=C(CH_3)$_2$), 0.97 (t, J = 7.5 Hz, 3 H, CH$_2$CH_3) ppm; ^{13}C NMR ($CDCl_3$) δ 170.5 (C=O), 163.7 (*C*=N), 132.0, 129.5, 128.5, 128.23, 128.22, 128.20, 128.05, 128.03, 127.99, 127.8, 127.6, 127.0, 32.9, 25.60 (3), 25.57, 25.5, 22.7, 21.9, 20.5, 16.9, 14.2 ppm.

4.4. 1,3-Dieicosapentaenoylglycerol **3**

To a mixture of glycerol (50 mg, 0.543 mmol), the EPA acetoxime ester derivative **1** (505 mg, 1.41 mmol) and 4 Å molecular sieves (350 mg) in dry dichloromethane (2 ml), immobilized *C. antarctica* lipase (83 mg) was added and the resulting mixture stirred for 4 h at 0–4 °C under nitrogen atmosphere. The lipase was separated off by filtration, the solvent removed *in vacuo* and the residue applied to a 4% boric acid impregnated flash silica gel column with chloroform/ethyl acetate (95:5) as an eluent, affording the product as a clear oil (305 mg, 85% yield); IR (ZnSe) 3465 (O—H), 3012 (=C—H *cis*), 1739 (C=O), 1654 (C=C) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{43}H_{64}O_5$ + H: 661.4827; found 661.4814 amu. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44–5.28 (m, 20 H, =CH), 4.19 (dd, J = 11.4, 4.3 Hz, 2 H, CH_2CHCH_2), 4.13 (dd, J = 11.4, 5.6 Hz, 2 H, CH_2CHCH_2), 4.12–4.04 (m, 1 H, CH$_2$C*H*CH$_2$), 2.88–2.77 (m, 16 H, =CCH_2C=), 2.39 (d, J = 7.0 Hz, 1 H, CHO*H*), 2.37 (t, J = 7.6 Hz, 4 H, CH_2COO), 2.15–2.04 (m, 8 H, =CCH_2CH$_2$ and =CCH_2CH$_3$), 1.72 (qn, J = 7.5 Hz, 4 H, CH_2CH$_2$COO), 0.97 (t, J = 7.5 Hz, 6 H, CH_3) ppm; ^{13}C NMR ($CDCl_3$) δ 173.6 (2) (*C*=O), 132.0 (2), 129.0 (2), 128.8 (2), 128.6 (2), 128.3 (2), 128.2 (2), 128.14 (2), 128.06 (2), 127.9 (2), 127.0 (2), 68.4 (CH$_2$*C*HCH$_2$), 65.1 (2) (*C*H$_2$CHCH$_2$), 33.4 (2), 26.5 (2), 25.6 (6), 25.5 (2), 24.7 (2), 20.6 (2), 14.3 (2) ppm.

4.5. 1,3-Didocosahexaenoylglycerol **4**

A procedure identical to that described above for **3** was followed using glycerol (70 mg, 0.760 mmol), DHA acetoxime ester **2** (758 mg, 1.98 mmol), 4 Å molecular sieves (500 mg), dry dichloromethane (2 ml) and immobilized *C. antarctica* lipase (124 mg) was added and the mixture stirred for 4 h at 0–4 °C under nitrogen atmosphere. The product **4** was afforded as a clear oil (460 mg, 85% yield); IR (ZnSe) 3465 (O—H), 3012 (=C—H *cis*), 1739 (C=O), 1653 (C=C) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{47}H_{68}O_5$ + H: 713.5140; found 713.5143 amu. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46–5.28 (m, 24 H, =CH), 4.19 (dd, J = 11.4, 4.3 Hz, 2 H, CH_2CHCH_2), 4.13 (dd, J = 11.4, 5.7 Hz, 2 H, CH_2CHCH_2), 4.13–4.04 (m, 1 H, CH$_2$C*H*CH$_2$), 2.88–2.78 (m, 20 H, =CCH_2C=) 2.44–2.37 (m, 8 H, CH_2CH$_2$COO), 2.42–2.39 (m, 1 H, CHO*H*), 2.11–2.04 (m, 4 H, =CCH_2CH$_3$), 0.97 (t, J = 7.5 Hz, 6 H, CH_3) ppm; ^{13}C NMR ($CDCl_3$) δ 173.1 (2) (*C*=O), 132.0 (2), 129.5 (2), 128.6 (2), 128.33 (2), 128.27 (2), 128.25 (2), 128.07 (2), 128.06 (2), 128.0 (2), 127.9 (2), 127.7 (2), 127.0 (2), 68.3 (CH$_2$*C*HCH$_2$), 65.2 (2) (*C*H$_2$CHCH_2), 34.0 (2), 25.63 (4), 25.62 (2), 25.6 (2), 25.5 (2), 22.7 (2), 20.6 (2), 14.27 (2) ppm.

