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Synthesis of enantiopure structured triacylglycerols

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ABSTRACT

The synthesis of nine enantiopure structured triacylglycerols (TAGs) of the AAB type is described, all possessing the (*S*)-absolute configuration. Six of them possess one saturated and two identical unsaturated fatty acyl chains, whereas the remaining three possess two identical saturated fatty acids along with one unsaturated fatty acid. The former group was synthesized by a five-step chemoenzymatic route involving a highly regioselective immobilized *Candida antarctica* lipase, starting from enantiopure (*R*)-solketal. The second group was prepared by a fully chemical five-step approach based on the same chiral precursor. Such enantiopure TAGs are strongly required as standards for the enantiospecific analysis of intact TAGs in fats and oils.

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1. Introduction

Triacylglycerols (TAGs) are a major class of nonpolar lipids that are ubiquitous in the fats and oils of plants and animals.[1–4] They constitute a glycerol backbone to which three fatty acyl groups are linked through carboxylic ester bonds. The variety of TAG molecular species is enormous in fats and oils. This relates to the high number of various fatty acids. The fatty acids differ in the length of the fatty acyl chain, which is commonly linear and possesses an even number of carbons from C4 to C22. However, branched and odd-numbered fatty acids do exist in the fats and oils of mainly microbial origin. The acyl chains can be saturated or unsaturated with the number of double bonds ranging from 0 to 6. The position of the double bonds may also vary within the hydrocarbon chain although they most commonly belong to the n-9, n-7, n-6 and n-3 fatty acid classes with the *cis*-configuration largely dominating, although the *trans*-configuration is known. In polyunsaturated fatty acids, the methylene interrupted skipped double-bond framework dominates although the double bonds may also be conjugated in the fatty acids and derived lipids.

The location of the fatty acids within the glycerol skeleton adds further to the great variety in terms of regioisomerism. Moreover, the glycerol molecule is prochiral, which means that when two different fatty acids are attached to the terminal primary alcohol groups of the glycerol backbone, a stereogenic centre is generated at the secondary alcohol mid-position of the glycerol skeleton. A stereospecific numbering designated by the prefix *sn*- is used to distinguish between the two enantiotopic terminal positions of the glycerol moiety, such that the *pro-S* hydroxymethyl group refers to the *sn*-1 position and the *pro-R* group refers to the *sn*-3 position with the remaining stereogenic carbon at the mid-position referred to as the *sn*-2 position.[1]

TAGs are important constituents of the human and animal diet. They are not only a source of energy but also provide essential fatty acids for various biological roles.[5–7] All the aforementioned varieties largely influence the physical, sensory, nutritional and biological properties of the TAGs. The situation is to an extent simplified by the fact that the fatty acids are not randomly positioned in the TAGs that are known to differ significantly in animals and plants from species to species. Classical examples of the non-random distribution of fatty acids into the stereospecific positions of TAGs include cocoa butter[8] used in chocolate manufacturing and human milk TAGs[9] while there are numerous reports on the stereospecific positioning of fatty acids in animals and plants.[10] On the other hand this should underline the urgent need for firm analytical methods to analyse the various regio- and enantiomeric TAGs in order to better understand their physicochemical properties, biological roles and the impact of the TAG structures on metabolism, fatty acid bioavailability, digestion, absorption efficiency, transport and human health.[11–13]

It is relatively straightforward to determine the total fatty acid composition of TAGs by GC after the release of their fatty acids and converting them into volatile methyl esters.[14] All information is, however, lost with regard to their positional distribution within the glycerol backbone. Atmospheric pressure chemical ionization mass spectrometry (APCI MS) provides information on the overall fatty acid composition of the 2-position as well as the 1, 3-positions, but this method does not distinguish between the enantiomeric *sn*-1 and *sn*-3 positions.[15,16] That type of information is available by enantiospecific analysis where multi-step procedures involving 1,3-regioselective lipase[17] or Grignard reagents[18] to form

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a mixture of diacylglycerols (DAGs) and monoacylglycerols (MAGs) undergoing further transformations involving enantioselective phospholipase A,[10,19] chiral derivatization[20] or chiral-phase HPLC[21,22] are combined with chromatography. Several drawbacks related to these processes are due to the tedious multi-step procedures and acyl-migration[23,24] side reactions where acyl groups migrate from one position to another within the glycerol framework, thus affecting the reliability of the stereospecific analysis.

The stereospecific analyses described above provide an insight into the overall fatty acid composition of the individual stereospecific *sn*-1, -2 and -3 positions for a large number of TAGs in a mixture rather than information on individual TAG enantiomers. The stereospecific analysis of intact TAGs in a natural oil mixture is far more of a challenge. Non-aqueous reversed phase and silver-ion HPLC/MS are the most commonly used analytical techniques for the separation and characterization of natural TAG molecular species,[15,25] but these methods cannot be used to separate TAG enantiomers. Accordingly, there is an urgent need for a non-destructive enantiospecific analysis of intact TAGs. This important challenge has been addressed in a few recent reports based on chiral HPLC in attempts to separate the TAG enantiomers.[26–28] A remaining obstacle here is the lack of appropriate TAG enantiomers as standards to distinguish between the intact TAG enantiomers in such analyses.[27,28]

Herein we address that impediment by providing nine enantiopure (*S*)-TAG enantiomers possessing fatty acids commonly present in plant oils and fats by chemical and chemoenzymatic synthesis starting from enantiomerically pure (*R*)-solketal. Their synthesis was based on previous reports of the chemoenzymatic synthesis of regiopure symmetrically structured ABA type TAGs,[29,30] enantiopure similarly structured diacylglycerol ethers (DAGE)[31] and enantiopure ABC type TAGs[32] by using immobilized *Candida antarctica* lipase (CAL-B), which showed excellent regioselectivity towards the terminal positions of the glycerol moiety.

2. Results and discussion

The enantiopure AAB type structured TAGs addressed herein all have an (*S*)-configuration with the term structured TAG[29,30] referring to selected fatty acids accommodating predetermined positions of the TAG glycerol skeleton. Each of the TAGs possesses two different types of fatty acids, one being saturated and the other unsaturated, thus allowing the TAGs to be detected by a UV detector in combination with chiral HPLC.

From a synthetic organic chemistry point of view the TAGs may be divided into two categories: (a) those possessing one saturated fatty acyl group located at the *sn*-3 position and two unsaturated fatty acyl groups at the remaining *sn*-1 and *sn*-2 positions of the glycerol backbone; (b) those possessing two saturated acyl groups at the *sn*-2 and *sn*-3 positions and one unsaturated acyl group at the *sn*-1 position. Of the TAGs synthesized herein, the saturated fatty acids belonging to the first category included decanoic (C10:0), hexadecanoic (C16:0), eicosanoic (C20:0) and docosanoic (C22:0) acids, whereas in the second category, the saturated fatty acids were limited to hexadecanoic and octadecanoic (C18:0) acids. The unsaturated fatty acids present in the first category included the mono-unsaturated *cis*-hexadec-9-enoic (C16:1) and *cis*-octadec-9-enoic (C18:1) acids and the di-unsaturated *cis*-octadec-9,12-dienoic (C18:2) acid with C18:1 and C18:2 belonging to the second category. The structures of these TAGs (*S*)-**1**–**9** are shown in Figure 1 with compounds (*S*)-**1**, **2**, **3**, **4**, **5** and **6** belonging to the first category and (*S*)-**7**, **8** and **9** belonging to the second one.

