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Article

Chemoenzymatic Synthesis of ABC-Type Enantiostructured Triacylglycerols by Use of the *p*-Methoxybenzyl Protective Group

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Abstract: This report demonstrates the first-time asymmetric synthesis of enantiopure structured triacylglycerols (TAGs) of the ABC type presenting three non-identical fatty acids, two of which unsaturated. The unsaturated fatty acids included the monounsaturated oleic acid (C18:1 n-9) and the polyunsaturated linoleic acid (C18:2 n-6). This was accomplished by a six-step chemoenzymatic approach starting from (*R*)- and (*S*)-solketals. The highly regioselective immobilized *Candida antarctica* lipase (CAL-B) played a crucial role in the regiocontrol of the synthesis. The synthesis also benefited from the use of the *p*-methoxybenzyl (PMB) ether protective group that enabled incorporation of two different unsaturated fatty acids to the glycerol skeleton. The total of six such TAGs were prepared, four constituting the unsaturated fatty acids in the *sn*-1 and *sn*-2 positions with a saturated fatty acid in the remaining *sn*-3 position of the glycerol backbone. In the two remaining TAGs the different unsaturated fatty acids accommodated the *sn*-1 and *sn*-3 end positions with the saturated fatty acid present in the *sn*-2 position. Enantiopure TAGs are urgently demanded as standards for enantiospecific analysis of intact TAGs in fats and oils.

Keywords: asymmetric synthesis; enantiostructured triacylglycerols; lipase; p-methoxybenzyl (PMB)-protective group; enantiospecific TAG analysis

1. Introduction

This report focuses on the synthesis of enantiopure structured triacylglycerols (TAGs), that we have named enantiostructured TAGs. Glycerol is prochiral, and the prerequisite for a TAG to become chiral is that the fatty acids accommodating the terminal 1,3-positions being different, regardless of the fatty acid occupying the central 2-position. Accordingly, chiral TAGs may be classified as AAB and ABC type TAGs constituting two or three different fatty acids, respectively. A prefix, *sn-*, pertains to a stereospecific numbering that is used to distinguish between the two enantiotopic terminal carbons of the glycerol backbone in TAGs [1,2]. The *pro-S* hydroxycarbon group refers to the *sn*-1 position, the *pro-R* group to the *sn*-3 position, and the central carbon to the *sn*-2 position.

Our interest relates to synthetic challenges as well as opening an access to a library of pure enantiostructured TAGs intended as standards for enantiospecific analysis of chiral TAGs present in natural fats and oils. A major obstacle in such analyses performed by chiral HPLC is the lack of enantiopure TAGs as reference compounds to confirm their elution behavior and retention order [3–8]. Ultimately, this may enable a more comprehensive analysis of complex natural TAG mixtures, that frequently contain remarkably high proportions of chiral ABC type TAGs [9–11]. The resulting TAGs may also find use in serving as model compounds to study enzyme activities and biological functions.

Recently, we have succeeded in the syntheses of enantiostructured TAGs of various types. This includes the AAB type TAGs, possessing only two different types of fatty acids. The AAB type TAGs

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are covered by four AAB subclasses. Our synthetic focus has been on the SSU category constituting two identical saturated fatty acids (S) and one unsaturated fatty acid (U), and the SUU category with two identical unsaturated fatty acids and one saturated fatty acid [7,12]. The remaining subclasses include the SSS' category possessing both fatty acids as saturated, and the UUU' category with both fatty acids as unsaturated. Likewise, we have also reported synthesis of ABC type enantiostructured TAGs possessing three different fatty acids with two different saturated fatty acids and one unsaturated fatty acid [13,14]. These TAGs belong to two (out of six) ABC type TAG subclasses, namely the SS'U and SUS' categories.