4.6. 3-O-Hexadecyl-sn-glycerol (R)-**5**

A procedure identical to that recently described for preparation of chimyl alcohol (*S*)-**5** (Magnusson et al., 2011) was followed using (*S*)-solketal (1.50 g, 11.3 mmol), 1-bromohexadecane (3.50 g, 11.3 mmol), tetra-*n*-butylammonium bromide (728 mg, 2.26 mmol), freshly grounded potassium hydroxide (1.60 g, 28.3 mmol) and *p*-toluenesulfonic acid (160 mg). The product was afforded as lustrous crystals (2.90 g, 80% yield). $[\alpha]_D^{20}$ −1.7 (*c* 4.96, chloroform); m.p. 65.4–66.1 °C. Its ^{1}H and ^{13}C NMR and IR spectroscopy data were identical to those obtained for (*S*)-**5**.

4.7. 3-O-Octadecyl-sn-glycerol (R)-**6**

A procedure identical to that recently described for preparation of batyl alcohol (*S*)-**6** (Magnusson et al., 2011) was followed using (*S*)-solketal (2.00 g, 15.0 mmol), 1-bromooctadecane (5.00 g, 15.0 mmol) and tetra-*n*-butylammonium bromide (1.00 g, 2.40 mmol), freshly grounded potassium hydroxide (2.10 g, 37.5 mmol) and *p*-toluenesulfonic acid (200 mg). The product was afforded as lustrous crystals (4.10 g, 77% yield). $[\alpha]_D^{20}$ −1.8 (*c* 4.71, chloroform); m.p. 70.3–71.0 °C. Its ^{1}H and ^{13}C NMR and IR spectroscopy data were identical to those obtained for (*S*)-**6**.

4.8. 3-O-cis-Octadec-9-enyl-sn-glycerol (R)-**7**

A procedure identical to that recently described for preparation of selachyl alcohol (*S*)-**7** (Magnusson et al., 2011) was followed using (*S*)-solketal (2.00 g, 15.1 mmol), *cis*-1-bromooctadec-9-ene (5.00 g, 15.1 mmol), tetra-*n*-butylammonium bromide (974 mg, 3.02 mmol), freshly grounded potassium hydroxide (2.12 g, 37.8 mmol) and *p*-toluenesulfonic acid (200 mg). The product was afforded as a clear viscous oil (4.0 g, 78% yield). $[\alpha]_D^{20}$ −1.4 (*c* 6.48, chloroform). Its ^{1}H and ^{13}C NMR and IR spectroscopy data were identical to those obtained for (*S*)-**7**.

4.9. 1-O-Hexadecyl-3-eicosapentaenoyl-sn-glycerol (R)-**8**

To a mixture of (*S*)-1-*O*-hexadecyl-*sn*-glycerol (*S*)-**5** (200 mg, 0.63 mmol), immobilized *C. antarctica* lipase (50 mg) and 4 Å molecular sieves (340 mg) in dry dichloromethane (6 ml), EPA acetoxime ester **1** (226 mg, 0.63 mmol) was added and the resulting mixture