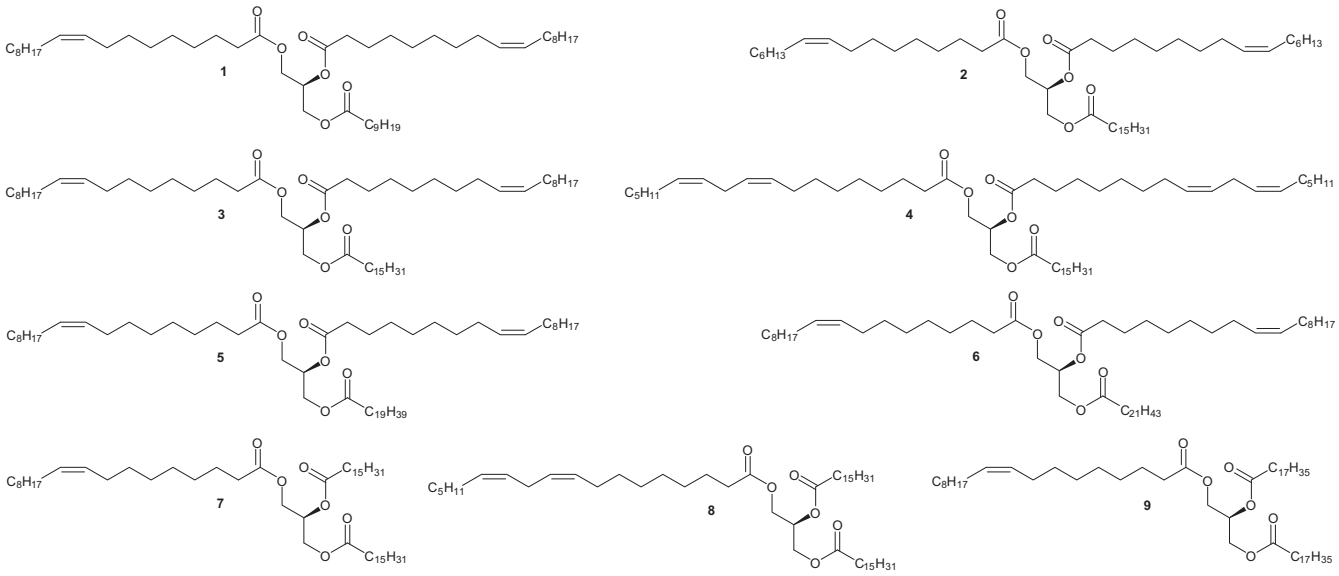
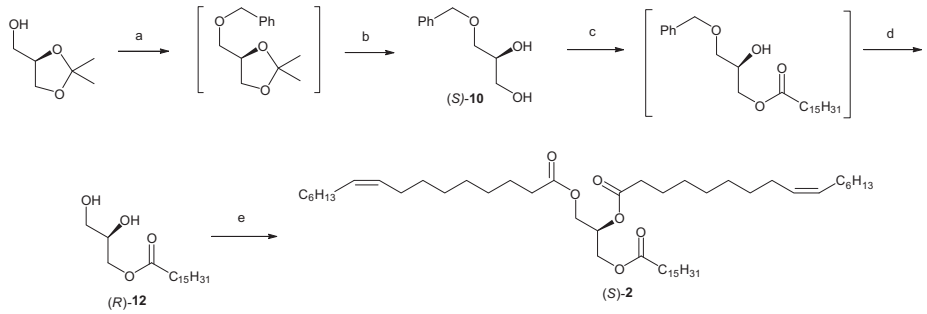


Figure 1. The enantiopure TAG products (*S*)-**1**–**9**.

The synthetic route to the TAGs (*S*)-**1**–**6** belonging to the first category is illustrated in Scheme 1 for (*S*)-**2**. It was based on a five-step chemoenzymatic process starting from enantiomerically pure 2,3-isopropylidene-*sn*-glycerol, (*R*)-solketal, as a chiral precursor. In the first four steps, a previously reported approach towards synthesis of 1-octadecanoyl-*sn*-glycerol obtained from (*S*)-solketal was followed.[32] 1-Octadecanoyl-*sn*-glycerol is an antipodal homologue of the required 3-acyl-*sn*-glycerols. Herein enantiomerically pure (*R*)-solketal was benzylated at the *sn*-3 position and the resulting benzylated adduct was subsequently deprotected without purification to remove the isopropylidene moiety. The enantiomerically pure key intermediate 1-*O*-benzyl-*sn*-glycerol (*S*)-**10** was obtained in 66% overall yield after purification.

The benzylated glycerol adduct (*S*)-**10** was acylated with activated esters (C10:0, C16:0, C20:0 and C22:0) by using highly regioselective immobilized *Candida antarctica* lipase (CAL-B from Novozymes) at rt in dichloromethane. For C10:0 and C16:0,



Scheme 1. Chemoenzymatic synthesis of the first category TAGs **1**–**6** [shown for (*S*)-**2**]. Reagents and conditions: (a) NaH, THF, then BnBr; (b) 1 M HCl, H_2O/EtOH, reflux 30 min; (c) vinyl hexanoate, CAL, CH_2Cl_2, rt; (d) H_2, 10% Pd/C, THF/hexane; (e) *cis*-hexadec-9-enoic acid, EDAC, DMAP, CH_2Cl_2, rt.

commercially available vinyl esters were used and the enzymatic reaction took only 90 min for completion. For C20:0 and C22:0, activation was needed and it was decided to activate these acids as acetoxime esters by using acetone oxime and EDAC coupling agent in the presence of DMAP by a method recently described for activation of the n-3 polyunsaturated fatty acids EPA and DHA.[33] The acetoxime esters were obtained in 99% and 64% yields, respectively, for C20:0 and C22:0. In all cases, the acylation exclusively took place at the *sn*-3 position as had been anticipated. Figure 2 illustrates the structure of the vinyl esters (shown for

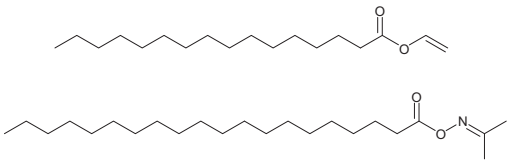


Figure 2. The structure of vinyl hexanoate (top) and eicosanoic acid acetoxime ester (bottom).

vinyl hexanoate) and the oxime esters (shown for eicosanoic acid acetoxime ester).

The acylated benzyl ether adducts were not purified but directly subjected to deprotection of the benzyl ether protecting group by catalytic hydrogenolysis using Pd/C catalyst in THF-hexane. The resulting MAGs (*R*)-**11**–**14** were obtained in moderate to good overall yields (38–75% in two steps) after crystallization. Table 1

Table 1
Yields[a] and specific activity of MAG and DAG intermediates (*R*)-**11**–**16** obtained in accordance with Schemes 1 and 2

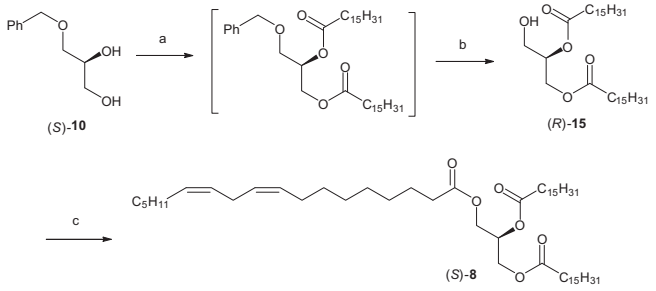
Entry	*sn*-1	*sn*-2	*sn*-3	Yield (%)	$[\alpha]_D^{20}$
(*R*)-**11**	OH	OH	C10:0	75	−3.46
(*R*)-**12**	OH	OH	C16:0	56	−2.58
(*R*)-**13**	OH	OH	C20:0	58	−1.89
(*R*)-**14**	OH	OH	C22:0	38	−1.61
(*R*)-**15**	OH	C16:0	C16:0	74	−4.23
(*R*)-**16**	OH	C18:0	C18:0	69	−3.87

[a] Overall yields for two steps from (*S*)-**10** in accordance with Schemes 1 and 2.

shows the yields obtained for these intermediate adducts along with their specific optical rotation values. The lower yields for the long-chain C20:0 (*R*)-**13** and particularly C22:0 (*R*)-**14** adducts were attributed to the hydrogenolysis step that required the addition of a drop of perchloric acid[34,35] to speed-up the reaction that would otherwise not have taken place at all. These intermediates were all obtained in excellent chemical and regiopurity, and the structures were firmly established by ^{1}H NMR spectroscopy at 400 MHz.[32]

The unsaturated fatty acids C16:1, C18:1 and C18:2 were introduced onto the free hydroxyl groups at the *sn*-1 and *sn*-2 positions of (*R*)-**11**–**14** using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) as a chemical coupling agent in the presence of dimethylaminopyridine (DMAP) following a previously described procedure.[29,30] The resulting first category products (*S*)-**1**–**6** were obtained in good to excellent yields (70–99%) after purification by flash chromatography. No acyl-migration was observed to take place during this reaction, as had been firmly established in previous studies.[29,30,32] Table 1 shows the type of products and the yields obtained along with their specific rotation values. As can be seen, these values are extremely low (vide infra) which could possibly be an indication of a loss in enantiopurity. However, that possibility can by ruled out by the absence of acyl migration and the establishment of the high enantiopurity of all of the synthesized TAGs by reversed phase chiral-HPLC.