In the work described herein our attention is focused on the synthesis of ABC type TAGs constituting two different unsaturated fatty acids and one saturated fatty acid. Accordingly, these TAGs belong to the UU'S and USU' subclass categories. The unsaturated fatty acids involved in this work are the monounsaturated oleic acid (C18:1 n-9) and the polyunsaturated linoleic acid (C18:2 n-6). The saturated fatty acids include lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (16:0). The synthetic target included a total of six TAG molecules, all having the (*S*)-configuration, four of which belong to the UU'S category, (*S*)-**1**-**4**, and two to the SUS' category, (*S*)-**5** and **6**. Their structure is revealed in Figure 1.

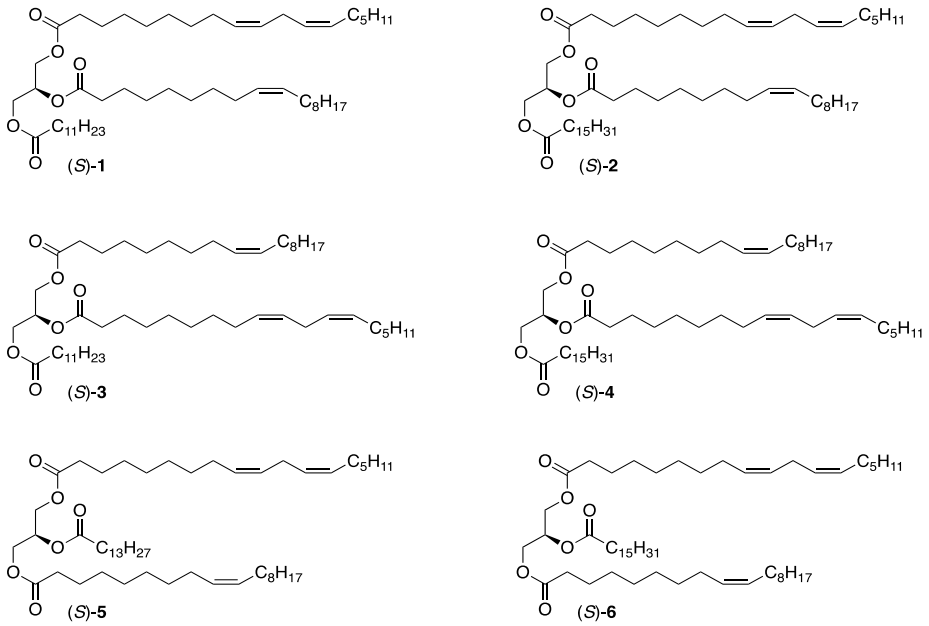

Figure 1. The structure of the six enantiostructured ABC-type TAG products synthesized, (*S*)-**1**, (*S*)-**2**, (*S*)-**3** and (*S*)-**4** belonging to the UU'S subclass category and (*S*)-**5** and (*S*)-**6** of the USU' subclass category.

To execute the synthesis of ABC type enantiostructured TAGs possessing two different unsaturated fatty acids, our previous strategy, that was based on the use of a benzyl ether as a protective group for the glycerol skeleton, needed a revision. With benzyl ether we can easily deal with TAGs possessing one unsaturated fatty acid and two different saturated fatty acids. This is no longer an option when dealing with two different unsaturated fatty acids. Our modification is based on replacing the benzyl group with a *p*-methoxybenzyl protective group. The results are described in the current report.

2. Results and Discussion

All these syntheses were dependent on enantiopure (*R*)- and (*S*)-solketals as chiral precursors, and a highly regioselective *Candida antarctica* lipase (CAL-B) to incorporate fatty acids exclusively into the primary alcohol 1,3-positions of the glycerol backbone to control the regiochemistry. The syntheses were also dependent on the use of a benzyl ether protective group to maintain the chirality of the glycerol skeleton after removal of the original isopropylidene protective moiety. The

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deprotection of the benzyl ether requires catalytic hydrogenolysis under which conditions unsaturated fatty acids present obviously will not survive. This means that all manipulations involving unsaturated fatty acids must be brought about after such deprotection and that introduction of unsaturated fatty acids must take place in the final step(s) of the synthesis.