stirred until the alcohol had completely dissolved. Then, additional EPA acetoxime ester (136 mg, 0.38 mmol) and lipase (34 mg) were added and the reaction mixture stirred for 3 h and 30 min at room temperature. The lipase was separated off by filtration, the solvent removed *in vacuo*, and the residue introduced to crystallization in methanol at −40 °C, affording the product as a white solid which melted into a clear oil when allowed to reach room temperature (342 mg, 90% yield). $[\alpha]_D^{20}$ −3.4 (*c* 1.00, benzene); IR (ZnSe) 3460 (O—H), 3012 (=C—H *cis*), 2923 (C—H), 1739 (C=O), 1653 (C=C), 1119 (C—O—C) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{39}H_{68}O_4$ + H: 601.5190; found 601.5188 amu. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44–5.28 (m, 10 H, =CH), 4.18 (dd, *J* = 11.5, 4.4 Hz, 1 H, C*H*$_2$OCO), 4.12 (dd, *J* = 11.5, 6.2 Hz, 1 H, C*H*$_2$OCO), 4.02–3.96 (m, 1 H, CH_2CHCH_2), 3.49 (dd, *J* = 9.7, 4.3 Hz, 1 H, CHC*H*$_2$O), 3.50–3.41 (2xdt, 9.2, 6.2 Hz, 2 H, OC*H*$_2$CH$_2$), 3.42 (dd, *J* = 9.7, 6.3 Hz, 1 H, CHC*H*$_2$O), 2.88–2.77 (m, 8 H, =CC*H*$_2$C=), 2.51–2.40 (s (br), 1 H, CHO*H*), 2.36 (t, *J* = 7.6 Hz, 2 H, C*H*$_2$COO), 2.15–2.04 (m, 4 H, =CC*H*$_2$CH$_2$ and =CC*H*$_2$CH$_3$ in EPA), 1.72 (qn, *J* = 7.5 Hz, 2 H, C*H*$_2$CH$_2$COO), 1.57 (qn (br), *J* = 6.9 Hz, 2 H, OCH$_2$C*H*$_2$), 1.37–1.20 (m, 26 H, C*H*$_2$), 0.98 (t, *J* = 7.5 Hz, 3 H, C*H*$_3$ in EPA), 0.88 (br t, *J* = 6.9 Hz, 3 H, C*H*$_3$ in 1-*O*-alkyl) ppm; ^{13}C NMR ($CDCl_3$) δ 173.7 (*C*=O), 132.0, 128.89, 128.87, 128.6, 128.3, 128.19, 128.16, 128.1, 127.9, 127.0, 71.8 (O*C*H$_2$CH$_2$), 71.4 (CH*C*H$_2$O), 68.8 (CH$_2$*C*HCH$_2$), 65.5 (*C*H$_2$OCO), 33.5, 31.9, 29.69 (3), 29.68, 29.67, 29.66, 29.62, 29.59 (2), 29.5, 29.4, 26.5, 26.1, 25.6 (3), 25.5, 24.8, 22.7, 20.6, 14.3, 14.1 ppm.

4.10. 3-O-Hexadecyl-1-eicosapentaenoyl-sn-glycerol (S)-*8*

A procedure identical to that described above for (*R*)-**8** was followed using (*R*)-3-*O*-hexadecyl-*sn*-glycerol (*R*)-**5** (100 mg, 0.32 mmol), immobilized *C. antarctica* lipase (25 mg), 4 Å molecular sieves (170 mg), dry dichloromethane (3 ml), EPA acetoxime ester **1** (114 mg, 0.32 mmol), additional acetoxime ester (69 mg, 0.19 mmol) and additional lipase (17 mg). The product (*S*)-**8** was afforded as a clear oil (173 mg, 90% yield). $[\alpha]_D^{20}$ +3.1 (*c* 1.00, benzene); HRMS (APCI): *m/z* calcd for $C_{39}H_{68}O_4$ + H: 601.5190; found 601.5199 amu. ^{1}H and ^{13}C NMR and IR spectroscopy data were identical to those obtained for (*R*)-**8**.