The second category products (*S*)-**7**–**9** were synthesized by the synthetic route shown in Scheme 2. No enzyme was required this



Scheme 2. Chemical synthesis of the second category TAGs **7**–**9** [shown for (*S*)-**7**]. Reagents and conditions: (a) hexanoic acid, EDAC, DMAP, CH_2Cl_2, rt; (b) H_2, 10% Pd/C, THF/hexane; (c) *cis*-octadec-9,12-dienoic acid, EDAC, DMAP, CH_2Cl_2, rt.

time since the role of the lipase in the first category TAG synthesis was to secure regiocontrol by introducing a saturated fatty acyl group exclusively at the *sn*-3 position of the glycerol skeleton leaving the *sn*-2 position free for an unsaturated fatty acid. This is a three-step chemical route based on the benzylated glycerol adduct (*S*)-**10** as a common precursor, shared with the synthesis of the first category TAG products. In the first step, two identical saturated fatty acids, C16:0 or C18:0, were introduced to the free hydroxyl groups at the *sn*-2 and *sn*-3 positions of the glycerol moiety using the EDAC coupling agent in the presence of DMAP in dichloromethane at rt by the same procedure as described above. This resulted in formation of the corresponding 1-*O*-benzyl-2,3-diacyl-*sn*-glycerols that were, without purification, subjected to

catalytic hydrogenolysis deprotection of the benzyl protecting group. This resulted in formation of the DAG intermediates (*R*)-**15** (C16:0) and (*R*)-**16** (C18:0), which were obtained in high overall yields (74% and 69%, respectively) after recrystallization (see Table 1). Their excellent regio- and enantiopurity was established by the fact that no acyl-migration was observed to take place during these reactions.

The 2,3-*sn*-DAG intermediates were then acylated with the unsaturated fatty acids using the same chemical coupling method as before, (*R*)-**15** with 18:1 and 18:2 to obtain the corresponding TAGs (*S*)-**7** and (*S*)-**8**, respectively, and (*R*)-**16** with 18:1 to obtain (*S*)-**9**. These products were obtained in high to excellent yields after recrystallization, as can be seen in Table 2. The specific rotation values for these enantiopure (as established by reversed phase chiral-HPLC) TAGs remained low (see Table 2).

Table 2
Yields[a] and specific activity of TAG products (*S*)-**1**–**9** obtained in accordance with Schemes 1 and 2

Entry	*sn*-1	*sn*-2	*sn*-3	Yield (%)	$[\alpha]_D^{20}$
(*S*)-**1**	C18:1	C18:1	C10:0	95	−0.111
(*S*)-**2**	C16:1	C16:1	C16:0	73	−0.055
(*S*)-**3**	C18:1	C18:1	C16:0	70	−0.041
(*S*)-**4**	C18:2	C18:2	C16:0	99	−0.045
(*S*)-**5**	C18:1	C18:1	C20:0	70	−0.028
(*S*)-**6**	C18:1	C18:1	C22:0	78	−0.027
(*S*)-**7**	C18:1	C16:0	C16:0	86	−0.035
(*S*)-**8**	C18:2	C16:0	C16:0	98	−0.053
(*S*)-**9**	C18:1	C18:0	C18:0	79	−0.044

[a] The yields are based on the acylation in the final step in Schemes 1 and 2.

The specific rotation values are extremely low and warrant a special comment. Enantiopure TAGs are known for their extremely low specific rotation values as was reported by Baer and Fischer in the late 1930s.[36,37] Such compounds are known for their cryptochirality[38] or cryptoactivity,[39,40] terms which refer to their specific rotation remaining close to zero, and which are hardly measureable. As can be seen from the results in Table 2, the specific rotation values for all of the enantiopure TAGs ranged from −0.027 to −0.055 except for (*S*)-**1**, which possessed the shortest saturated C10:0 chain, where the absolute value was higher although still low at −0.111. The specific rotation values for the intermediates (*R*)-MAGs and (*R*)-DAGs displayed in Table 1 are significantly higher, or roughly two orders of magnitude higher. The trend towards lower activity values when the acyl chain gets shorter is noteworthy.

All intermediate adducts including the *sn*-3-MAGs and *sn*-2,3-DAGs and the final TAG products were obtained in high chemical purity and were all fully characterized by synthetic organic chemistry methods including high-resolution ^{1}H (400 MHz) and ^{13}C NMR and IR spectroscopy methods as well as satisfactory high-resolution accurate mass spectrometry analyses. The specific rotation was determined for all of the chiral compounds involved. Full regiocontrol and therefore enantiocontrol in all of the reactions was firmly established as based on detailed studies by high-resolution ^{1}H and ^{13}C NMR spectroscopy in accordance with previous reports[29,30,32] which was fully confirmed by chiral HPLC measurements.

The synthetic strategy used is suitable for preparing enantiopure TAGs of the AAB type possessing two different fatty acids with the prerequisite that at least one of them is saturated. This relates to the use of catalytic hydrogenolysis to remove the benzyl protecting group from the mono- and diacylated 1-O-benzylglycerol intermediates that essentially need to be saturated. TAGs possessing one saturated and two identical unsaturated fatty acids or two identical saturated and one unsaturated fatty acids of the type addressed herein as well as TAGs possessing two different types of saturated fatty acids belong to this ABB type structured TAG category that are readily accessible by the current synthetic approach.

3. Conclusion

One of the main obstacles in the enantiospecific analysis of intact TAGs in fats and oils of plant and animal origin is the lack of enantiopure structured TAG enantiomers as standards for such analysis. That impediment has been addressed to an extent here where we have described the synthesis of nine enantiopure AAB type structured (*S*)-TAGs. Six of them possess one saturated and two identical unsaturated fatty acyl chains and were synthesized by a five-step chemoenzymatic route involving immobilized *Candida antarctica* lipase, and starting from enantiopure (*R*)-solketal. The three remaining TAGs possess two identical unsaturated fatty acids along with one saturated fatty acid and were prepared by a similar, but fully chemical, five-step approach based on the same chiral precursor. This highly efficient synthetic strategy should be useful in the synthesis of a variety of related enantiopure AAB type structured TAGs to be applied as standards in the stereospecific analysis of intact TAGs in fats and oils.

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; dd, doublet of doublets; m, multiplet. The number of carbon nuclei behind each ^{13}C signal is indicated in parentheses after each chemical shift value, when there is more than one carbon responsible for the peak. Infrared spectra were conducted on a Thermo Nicolet FT-IR iS10 Spectrophotometer on a KBr pellet (crystalline material) or as a neat liquid (oils). Melting points were determined on a Büchi 520 melting point apparatus and are uncorrected. The high-resolution mass spectra (HRMS) were acquired on a Bruker micrOTOF-Q mass spectrometer. All data analyses were done on Bruker software. Optical activity measurements were performed on an Autopol^R V Automatic Polarimeter from Rudolph Research Analytical using a 40T-2.5-100-0.7 Temp Trol™ polarimetric cell with 2.5 mm inside diameter, 100 mm optical path length and 0.7 ml volume, with c referring to g sample/100 ml.