This is not a problem when dealing with the synthesis of the above AAB and ABC type enantiostructured TAGs substituted with only one type of an unsaturated fatty acid. But once dealing with the introduction of two unsaturated fatty acids of different type, which is the case with the TAGs belonging to the SUU' and USU' subclass categories, the task becomes more challenging. That task requires an alternative protective group to the benzyl group of which removal is tolerated by the unsaturated fatty acids. In that context we came up with the idea of using a *p*-methoxybenzyl (PMB) ether to protect the end position of the glyceryl backbone that may be cleaved under mild oxidative conditions by use of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) [15,16]. The question remained here as to whether not only mono- but also di- or even higher polyunsaturated fatty acids might possibly survive such oxidative treatment. It should also be emphasized that challenges related to acyl migration promoted by parameters including temperature, the presence of an acid or base and the use of silica gel in relation to chromatography treatments, must also be kept under control to maintain the regiocontrol of the synthesis [17–19].

2.1. Chemoenzymatic Synthesis of the SUU' Subclass Category TAGs (S)-1 and 2

A six-step chemoenzymatic approach was designed for the synthesis of the SUU' subclass category TAGs (*S*)-**1**-**4** that is depicted in Figure 2 (as shown for the (*S*)-**1**). It is based on the use of (*R*)-solketal as a chiral precursor of which the *sn*-1 position is protected as a PMB ether in the first step. This is followed by removal of the isopropylidene protective moiety, and a subsequent lipase promoted regioselective acylation of the *sn*-3 hydroxyl group of the resulting diol with a saturated fatty acid. The fourth step involves an introduction of the first unsaturated fatty acid into the remaining *sn*-2 position. This is followed by removal of the PMB protective group, and in the final step the second unsaturated fatty acid is incorporated into the *sn*-3 position of the glycerol backbone to complete the synthesis.

In the first step the PMB protective group was attached to the free *sn*-1 hydroxyl group of (*R*)-solketal by treating it with *p*-methoxybenzyl chloride (PMB-Cl) in THF under reflux for 22 hours using sodium hydride as a base. The PMB-protected solketal (*R*)-**7** was obtained in 76% yield. The reaction required a significantly longer reaction time than the corresponding reaction with benzyl chloride (4 hours) under identical conditions [7,12,13] which relates to the electron donating properties of the *p*-methoxyl group. That electron donating effect also created challenges in the subsequent removal of the isopropylidene protective moiety from the (*R*)-**7** product.

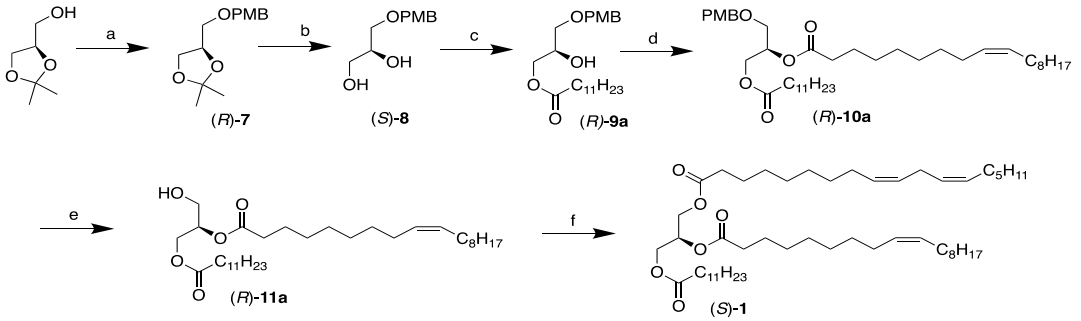

Figure 2. Chemoenzymatic synthesis of the SUU' subclass category TAGs (*S*)-**1**, **2**, **3** and **4** (shown for (*S*)-**1**). *Reagents and conditions*: (a) NaH, THF, then PMB-Cl, reflux (76%); (b) I_2, H_2O, acetonitrile r.t. (97%); (c) Vinyl dodecanoate, CAL, CH_2Cl_2, r.t. (>99%); (d) Oleic acid, EDCI, DMAP, CH_2Cl_2, r.t. (99%); (e) DDQ, CH_2Cl_2, H_2O, 0 °C to r.t. (91%); (f) Linoleic acid, EDCI, DMAP, CH_2Cl_2, r.t. (85%).