4.11. 1-O-Hexadecyl-3-docosahexaenoyl-sn-glycerol (R)-*9*

A procedure identical to that described above for (*R*)-**8** was followed using (*S*)-1-*O*-hexadecyl-*sn*-glycerol (***S***)-**5** (150 mg, 0.47 mmol), immobilized *C. antarctica* lipase (40 mg), 4 Å molecular sieves (260 mg), dry dichloromethane (4.5 ml), DHA acetoxime ester **2** (182 mg, 0.47 mmol), additional acetoxime ester (109 mg, 0.28 mmol) and additional lipase (26 mg). The product (*R*)-**9** was afforded as a clear oil (270 mg, 91% yield). $[\alpha]_D^{20}$ −2.5 (*c* 1.00, benzene); IR (ZnSe) 3460 (O—H), 3014 (=C—H *cis*), 2923 (C—H), 1740 (C=O), 1654 (C=C), 1117 (C—O—C) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{41}H_{70}O_4$ + H: 627.5347; found 627.5349 amu. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45–5.28 (m, 12 H, =CH), 4.18 (dd, *J* = 11.5, 4.4 Hz, 1 H, C*H*$_2$OCO), 4.12 (dd, *J* = 11.5, 6.2 Hz, 1 H, C*H*$_2$OCO), 4.02–3.96 (m, 1 H, CH_2CHCH_2), 3.49 (dd, *J* = 9.7, 4.3 Hz, 1 H, CHC*H*$_2$O), 3.49–3.41 (2xdt, 9.1, 6.1 Hz, 2 H, OC*H*$_2$CH$_2$), 3.42 (dd, *J* = 9.7, 6.3 Hz, 1 H, CHC*H*$_2$O), 2.91–2.77 (m, 10 H, =CC*H*$_2$C=), 2.43–2.38 (m, 4 H, C*H*$_2$CH$_2$COO), 2.08 (qn (br), *J* = 7.4 Hz, 2 H, =CC*H*$_2$CH$_3$), 1.57 (qn (br), *J* = 6.9 Hz, 2 H, OCH$_2$C*H*$_2$), 1.37–1.18 (m, 26 H, C*H*$_2$), 0.97 (t, *J* = 7.5 Hz, 3 H, C*H*$_3$ in DHA), 0.88 (br t, *J* = 6.8 Hz, 3 H, C*H*$_3$ in 1-*O*-alkyl) ppm; ^{13}C NMR ($CDCl_3$) δ 173.2 (C=O), 132.0, 129.4, 128.6, 128.29, 128.27, 128.24, 128.09, 128.07, 128.0, 127.9, 127.8, 127.0, 71.8 (O*C*H$_2$CH$_2$), 71.4 (CH*C*H$_2$O), 68.8 (CH$_2$*C*HCH$_2$), 65.6 (*C*H$_2$OCO), 34.0, 31.9, 29.69 (3), 29.68, 29.67 (2), 29.62, 29.59 (2), 29.5, 29.4, 26.1, 25.64 (3), 25.60, 25.5, 22.74, 22.69, 20.6, 14.3, 14.1 ppm.

4.12. 3-O-Hexadecyl-1-docosahexaenoyl-sn-glycerol (S)-*9*

A procedure identical to that described above for (*R*)-**8** was followed using (*R*)-3-*O*-hexadecyl-*sn*-glycerol (*R*)-**5** (150 mg, 0.47 mmol), immobilized *C. antarctica* lipase (40 mg), 4 Å molecular sieves (260 mg), dry dichloromethane (4.5 ml), DHA acetoxime ester **2** (182 mg, 0.47 mmol), additional acetoxime ester (109 mg, 0.28 mmol) and additional lipase (26 mg). The product (*S*)-**9** was afforded as a clear oil (270 mg, 91% yield). $[\alpha]_D^{20}$ +2.5 (*c* 1.00, benzene); HRMS (APCI): *m/z* calcd for $C_{41}H_{70}O_4$ + H: 627.5347; found 627.5350 amu. ^{1}H and ^{13}C NMR and IR spectroscopy data were identical to those obtained for (*R*)-**9**.