The immobilized *Candida antarctica* lipase (Novozym 435; CAL-B) was supplied as a gift from Novozymes A/S (Bagsvaerd, Denmark). All chemicals and solvents were used without further purification unless otherwise stated. (*R*)-(−)-2,2-Dimethyl-1,3-dioxolane-4-methanol [(*R*)-solketal, 98% purity and 99% ee] was purchased from Sigma–Aldrich (Steinheim, Germany). Vinyl capriate (>99%) and vinyl palmitate (>96%) were purchased from TCI Europe (Zwindrecht, Belgium. Eicosanoic (arachidic), docosanoic (behenic), octadecanoic (stearic), hexadecanoic (palmitic), *cis*-hexadec-9-enoic (palmitoleic), *cis*-octadec-9-enoic (oleic) and *cis*-octadec-9,12-dienoic (linoleic) acids were all obtained in high (>99%) purity from Larodan Fine Chemicals (Malmö, Sweden). Anhydrous magnesium sulfate was obtained from Merck (Darmstadt, Germany), benzyl bromide (98%) and sodium hydride (60% dispersion in mineral oil) from Sigma-Aldrich (Steinheim) and

10% palladium on carbon catalyst from Merck (Munich, Germany). Dichloromethane and ethyl acetate were obtained HPLC grade from Sigma–Aldrich (Steinheim, Germany), *n*-hexane as p.a. from Merck (Darmstadt, Germany), pet. ether, boiling range 40–60 °C, and diethyl ether as p.a. from Riedel-de Haën (Seelze, Germany). Dichloromethane was dried over calcium hydride under a dry nitrogen atmosphere. Tetrahydrofuran for analysis was obtained from Acros Organics (Geel, Belgium) and dried over Na wire in the presence of benzophenone under a dry nitrogen atmosphere. Silica gel (Silica gel 60) and analytical TLC plates (DC Alufolien Kieselgel 60 F_{254}) were obtained from Merck (Darmstadt, Germany). EDAC was obtained of commercial grade from Sigma–Aldrich (Steinheim, Germany) and DMAP (99%) and acetone oxime (98%) from Acros Organics (Geel, Belgium).

4.2. Determination of the enantiopurity by HPLC

The retained enantiopurity (99% ee) of all the synthetic TAG enantiomers (*S*)-**1**–**9** was confirmed on reversed phase chiral HPLC by using two identical CHIRALCEL OD-RH (150 × 4.6 mm, 5 μm) columns obtained from Chiral Technologies Europe (Illkirch, France), which were connected in a series. The nine corresponding racemic TAGs **1**–**9** purchased from Larodan Fine Chemicals (Malmö, Sweden) were used as standards and recycled until adequate separation was obtained as detected by UV, where the (*S*)-enantiomers were observed to elute faster than the (*R*)-enantiomers. The elution order of the enantiomers was determined by the co-injection of enantiopure and racemic TAGs. All details of the relevant methodology that benefited from the current TAGs (*S*)-**1**–**9** being used as enantiopure standards will be published separately.[41]

4.3. 1-*O*-Benzyl-*sn*-glycerol (*S*)-10

Prior to use, all glassware was flame-dried and allowed to cool under dry nitrogen atmosphere. Next, NaH (60% mineral oil dispersion; 1.00 g, 25.0 mmol) was added to a three-necked r.b. flask and treated several times with dry pet. ether using a syringe to remove the mineral oil prior to the addition of freshly dried THF (20 ml). (*R*)-Solketal (2.20 g, 16.7 mmol) dissolved in THF was added via syringe at a rate appropriate to keep the heat formation under control. The resulting suspension was stirred for a few minutes before benzyl bromide (3.00 g, 17.5 mmol) dissolved in dry THF was added via syringe. The mixture was refluxed for 4 h after which all of the solketal had been consumed. The reaction mixture was allowed to cool to rt and 1 M HCl (30 ml) was added together with ethanol sufficient to make the solution homogeneous. The resulting mixture was refluxed for 30 min or until all of 1-*O*-benzyl-2,3-ispropylidene-*sn*-glycerol intermediate had reacted. After cooling to rt the reaction mixture was extracted with diethyl ether and the organic phase was treated several times with 14% $NaHCO_3$ until pH >7 and finally dried over $MgSO_4$. The solvent was removed in vacuo on a rotary evaporator and the residue was introduced to a kugelrohr distillation at 2.8 Torr. A first fraction was collected at 50 °C and discarded. The desired product (*S*)-**10** was collected as a colourless liquid (2.00 g, 11.1 mmol) of high purity as indicated by TLC (1:1 ethyl acetate/pet. ether) in 66% overall yield. $[\alpha]_D^{20} = -5.48$ (*c* 20, chloroform); −2.42 (*c* 5.0, THF). ^{1}H NMR δ 7.38–7.30 (m, 5H, Ph–H), 4.55 (s, 2H, Ph–CH_2), 3.92–3.87 (m, 1H, –C*H*–OH), 3.70 (dd, *J* = 11.6 Hz, *J* = 4.0 Hz, 1H, –C*H*$_2$–OH), 3.62 (dd, *J* = 11.6 Hz, *J* = 5.6 Hz, 1H, –C*H*$_2$–OH), 3.58 (dd, *J* = 9.6 Hz, *J* = 4.4 Hz, 1H, –C*H*$_2$–OCH_2Ph), 3.53 (dd, *J* = 9.6 Hz, *J* = 6.4 Hz, 1H, –C*H*$_2$–OCH_2Ph), 2.81 (br s, 1H, –OH), 2.40 (br s, 1H, OH) ppm. ^{13}C δ 137.6, 128.5 (2), 127.9, 127.8 (2), 73.5, 71.7, 70.7 and 64.0 ppm. IR ν_{max} 3150-3600 (br, O-H), 2926 (vs, C–H) and 2868 (vs, C–H) cm^{-1}. HRMS (ESI): calcd for $C_{10}H_{14}O_3Na^+$ *m*/*z* 205.0835; found 205.0841 amu.

4.4. Eicosanoic acid acetoxime ester

To a solution of eicosanoic acid (710 mg, 2.27 mmol), DMAP (20 mg, 0.16 mmol) and EDAC (463 mg, 2.42 mmol) in dried dichloromethane (10 ml), acetone oxime (193 mg, 2.64 mmol) was added and the resulting solution was stirred overnight at rt. The reaction solution was passed through a short silica gel column with ethyl acetate/petroleum ether (60:40) as an eluent, affording the product as white crystalline material after evaporation of the solvents and recrystallization from petroleum ether (826 mg, 2.25 mmol) in 99% yield. Mp = 60.6–62.0 °C. ^{1}H NMR δ 2.39 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.04 (s, 3H, N=C(CH_3)$_2$), 2.00 (s, 3H, N=C(CH_3)$_2$), 1.68 (quin, *J* = 7.6 Hz, 2H, C*H*$_2$$CH_2COO$), 1.38–1.18 (m, 32H, CH_2), and 0.87 (t, *J* = 6.8 Hz, 3H, –C*H*$_3$) ppm. ^{13}C δ 171.2 (C=O), 163.6 (C=N), 33.0, 31.9, 29.7 (10), 29.6, 29.3, 29.2, 29.1, 25.0, 22.7, 22.0, 16.9 and 14.1 ppm. IR ν_{max} 2917 (vs, C–H), 2848 (vs, C–H) and 1757 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{45}NO_2Na^+$ *m*/*z* 390.3343; found 390.3358 amu.

4.5. Docosanoic acid acetoxime ester

A procedure identical to that described above for eicosanoic acid acetoxime ester was followed using docosanoic acid (564 mg, 1.66 mmol), DMAP (20 mg, 0.16 mmol), EDAC (400 mg, 2.09 mmol), dichloromethane (10 ml) and acetoxime (186 mg, 2.54 mmol). The product was afforded as white crystals after recrystallization from petroleum ether (420 mg, 1.06 mmol) in 64% yield. Mp = 69.0–70.0 °C. ^{1}H NMR δ 2.41 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.06 (s, 3H, N=C(CH_3)$_2$), 2.00 (s, 3H, N=C(CH_3)$_2$), 1.70 (quin, *J* = 7.6 Hz, 2H, C*H*$_2$$CH_2COO$), 1.38–1.18 (m, 36H, CH_2), and 0.89 (t, *J* = 6.8 Hz, 3H, –C*H*$_3$) ppm. ^{13}C δ 171.3 (C=O), 163.6 (C=N), 33.1, 32.0, 29.7 (11), 29.6, 29.5, 29.4, 29.3, 29.2, 25.0, 22.0, 22.1, 17.0 and 14.2 ppm. IR ν_{max} 2923 (vs, C–H), 2849 (vs, C–H) and 1758 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{25}H_{49}NO_2Na^+$ *m*/*z* 418.3656; found 418.3670 amu.