4.13. 1-O-Octadecyl-3-eicosapentaenoyl-sn-glycerol (R)-*10*

A procedure identical to that described above for (*R*)-**8** was followed using (*S*)-1-*O*-octadecyl-*sn*-glycerol (*S*)-**6** (250 mg, 0.73 mmol), immobilized *C. antarctica* lipase (60 mg), 4 Å molecular sieves (400 mg), dry dichloromethane (7 ml), EPA acetoxime ester **1** (260 mg, 0.73 mmol), additional acetoxime ester (156 mg, 0.436 mmol) and additional lipase (40 mg). The product (*R*)-**10** was afforded as a clear oil (425 mg, 93% yield). $[\alpha]_D^{20}$ −2.8 (*c* 1.01, benzene); IR (ZnSe) 3458 (O—H), 3013 (=C—H *cis*), 2922 (C—H), 1739 (C=O), 1653 (C=C), 1120 (C—O—C) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{41}H_{72}O_4$ + H: 629.5503; found 629.5533 amu. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44–5.28 (m, 10 H, =CH), 4.18 (dd, *J* = 11.5, 4.4 Hz, 1 H, C*H*$_2$OCO), 4.12 (dd, *J* = 11.5, 6.2 Hz, 1 H, C*H*$_2$OCO), 4.02–3.96 (m, 1 H, CH_2CHCH_2), 3.49 (dd, *J* = 9.7, 4.3 Hz, 1 H, CHC*H*$_2$O), 3.50–3.41 (2xdt, 9.1, 6.3 Hz, 2 H, OC*H*$_2$CH$_2$), 3.42 (dd, *J* = 9.7, 6.3 Hz, 1 H, CHC*H*$_2$O), 2.89–2.77 (m, 8 H, =CC*H*$_2$C=), 2.36 (t, *J* = 7.6 Hz, 2 H, C*H*$_2$COO), 2.15–2.04 (m, 4 H, =CC*H*$_2$CH$_2$ and =CC*H*$_2$CH$_3$ in EPA), 1.72 (qn, *J* = 7.5 Hz, 2 H, C*H*$_2$CH$_2$COO), 1.57 (qn (br), *J* = 6.9 Hz, 2 H, OCH$_2$C*H*$_2$), 1.37–1.20 (m, 30 H, C*H*$_2$), 0.97 (t, *J* = 7.5 Hz, 3 H, C*H*$_3$ in EPA), 0.88 (br t, *J* = 6.9 Hz, 3 H, C*H*$_3$ in 1-*O*-alkyl) ppm; ^{13}C NMR ($CDCl_3$) δ 173.7 (*C*=O), 132.0, 128.90, 128.87, 128.6, 128.3, 128.19, 128.17, 128.1, 127.9, 127.0, 71.8 (O*C*H$_2$CH$_2$), 71.4 (CH*C*H$_2$O), 68.8 (CH$_2$*C*HCH$_2$), 65.5 (*C*H$_2$OCO), 33.5, 31.9, 29.70 (6), 29.67, 29.66, 29.63, 29.60, 29.58, 29.5, 29.4, 26.5, 26.1, 25.64 (3), 25.55, 24.8, 22.7, 20.6, 14.3, 14.1 ppm.

4.14. 3-O-Octadecyl-1-eicosapentaenoyl-sn-glycerol (S)-*10*

A procedure identical to that described above for (*R*)-**8** was followed using (*R*)-3-*O*-octadecyl-*sn*-glycerol (*R*)-**6** (100 mg, 0.29 mmol), immobilized *C. antarctica* lipase (25 mg), 4 Å molecular sieves (160 mg), dry dichloromethane (3 ml), EPA acetoxime ester **1** (115 mg, 0.322 mmol), additional acetoxime ester (55 mg, 0.154 mmol) and additional lipase (15 mg). The product was afforded as a clear oil at room temperature (165 mg, 90% yield). $[\alpha]_D^{20}$ +2.9 (*c* 0.85, benzene); HRMS (APCI): *m/z* calcd for $C_{41}H_{72}O_4$ + H: 629.5503; found 629.5496 amu. ^{1}H and ^{13}C NMR and IR spectroscopy data were identical to those obtained for (*R*)-**10**.

4.15. 1-O-Octadecyl-3-docosahexaenoyl-sn-glycerol (R)-*11*

A procedure identical to that described above for (*R*)-**8** was followed using (*S*)-1-*O*-octadecyl-*sn*-glycerol (*S*)-**6** (200 mg, 0.58 mmol), immobilized *C. antarctica* lipase (50 mg), 4 Å molecular sieves (330 mg), dry dichloromethane (6 ml), DHA acetoxime ester **2** (223 mg, 0.58 mmol), additional acetoxime ester (134 mg, 0.35 mmol) and additional lipase (30 mg). The product was afforded as a clear oil at room temperature (340 mg, 89% yield). $[\alpha]_D^{20}$ −2.3 (*c* 1.00, benzene); IR (ZnSe) 3460 (O—H), 3013 (=C—H *cis*), 2923 (C—H), 1740 (C=O), 1653 (C=C), 1119 (C—O—C) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{43}H_{74}O_4$ + H: 655.5660; found 655.5686 amu. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45–5.28 (m, 12 H, =CH), 4.18

(dd, J = 11.5, 4.4 Hz, 1 H, CH_2OCO), 4.12 (dd, J = 11.5, 6.2 Hz, 1 H, CH_2OCO), 4.02–3.96 (m, 1 H, CH$_2$CHCH$_2$), 3.49 (dd, J = 9.6, 4.3 Hz, 1 H, CHCH_2O), 3.50–3.41 (2xdt, 9.2, 6.2 Hz, 2 H, OCH_2CH$_2$), 3.42 (dd, J = 9.7, 6.3 Hz, 1 H, CHCH_2O), 2.90–2.78 (m, 10 H, =CCH_2C=), 2.43–2.38 (m, 4 H, CH_2CH_2COO), 2.08 (qn (br), J = 7.4 Hz, 2 H, =CCH_2CH$_3$), 1.57 (qn (br), J = 6.9 Hz, 2 H, OCH$_2$CH_2), 1.37–1.19 (m, 30 H, CH_2), 0.97 (t, J = 7.5 Hz, 3 H, CH_3 in DHA), 0.88 (br t, J = 6.8 Hz, 3 H, CH_3 in 1-*O*-alkyl) ppm; ^{13}C NMR (CDCl$_3$) δ 173.2 (C=O), 132.0, 129.4, 128.6, 128.29, 128.27, 128.24, 128.10, 128.07, 128.0, 127.9, 127.8, 127.0, 71.8 (OCH$_2$CH$_2$), 71.4 (CHCH$_2$O), 68.8 (CH$_2$CHCH$_2$), 65.6 (CH$_2$OCO), 34.0, 31.9, 29.70 (6), 29.67, 29.66, 29.62, 29.60, 29.58, 29.5, 29.4, 26.1, 25.64 (3), 25.60, 25.5, 22.74, 22.69, 20.6, 14.3, 14.1 ppm.

4.16. 3-*O*-Octadecyl-1-docosahexaenoyl-sn-glycerol (*S*)-**11**

A procedure identical to that described above for (*R*)-**8** was followed using (*R*)-3-*O*-octadecyl-*sn*-glycerol (*R*)-**6** (200 mg, 0.58 mmol), immobilized *C. antarctica* lipase (50 mg), 4 Å molecular sieves (330 mg), dry dichloromethane (6 ml), DHA acetoxime ester **2** (223 mg, 0.58 mmol), additional acetoxime ester (134 mg, 0.35 mmol) and additional lipase (30 mg). The product was afforded as a clear oil at room temperature (347 mg, 90% yield). $[\alpha]_D^{20}$ +2.1 (*c* 0.99, benzene); HRMS (APCI): *m/z* calcd for $C_{43}H_{74}O_4$ + H: 655.5660; found 655.5674 amu. ^{1}H and ^{13}C NMR and IR spectroscopy data were identical to those obtained for (*R*)-**11**.