4.6. 3-Decanoyl-*sn*-glycerol (*R*)-11

To a solution of 1-*O*-benzyl-*sn*-glycerol (*S*)-**10** (305 mg, 1.67 mmol) and vinyl decanoate (408 mg, 2.06 mmol) in dichloromethane (3 ml) was added immobilized CAL (30 mg). The resulting suspension was stirred at rt for approx. 90 min when TLC monitoring (1:1 ethyl acetate/pet. ether) indicated a complete reaction. The lipase preparation was separated by filtration and the solvent was removed in vacuo on a rotary evaporator. The resulting residue was dissolved in THF (30 ml) without further purification, followed by the addition of n-hexane (70 ml) and Pd/C catalyst (15 mg). The reaction was performed in a PARR reactor under hydrogen pressure (5 bar) during which time the product precipitated. When the reaction had proceeded to completion (approximately 2 h), THF was added until all of the product had been dissolved. The catalyst was separated off by filtration with the aid of Celite and the solvent was removed in vacuo on a rotary evaporator. The residue was redissolved in the minimum amount of THF and a four-fold volume of *n*-hexane was added. The resulting mixture was allowed to stand at rt overnight to afford product (*R*)-**11** as white crystals (307 mg, 1.25 mmol) in 75% overall yields. Mp = 43.5–44.7 °C. $[\alpha]_D^{20} = -3.46$ (*c* 5.1, THF). ^{1}H NMR δ 4.22 (dd, *J* = 11.6 Hz, *J* = 4.8 Hz, 1H, –CH_2–OCO), 4.16 (dd, *J* = 11.6 Hz, *J* = 6.0 Hz, 1H, –CH_2–OCO), 3.96–3.90 (m, 1H, –C*H*–OH), 3.72–3.67 (m, 1H, –C*H*$_2$–OH), 3.63–3.57 (m, 1H, –C*H*$_2$–OH), 2.52 (br d, 1H, –CH–O*H*), 2.36 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.08 (br s, 1H, –CH_2–O*H*), 1.64 (quin, *J* = 7.2 Hz, 2H, C*H*$_2$$CH_2COO$), 1.37–1.18 (m, 12H, CH_2), and 0.88 (t, *J* = 6.8 Hz, 3H, –C*H*$_3$) ppm. ^{13}C δ 174.4, 70.3, 65.1, 63.3, 34.1, 31.8, 29.4, 29.2 (2), 29.1, 24.9, 22.6 and 14.1 ppm. IR ν_{max} 3150-3600 (br, O–H), 2918 (vs, C–H), 2850 (vs, C–H) and 1733

(vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{13}H_{26}O_4Na^+$ m/z 269.1732; found 269.1719 amu.

4.7. 3-Hexadecanoyl-*sn*-glycerol (*R*)-12

The same procedure was followed as described for (*R*)-**11** using 1-*O*-benzyl-*sn*-glycerol (*S*)-**10** (505 mg, 2.77 mmol), vinyl hexadecanoate (1028 mg, 3.64 mmol), dichloromethane (5 ml), immobilized CAL (60 mg) and Pd/C catalyst (30 mg). The product (*R*)-**12** (510 mg, 0.212 mmol) was afforded as white crystals in 56% overall yield. Mp = 70.8–72.0 °C. $[\alpha]_D^{20} = -2.58$ (*c* 5.2, THF). ^{1}H NMR δ 4.21 (dd, *J* = 11.6 Hz, *J* = 4.4 Hz, 1H, –CH_2–OCO), 4.15 (dd, *J* = 11.6 Hz, *J* = 6.0 Hz, 1H, –CH_2–OCO), 3.96–3.90 (m, 1H, –C*H*–OH), 3.70 (dd, *J* = 11.6 Hz, *J* = 4.0 Hz, 1H, –CH_2–OH), 3.60 (dd, *J* = 11.6 Hz, *J* = 6.0 Hz, 1H, –CH_2–OH), 2.54 (br s, 1H, –CH–O*H*), 2.35 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.10 (br s, 1H, –CH_2–O*H*), 1.63 (quin, *J* = 7.2 Hz, 2H, CH_2CH_2COO), 1.37–1.18 (m, 24H, CH_2), and 0.88 (t, *J* = 6.8 Hz, 3H, –CH_3) ppm. ^{13}C δ 174.4, 70.3, 65.1, 63.3, 34.1, 31.9, 29.7 (3), 29.6 (3), 29.4, 29.3, 29.2, 29.1, 24.9, 22.7 and 14.1 ppm. IR ν_{max} 3150-3600 (br, O-H), 2919 (vs, C–H), 2849 (vs, C–H) and 1735 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{19}H_{38}O_4Na^+$ m/z 353.2662; found 353.2672 amu.

4.8. 3-Eicosanoyl-*sn*-glycerol (*R*)-13

The same procedure was followed as descibed for (*R*)-**11** using 1-*O*-benzyl-*sn*-glycerol (*S*)-**10** (250 mg, 1.37 mmol), eicosanoic acid acetoxime ester (550 mg, 1.50 mmol), dichloromethane (4 ml), immobilized CAL (35 mg) and Pd/C catalyst (15 mg). The hydrogenolysis reaction required a drop of perchloric acid to be added to the reaction mixture as a promoter.[34,35] The product (*R*)-**13** (306 mg, 0.791 mmol) was afforded as white crystals in 58% overall yield. Mp = 78.2–79.7 °C. $[\alpha]_D^{20} = -1.89$ (*c* 5.2, THF). ^{1}H NMR δ 4.21 (dd, *J* = 11.6 Hz, *J* = 4.8 Hz, 1H, OCO–CH_2–), 4.15 (dd, *J* = 11.6 Hz, *J* = 6.0 Hz, 1H, OCO–CH_2–), 3.96–3.90 (m, 1H, –C*H*–OH), 3.70 (dd, *J* = 11.6 Hz, *J* = 4.0 Hz, 1H, –CH_2–OH), 3.60 (dd, *J* = 11.6 Hz, *J* = 6.0 Hz, 1H, –CH_2–OH), 2.57 (br s, 1H, –CH–O*H*), 2.35 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.14 (br s, 1H, –CH_2–O*H*), 1.63 (quin, *J* = 7.2 Hz, 2H, CH_2CH_2COO), 1.37–1.18 (m, 32H, CH_2), and 0.88 (t, *J* = 6.8 Hz, 3H, –CH_3) ppm. ^{13}C δ 174.3, 70.3, 65.2, 63.3, 34.2, 31.9, 29.7 (10), 29.4 (2), 29.2, 29.1, 24.9, 22.7 and 14.1 ppm. IR ν_{max} 3150–3600 (br, O–H), 2919 (vs, C–H), 2849 (vs, C–H) and 1735 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{46}O_4Na^+$ m/z 409.3288; found 409.3299 amu.

4.9. 3-Docosanoyl-*sn*-glycerol (*R*)-14

The same procedure was followed as described for (*R*)-**13** using 1-*O*-benzyl-*sn*-glycerol (*S*)-**10** (149 mg, 0.818 mmol), docosanoic acid acetoxime ester (330 mg, 0.834 mmol), dichloromethane (4 ml), immobilized CAL (18 mg) and Pd/C catalyst (10 mg). The product (*R*)-**14** (130 mg, 0.314 mmol) was afforded as white crystals in 38% overall yield. Mp = 79.2–80.0 °C. $[\alpha]_D^{20} = -1.61$ (*c* 5.1, THF). ^{1}H NMR δ 4.21 (dd, *J* = 11.6 Hz, *J* = 4.8 Hz, 1H, OCO–CH_2–), 4.15 (dd, *J* = 11.6 Hz, *J* = 6.0 Hz, 1H, OCO–CH_2–), 3.96–3.90 (m, 1H, –C*H*–OH), 3.72–3.67 (m, 1H, –CH_2–OH), 3.63–3.57 (m, 1H, –CH_2–OH), 2.50 (br s, 1H, –CH–O*H*), 2.35 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.04 (br s, 1H, –CH_2–O*H*), 1.63 (quin, *J* = 7.2 Hz, 2H, CH_2CH_2COO), 1.37–1.18 (m, 36H, CH_2), and 0.88 (t, *J* = 6.8 Hz, 3H, –CH_3) ppm. ^{13}C δ 174.3, 70.3, 65.2, 63.3, 34.2, 31.9, 29.7 (11), 29.6, 29.4 (2), 29.2, 29.1, 24.9, 22.7 and 14.1 ppm. IR ν_{max} 3150–3600 (br, O–H), 2918 (vs, C–H), 2849 (vs, C–H) and 1735 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{25}H_{50}O_4Na^+$ m/z 437.3601; found 437.3619 amu.

4.10. 2,3-Dihexadecanoyl-*sn*-glycerol (*R*)-15

To a solution of 1-*O*-benzyl-*sn*-glycerol (*S*)-**10** (300 mg, 1.65 mmol) and hexanoic acid (928 mg, 3.62 mmol) in dried dichloromethane (4.0 ml) were added DMAP (95 mg, 0.78 mmol) and EDAC (695 mg, 3.63 mmol). The resulting solution was stirred magnetically at rt until TLC monitoring (80:20:1 pet. ether/ether/acetic acid) indicated complete reaction. The reaction was stopped by passing the reaction mixture through a short column packed with silica gel using petroleum ether as an eluent. The solvent was removed in vacuo and the resulting residue without further purification was dissolved in n-hexane (50 ml) and transferred to a hydrogenation flask followed by the addition of Pd/C catalyst (25 mg). The reaction was performed at rt in a PARR reactor under hydrogen pressure (5 bar). When the reaction was complete (2 h), the catalyst was separated off by filtration with the aid of Celite and the solvent was removed in vacuo on a rotary evaporator. Recrystallization from n-hexane or petroleum ether afforded the product (*R*)-**15** as white crystals (695 mg, 1.22 mmol) in 74% overall yield. Mp = 71.0–72.2 °C. $[\alpha]_D^{20} = -4.23$ (*c* 5.1, THF). ^{1}H NMR δ 5.11–5.06 (m, 1H, CH–O–CO), 4.32 (dd, *J* = 12.0 Hz, *J* = 4.8 Hz, 1H, –CH_2–OCO), 4.23 (dd, *J* = 12.0 Hz, *J* = 5.6 Hz, 1H, –CH_2–OCO), 3.75–3.72 (m, 2H, –CH_2–OH), 2.34 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.33 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.10 (t, *J* = 6.4 Hz, 1H, –CH_2–O*H*), 1.64–1.58 (m, 4H, CH_2CH_2COO), 1.38–1.17 (m, 48H, CH_2), and 0.87 (t, *J* = 6.8 Hz, 6H, –CH_3) ppm. ^{13}C δ 173.8 (α C=O), 173.4 (β C=O), 72.1, 62.0, 61.6, 34.3, 34.1, 31.9 (2), 29.7 (10), 29.6 (2), 29.5 (2), 29.4 (2), 29.3 (2), 29.1 (2), 24.9 (2), 22.7 (2) and 14.1 (2) ppm. IR ν_{max} 3300–3600 (br, O–H), 2957 (vs, C–H), 2918 (vs, C–H), 2850 (vs, C–H), 1732 (vs, C=O) and 1709 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{35}H_{68}O_5Na^+$ m/z 591.4959 found 591.4962 amu.

4.11. 2,3-Dioctadecanoyl-*sn*-glycerol (*R*)-16

The same procedure was followed as described for (*R*)-**15** using 1-*O*-benzyl-*sn*-glycerol (*S*)-**10** (150 mg, 0.823 mmol), octadecanoic acid (490 mg, 1.72 mmol), dichloromethane (4 ml), DMAP (60 mg, 0.49 mmol), EDAC (347 mg, 1.81 mmol) and Pd/C (12 mg). The product (*R*)-**16** (354 mg, 0.566 mmol) was afforded as white crystals in 69% overall yield. Mp = 76.3–77.0 °C. $[\alpha]_D^{20} = -3.87$ (*c* 5.1, THF). ^{1}H NMR δ 5.11–5.06 (m, 1H, CH–O–CO), 4.32 (dd, *J* = 12.0 Hz, *J* = 4.4 Hz, 1H, –CH_2–OCO), 4.24 (dd, *J* = 12.0 Hz, *J* = 5.6 Hz, 1H, –CH_2–OCO), 3.75–3.71 (m, 2H, –CH_2–OH), 2.34 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.32 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.06 (t, *J* = 6.4 Hz, 1H, –CH_2–O*H*), 1.66–1.57 (m, 4H, CH_2CH_2COO), 1.38–1.17 (m, 56H, CH_2), and 0.87 (t, *J* = 6.8 Hz, 6H, –CH_3) ppm. ^{13}C δ 173.8 (α C=O), 173.4 (β C=O), 72.1, 62.0, 61.6, 34.3, 34.1, 31.9 (2), 29.7 (14), 29.6 (2), 29.5 (2), 29.4 (2), 29.3 (2), 29.1 (2), 24.9 (2), 22.7 (2) and 14.1 (2) ppm. IR ν_{max} 3300–3600 (br, O-H), 2957 (vs, C–H), 2918 (vs, C–H), 2849 (vs, C–H), 1732 (vs, C=O) and 1709 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{39}H_{76}O_5Na^+$ m/z 647.5585; found 647.5576 amu.

4.12. 3-Decanoyl-1,2-di(*cis*-octadec-9-enoyl)-*sn*-glycerol (*S*)-1

To a solution of 3-decanoyl-*sn*-glycerol (*R*)-**11** (103 mg, 0.418 mmol) and *cis*-octadec-9-enoic acid (295 mg, 1.04 mmol) in dried dichloromethane (4.0 ml) were added DMAP (40 mg, 0.33 mmol) and EDAC (200 mg, 1.04 mmol). The resulting solution was stirred magnetically at rt until TLC monitoring (80:20:1 pet. ether/ether/acetic acid) indicated complete reaction. The reaction was stopped by passing the reaction mixture through a short column packed with silica gel using petroleum ether as an eluent and the solvent was removed in vacuo on a rotary evaporator.

The pure product (*S*)-**1** was afforded as a colourless oil (311 mg, 0.401 mmol) in 95% yield. $[\alpha]_D^{20} = -0.111$ (*c* 9.0, benzene). ^{1}H NMR δ 5.39–5.30 (m, 4H, H—C=C), 5.29–5.23 (m, 1H, CH–OCO), 4.29 (dd, *J* = 12.0 Hz, *J* = 4.4 Hz, 2H, –CH_2–OCO), 4.14 (dd, *J* = 12.0 Hz, *J* = 6.0 Hz, 2H, –CH_2–OCO), 2.31 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.31 (t, *J* = 7.6 Hz, 4H, CH_2COO), 2.05–1.97 (m, 8H, —CH_2—CH=), 1.66–1.57 (m, 6H, CH_2CH_2COO), 1.38–1.18 (m, 32H, CH_2), and 0.88 (t, *J* = 6.8 Hz, 9H, –CH_3) ppm. ^{13}C δ 173.2 (2), 172.8, 130.0 (2), 129.7 (2), 68.9, 62.1 (2), 34.2, 34.0 (2) 31.9 (3), 29.8–29.1 (21), 27.2 (3), 24.9 (3), 22.7 (3) and 14.1 (3) ppm. IR ν_{max} 2925 (vs, C–H), 2854 (vs, C–H) and 1747 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{49}H_{90}O_6Na^+$ *m*/*z* 797.6630; found 797.6667 amu.