4.17. 1-*O*-cis-Octadec-9-enyl-3-eicosapentaenoyl-sn-glycerol (*R*)-**12**

To a mixture of (*S*)-1-*O*-*cis*-octadec-9-enyl-*sn*-glycerol (*S*)-**7** (150 mg, 0.438 mmol), EPA acetoxime ester **1** (188 mg, 0.526 mmol) in a 10 ml flask, immobilized *C. antarctica* lipase (51 mg) was added, the flask connected to an oil vacuum pump system (10^{-2} mmHg) and the resulting mixture stirred over a hot plate at 25–30 °C for 1 h and 30 min. Then, the vacuum was disconnected, dichloromethane added and the lipase separated off by filtration. After solvent evaporation the crude residue was applied to a 4% boric acid impregnated flash silica gel column with gradient elution of petroleum ether/ethyl acetate (90:10 and 80:20) affording the product (*R*)-**12** as a clear oil (245 mg, 89% yield). $[\alpha]_D^{20}$ −2.3 (*c* 1.28, benzene); IR (ZnSe) 3465 (O—H), 3012 (=C—H *cis*), 2925 (C—H), 1739 (C=O), 1654 (C=C), 1120 (C—O—C) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{41}H_{70}O_4$ + H: 627.5347; found 627.5363 amu. ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.44–5.28 (m, 12 H, =CH), 4.17 (dd, J = 11.5, 4.4 Hz, 1 H, CH_2OCO), 4.12 (dd, J = 11.5, 6.2 Hz, 1 H, CH_2OCO), 4.02–3.96 (m, 1 H, CH$_2$CHCH$_2$), 3.49 (dd, J = 9.7, 4.3 Hz, 1 H, CHCH_2O), 3.50–3.41 (2xdt, 9.2, 6.2 Hz, 2 H, OCH_2CH$_2$), 3.41 (dd, J = 9.7, 6.3 Hz, 1 H, CHCH_2O), 2.88–2.77 (m, 8 H, =CCH_2C=), 2.36 (t, J = 7.6 Hz, 2 H, CH_2COO), 2.14–2.04 (m, 4 H, =CCH_2CH$_2$ and =CCH_2CH$_3$ in EPA), 2.01 (qr (br), J = 6.5 Hz, 4 H, =CHCH_2 in 1-*O*-alkyl), 1.72 (qn, J = 7.5 Hz, 2 H, CH_2CH$_2$COO), 1.57 (qn (br), J = 6.9 Hz, 2 H, OCH$_2$CH_2), 1.39–1.21 (m, 22 H, CH_2), 0.97 (t, J = 7.5 Hz, 3 H, CH_3 in EPA), 0.88 (t (br), J = 6.9 Hz, 3 H, CH_3 in 1-*O*-alkyl) ppm; ^{13}C NMR (CDCl$_3$) δ 173.7 (*C*=O), 132.0, 129.9, 129.8, 128.89, 128.86, 128.6, 128.3, 128.18, 128.15, 128.1, 127.9, 127.0, 71.8 (OCH$_2$CH$_2$), 71.4 (CHCH$_2$O), 68.8 (CH$_2$CHCH$_2$), 65.5 (CH$_2$OCO), 33.5, 31.9, 29.76, 29.75, 29.6, 29.52, 29.48, 29.4, 29.32 (2), 29.26, 27.21, 27.20, 26.5, 26.1, 25.6 (3), 25.5, 24.8, 22.7, 20.6, 14.3, 14.1 ppm.

4.18. 3-*O*-cis-Octadec-9-enyl-1-eicosapentaenoyl-sn-glycerol (*S*)-**12**

A procedure identical to that described above for (*R*)-**12** was followed using (*R*)-3-*O*-*cis*-octadec-9-enyl-*sn*-glycerol (*R*)-**7** (150 mg, 0.438 mmol), EPA acetoxime ester **1** (188 mg, 0.526 mmol) and immobilized *C. antarctica* lipase (51 mg). The product (*S*)-**12** was afforded as a clear oil (242 mg, 88% yield). $[\alpha]_D^{20}$ +2.7 (*c* 1.32, benzene); HRMS (APCI): *m/z* calcd for $C_{41}H_{70}O_4$ + H: 627.5347; found 627.5366 amu. ^{1}H and ^{13}C NMR and IR spectroscopy data were identical to those obtained for (*R*)-**12**.