4.13. 3-Hexadecanoyl-1,2-di(*cis*-hexadec-9-enoyl)-*sn*-glycerol (*S*)-2

The same procedure was followed as described for (*S*)-**1** using 3-hexadecanoyl-*sn*-glycerol (*R*)-**12** (100 mg, 0.303 mmol), *cis*-hexadec-9-enoic acid (169 mg, 0.664 mmol), dichloromethane (4.0 ml), DMAP (20 mg, 0.16 mmol) and EDAC (128 mg, 0.668 mmol). The pure product (*S*)-**2** was afforded as a colourless oil (174 mg, 0.217 mmol) in 73% yield. $[\alpha]_D^{20} = -0.055$ (*c* 9.1, benzene). ^{1}H NMR δ 5.40–5.31 (m, 4H, H—C=C), 5.30–5.24 (m, 1H, CH–OCO), 4.30 (dd, *J* = 11.6 Hz, *J* = 4.4 Hz, 2H, –CH_2–OCO), 4.15 (dd, *J* = 11.6 Hz, *J* = 6.0 Hz, 2H, –CH_2–OCO), 2.32 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.32 (t, *J* = 7.6 Hz, 4H, CH_2COO), 2.08–1.99 (m, 8H, —CH_2—CH=), 1.65–1.57 (m, 6H, CH_2CH_2COO), 1.38–1.18 (m, 56H, CH_2), and 0.88 (t, *J* = 6.8 Hz, 9H, –CH_3) ppm. ^{13}C δ 173.2 (2), 172.8, 130.0 (2), 129.7 (2), 68.9, 62.1 (2), 34.2, 34.0 (2) 31.9 (2), 31.8, 29.7–29.0 (23), 27.2 (3), 24.9 (3), 22.7 (3) and 14.1 (3) ppm. IR ν_{max} 3004 (s, C–H), 2925 (vs, C–H), 2854 (vs, C–H) and 1746 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{51}H_{94}O_6Na^+$ *m*/*z* 825.6943; found 825.6924 amu.

4.14. 3-Hexadecanoyl-1,2-di(*cis*-octadec-9-enoyl)-*sn*-glycerol (*S*)-3

The same procedure was followed as described for (*S*)-**1** using 3-hexadecanoyl-*sn*-glycerol (*R*)-**12** (100 mg, 0.303 mmol), *cis*-octadec-9-enoic acid (188 mg, 0.666 mmol), dichloromethane (4.0 ml), DMAP (20 mg, 0.16 mmol) and EDAC (128 mg, 0.668 mmol). The pure product (*S*)-**3** was afforded as a colourless oil (181 mg, 0.211 mmol) in 70% yield. $[\alpha]_D^{20} = -0.041$ (*c* 8.7, benzene). ^{1}H NMR δ 5.39–5.30 (m, 4H, H—C=C), 5.30–5.24 (m, 1H, CH–OCO), 4.29 (dd, *J* = 11.6 Hz, *J* = 4.4 Hz, 2H, –CH_2–OCO), 4.14 (dd, *J* = 11.6 Hz, *J* = 6.0 Hz, 2H, –CH_2–OCO), 2.31 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.31 (t, *J* = 7.6 Hz, 4H, CH_2COO), 2.05–1.97 (m, 8H, —CH_2—CH=), 1.66–1.57 (m, 6H, CH_2CH_2COO), 1.38–1.18 (m, 64H, CH_2), and 0.88 (t, *J* = 6.8 Hz, 9H, –CH_3) ppm. ^{13}C δ 173.2 (2), 172.8, 130.0 (2), 129.7 (2), 68.9, 62.1 (2), 34.2, 34.0 (2) 31.9 (3), 29.8–29.1 (27), 27.2 (3), 24.9 (3), 22.7 (3) and 14.1 (3) ppm. IR ν_{max} 2924 (vs, C–H), 2854 (vs, C–H) and 1747 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{55}H_{102}O_6Na^+$ *m*/*z* 881.7569; found 881.7567 amu.

4.15. 3-Hexadecanoyl-1,2-di(*cis*-octadec-9,12-dienoyl)-*sn*-glycerol (*S*)-4

The same procedure was followed as described for (*S*)-**1** using 3-hexadecanoyl-*sn*-glycerol (*R*)-**12** (100 mg, 0.303 mmol), *cis*-octadec-9,12-dienoic acid (190 mg, 0.677 mmol), dichloromethane (4.0 ml), DMAP (20 mg, 0.16 mmol) and EDAC (128 mg, 0.668 mmol). The pure product (*S*)-**4** was afforded as a colourless oil (257 mg, 0.300 mmol) in 99% yield. $[\alpha]_D^{20} = -0.045$ (*c* 9.0, benzene). ^{1}H NMR δ 5.42–5.29 (m, 8H, H—C=C), 5.28–5.24 (m, 1H, CH–OCO), 4.29 (dd, *J* = 11.6 Hz, *J* = 4.4 Hz, 2H, –CH_2–OCO), 4.14 (dd, *J* = 11.6 Hz, *J* = 6.0 Hz, 2H, –CH_2–OCO), 2.77 (t, *J* = 6.4 Hz, 4H, =CH—CH_2—CH=), 2.31 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.31 (t, *J* = 7.6 Hz, 4H, CH_2COO), 2.07–1.99 (m, 8H, —CH_2—CH=), 1.66–1.57 (m, 6H, CH_2CH_2COO), 1.38–1.18 (m, 52H, CH_2), and 0.88 (t, *J* = 6.8 Hz, 9H, –CH_3) ppm. ^{13}C δ 173.3 (2), 172.9, 130.3 (2), 130.0 (2), 128.1 (2), 127.9 (2), 68.9, 62.1 (2), 34.2, 34.1 (2) 32.0, 31.6 (2), 29.7–22.6 (32) and 14.1 (3) ppm. IR ν_{max} 3009 (s, C–H), 2925 (vs, C–H), 2854 (vs, C–H) and 1747 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{55}H_{98}O_6Na^+$ *m*/*z* 877.7256; found 877.7316 amu.

4.16. 3-Eicosanoyl-1,2-di(*cis*-octadec-9-enoyl)-*sn*-glycerol (*S*)-5

The same procedure was followed as described for (*S*)-**1** using 3-eicosanoyl-*sn*-glycerol (*R*)-**13** (200 mg, 0.517 mmol), *cis*-octadec-9-enoic acid (162 mg, 0.573 mmol), dichloromethane (4.0 ml), DMAP (40 mg, 0.33 mmol) and EDAC (190 mg, 0.991 mmol). The pure product (*S*)-**5** was afforded as a white wax (186 mg, 0.203 mmol) in 70% yield (as based on the fatty acid). $[\alpha]_D^{20} = -0.028$ (*c* 7.2, benzene). ^{1}H NMR δ 5.39–5.29 (m, 4H, H—C=C), 5.29–5.23 (m, 1H, CH–OCO), 4.29 (dd, *J* = 12.0 Hz, *J* = 4.4 Hz, 2H, –CH_2–OCO), 4.14 (dd, *J* = 12.0 Hz, *J* = 6.0 Hz, 2H, –CH_2–OCO), 2.31 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.31 (t, *J* = 7.6 Hz, 4H, CH_2COO), 2.06–1.95 (m, 8H, —CH_2—CH=), 1.66–1.57 (m, 6H, CH_2CH_2COO), 1.38–1.18 (m, 72H, CH_2), and 0.88 (t, *J* = 6.8 Hz, 9H, –CH_3) ppm. ^{13}C δ 173.2 (2), 172.8, 130.0 (2), 129.7 (2), 68.9, 62.1 (2), 34.2, 34.0 (2) 31.9 (3), 29.7–29.1 (31), 27.2 (3), 24.9 (3), 22.7 (3) and 14.1 (3) ppm. IR ν_{max} 2924 (vs, C–H), 2853 (vs, C–H) and 1747 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{59}H_{110}O_6Na^+$ *m*/*z* 937.8195; found 937.8196 amu.

4.17. 3-Docosanoyl-1,2-di(*cis*-octadec-9-enoyl)-*sn*-glycerol (*S*)-6

The same procedure was followed as described for (*S*)-**1** using 3-docosanoyl-*sn*-glycerol (*R*)-**14** (104 mg, 0.251 mmol), *cis*-octadec-9-enoic acid (168 mg, 0.595 mmol), dichloromethane (4.0 ml), DMAP (20 mg, 0.16 mmol) and EDAC (105 mg, 0.548 mmol). The pure product (*S*)-**6** was afforded as a white wax (186 mg, 0.197 mmol) in 78% yield. $[\alpha]_D^{20} = -0.027$ (*c* 5.1, benzene). ^{1}H NMR δ 5.39–5.29 (m, 4H, H—C=C), 5.29–5.23 (m, 1H, CH–OCO), 4.29 (dd, *J* = 12.0 Hz, *J* = 4.4 Hz, 2H, –CH_2–OCO), 4.14 (dd, *J* = 12.0 Hz, *J* = 6.0 Hz, 2H, –CH_2–OCO), 2.31 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.31 (t, *J* = 7.6 Hz, 4H, CH_2COO), 2.06–1.96 (m, 8H, —CH_2—CH=), 1.66–1.57 (m, 6H, CH_2CH_2COO), 1.38–1.18 (m, 76H, CH_2), and 0.88 (t, *J* = 6.8 Hz, 9H, –CH_3) ppm. ^{13}C δ 173.2 (2), 172.8, 130.0 (2), 129.7 (2), 68.9, 62.1 (2), 34.2, 34.1 (2) 31.9 (3), 30.0–29.1 (33), 27.2 (3), 24.9 (3), 22.7 (3) and 14.1 (3) ppm. IR ν_{max} 3004 (s, C–H), 2922 (vs, C–H), 2852 (vs, C–H) and 1743 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{61}H_{114}O_6Na^+$ *m*/*z* 965.8508; found 965.8528 amu.

4.18. 2,3-Dihexadecanoyl-1-*cis*-octadec-9-enoyl-*sn*-glycerol (*S*)-7

To a solution of 2,3-dihexadecanoyl-*sn*-glycerol (*R*)-**15** (151 mg, 0.265 mmol) and *cis*-octadec-9-enoic acid (104 mg, 0.368 mmol) in dried dichloromethane (4.0 ml) were added DMAP (24 mg, 0.20 mmol) and EDAC (62 mg, 0.32 mmol). The resulting solution was stirred magnetically at rt until TLC monitoring (80:20:1 pet. ether/ether/acetic acid) indicated complete reaction. The reaction was stopped by passing the reaction mixture through a short column packed with silica gel using petroleum ether as an eluent and the solvent was removed in vacuo on a rotary evaporator. The pure product (*S*)-**7** was afforded as a white wax (190 mg, 0.228 mmol) in 86% yield. $[\alpha]_D^{20} = -0.035$ (*c* 8.7, benzene). ^{1}H NMR δ 5.39–5.30 (m, 2H, H—C=C), 5.29–5.24 (m, 1H, CH–OCO), 4.29 (dd, *J* = 11.6 Hz, *J* = 4.4 Hz, 2H, –CH_2–OCO), 4.14 (dd, *J* = 11.6 Hz, *J* = 6.0 Hz, 2H, –CH_2–OCO), 2.31 (t, *J* = 7.6 Hz, 2H, CH_2COO), 2.31 (t, *J* = 7.6 Hz, 4H, CH_2COO), 2.06–1.99 (m, 4H,

—CH_2—CH=), 1.65–1.57 (m, 6H, CH_2CH_2COO), 1.39–1.19 (m, 68H, CH_2), and 0.88 (t, J = 6.8 Hz, 9H, –CH_3) ppm. ^{13}C δ 173.2 (2), 172.8, 130.0, 129.7, 68.9, 62.1 (2), 34.2, 34.0 (2) 31.9 (3), 29.7–24.9 (30), 24.9 (3), 22.7 (3) and 14.1 (3) ppm. IR ν_{max} 2954 (s, C–H), 2918 (vs, C–H), 2850 (vs, C–H) and 1734 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{53}H_{100}O_6Na^+$ m/z 855.7412; found 855.7436 amu.

4.19. 2,3-Dihexadecanoyl-1–*cis*-octadec-9,12-dienoyl-*sn*-glycerol (*S*)-8

The same procedure was followed as described for (*S*)-**7** using 2,3-dihexadecanoyl-*sn*-glycerol (*R*)-**15** (155 mg, 0.272 mmol), *cis*-octadec-9,12-dienoic acid (106 mg, 0.378 mmol), dichloromethane (4.0 ml), DMAP (21 mg, 0.17 mmol) and EDAC (60 mg, 0.31 mmol). The pure product (*S*)-**8** was afforded as a white wax (211 mg, 0.266 mmol) in 98% yield. $[\alpha]_D^{20} = -0.053$ (*c* 9.5, benzene). ^1H NMR δ 5.42–5.29 (m, 4H, H—C=C), 5.28–5.23 (m, 1H, CH–OCO), 4.29 (dd, J = 12.0 Hz, J = 4.4 Hz, 2H, –CH_2–OCO), 4.14 (dd, J = 12.0 Hz, J = 6.0 Hz, 2H, –CH_2–OCO), 2.77 (t, J = 6.4 Hz, 2H, =CH—CH_2—CH=), 2.31 (t, J = 7.6 Hz, 2H, CH_2COO), 2.31 (t, J = 7.6 Hz, 4H, CH_2COO), 2.07–2.00 (m, 4H, —CH_2—CH=), 1.66–1.57 (m, 6H, CH_2CH_2COO), 1.40–1.19 (m, 62H, CH_2), and 0.88 (t, J = 6.8 Hz, 9H, –CH_3) ppm. ^{13}C δ 173.3 (2), 172.9, 130.2, 130.0, 128.1, 127.9, 68.9, 62.1 (2), 34.2, 34.1 (2) 32.0 (2), 31.5, 29.7–24.9 (34) and 14.1 (3) ppm. IR ν_{max} 3009 (s, C–H), 2918 (vs, C–H), 2850 (vs, C–H) and 1735 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{53}H_{98}O_6Na^+$ m/z 853.7256; found 853.7212 amu.

4.20. 2,3-Dioctadecanoyl-1-*cis*-octadec-9-enoyl-*sn*-glycerol (*S*)-9

The same procedure was followed as described for (*S*)-**7** using 2,3-dioctadecanoyl-*sn*-glycerol (*R*)-**9** (178 mg, 0.285 mmol), *cis*-octadec-9-enoic acid (101 mg, 0.358 mmol), dichloromethane (4.0 ml), DMAP (42 mg, 0.34 mmol) and EDAC (66 mg, 0.34 mmol). The pure product (*S*)-**9** was afforded as a white wax (199 mg, 0.224 mmol) in 79% yield. $[\alpha]_D^{20} = -0.044$ (*c* 9.1, benzene). ^1H NMR δ 5.40–5.30 (m, 2H, H—C=C), 5.29–5.23 (m, 1H, CH–OCO), 4.29 (dd, J = 12.0 Hz, J = 4.4 Hz, 2H, –CH_2–OCO), 4.14 (dd, J = 12.0 Hz, J = 6.0 Hz, 2H, –CH_2–OCO), 2.31 (t, J = 7.6 Hz, 2H, CH_2COO), 2.31 (t, J = 7.6 Hz, 4H, CH_2COO), 2.05–1.97 (m, 4H, —CH_2—CH=), 1.65–1.57 (m, 6H, CH_2CH_2COO), 1.39–1.18 (m, 76H, CH_2), and 0.88 (t, J = 6.8 Hz, 9H, –CH_3) ppm. ^{13}C δ 173.2 (2), 172.9, 130.0, 129.7, 68.9, 62.1 (2), 34.2, 34.0 (2) 31.9 (3), 29.7–24.9 (34), 24.9 (3), 22.7 (3) and 14.1 (3) ppm. IR ν_{max} 2923 (vs, C–H), 2853 (vs, C–H) and 1747 (vs, C=O) cm^{-1}. HRMS (ESI): calcd for $C_{57}H_{108}O_6Na^+$ m/z 911.8038; found 911.8028 amu.

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