4.19. 1-*O*-cis-Octadec-9-enyl-3-docosahexaenoyl-sn-glycerol (*R*)-**13**

A procedure identical to that described above for (*R*)-**12** was followed using (*S*)-1-*O*-*cis*-octadec-9-enyl-*sn*-glycerol (*S*)-**7** (200 mg, 0.584 mmol), DHA acetoxime ester **2** (269 mg, 0.701 mmol) and immobilized *C. antarctica* lipase (70 mg). The product (*R*)-**13** was afforded as a clear oil (350 mg, 92% yield). $[\alpha]_D^{20}$ −2.7 (*c* 1.10, benzene); IR (ZnSe) 3475 (O—H), 3012 (=C—H *cis*), 2923 (C—H), 1740 (C=O), 1655 (C=C), 1121 (C—O—C) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{43}H_{72}O_4$ + H: 653.5503; found 653.5495 amu. ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.45–5.28 (m, 14 H, =CH), 4.18 (dd, J = 11.5, 4.4 Hz, 1 H, CH_2OCO), 4.12 (dd, J = 11.5, 6.2 Hz, 1 H, CH_2OCO), 4.02–3.97 (m, 1 H, CH$_2$CHCH$_2$), 3.49 (dd, J = 9.6, 4.3 Hz, 1 H, CHCH_2O), 3.50–3.41 (2xdt, 9.1, 6.2 Hz, 2 H, OCH_2CH$_2$), 3.42 (dd, J = 9.7, 6.3 Hz, 1 H, CHCH_2O), 2.88–2.77 (m, 10 H, =CCH_2C=), 2.43–2.39 (m, 4 H, CH_2CH_2COO), 2.11–2.04 (m, 2 H, =CCH_2CH$_3$), 2.01 (qr (br), J = 6.4 Hz, 4 H, =CHCH_2 in 1-*O*-alkyl), 1.57 (qn (br), J = 6.9 Hz, 2 H, OCH$_2$CH_2), 1.38–1.22 (m, 22 H, CH_2), 0.97 (t, J = 7.5 Hz, 3 H, CH_3 in DHA), 0.88 (br t, J = 6.9 Hz, 3 H, CH_3 in 1-*O*-alkyl) ppm; ^{13}C NMR (CDCl$_3$) δ 173.2 (C=O), 132.0, 130.0, 129.8, 129.4, 128.6, 128.29, 128.27, 128.2, 128.10, 128.08, 128.0, 127.9, 127.8, 127.0, 71.8 (OCH$_2$CH$_2$), 71.4 (CHCH$_2$O), 68.8 (CH$_2$CHCH$_2$), 65.6 (CH$_2$OCO), 34.1, 31.9, 29.77, 29.76, 29.6, 29.52, 29.49, 29.4, 29.32 (2), 29.26, 27.22, 27.20, 26.1, 25.64 (3), 25.60, 25.5, 22.74, 22.7, 20.6, 14.3, 14.1 ppm.

4.20. 3-*O*-cis-Octadec-9-enyl-1-docosahexaenoyl-sn-glycerol (*S*)-**13**

A procedure identical to that described above for (*R*)-**12** was followed using (*R*)-3-*O*-*cis*-octadec-9-enyl-*sn*-glycerol (*R*)-**7** (200 mg, 0.584 mmol), DHA acetoxime ester **2** (269 mg, 0.701 mmol) and immobilized *C. antarctica* lipase (70 mg). The product (*S*)-**13** was afforded as a clear oil (343 mg, 90% yield). $[\alpha]_D^{20}$ +2.4 (*c* 1.09, benzene); HRMS (APCI): *m/z* calcd for $C_{43}H_{72}O_4$ + H: 653.5503; found 653.5494 amu. ^{1}H and ^{13}C NMR and IR spectroscopy data were identical to those obtained for (*R*)-**13**.

Supporting information

^{1}H and ^{13}C NMR spectra of compounds **1**–**4**, (***R***)-**5**–**13** and **(*S*)-8**–**13**.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemphyslip.2012.07.005.

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