In our previous TAG synthesis cases involving the benzyl group protection of the solketal the isopropylidene group deprotection was smoothly brought about by hydrolysis using aqueous 1 M

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HCl in ethanol [7,12,13]. However, when the PMB-protected solketal (*R*)-**7** was gently refluxed with aqueous HCl in ethanol no PMB-protected glycerol (*S*)-**8** was obtained. The reaction was attempted several times under milder conditions at room temperature, with differing reaction time and acid concentration. That resulted in the desired diol in poor yields (20-35%). It appears that the electron donating properties of the *p*-methoxyl group were strong enough to induce an S_N1 type cleavage of the PMB group, causing the reaction to yield mostly a free glycerol. Hence it was clear that a different approach was needed.

Molecular iodine in the presence of water in acetonitrile as a solvent has been used to cleave isopropylidene acetals in the presence of several acid sensitive protecting groups, including PMB [20,21]. This iodine-water based method was adopted to remove the isopropylidene group from solketal in the above synthesis to give excellent results with the PMB-protected diol (*S*)-**8** being obtained in 97% yields.

Having the chemoselective acetal protective group removal successfully sorted out and the resulting PMB ether protected glycerol (*S*)-**8** in hand the next reaction involved an introduction of fatty acids into the free end position. The selected fatty acids included the three saturated lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids. All the three saturated fatty acids were commercially available as activated vinyl esters. Their activation ensures a fast reaction with the enol leaving group undergoing tautomerization to form acetaldehyde securing an irreversible process. A fast irreversible process favours the excellent regioselectivity of the lipase [22,23].

The highly regioselective *Candida antarctica* lipase B (CAL-B) was used to exclusively acylate the primary *sn*-3 rather than the secondary *sn*-2 position of the glycerol backbone. Mildness in terms of temperature is another essential parameter that is offered by the lipase to avoid acyl migration [17–19] of the fatty acid once it has been introduced to a predetermined position of the glycerol backbone. We have demonstrated that under the above mild conditions offered by the lipase no such acyl migration took place to disturb the regiocontrol of the acylation and that was also the case in the current reactions [22,23].

Besides that, in all previous and current acylation steps involving the lipase, and when dealing with all intermediates possessing an acyl group adjacent to a free hydroxyl group on the glycerol skeleton, an extra care had to be taken to avoid acyl migration. That includes when performing the reactions, during their work-up, and the purification of the products by use of silica gel-based chromatography. The use of silica gel is known to promote acyl migration and to get around that a silica gel impregnated with 4% boric acid was used [24,25].

The lipase promoted reactions of the vinyl esters were performed in dry dichloromethane at room temperature and were completed in 4 hours to afford the (*R*)-**9a**-**c** PMB-protected monoacylglycerols (MAGs) in excellent to quantitative yields. The (*R*)-**9a** product with lauric was obtained as a colourless oil while those of myristic and palmitic acids, (*R*)-**9b** and (*R*)-**9c**, precipitated as white lightweight powders. Table 1 shows the identity, yields and specific optical activity of these PMB-protected *sn*-3-MAG derivatives. The corresponding (*R*)-**14** from the USU' subclass category TAG synthesis (see section 2.2 below) has been included in the table.

Table 1. Summary of yields and specific rotation of intermediates (*R*)-**9a**-**c** and (*R*)-**14**.

Compound	*sn*-1	*sn*-2	*sn*-3	Yields	$[\alpha]^{20}_D$
(*R*)-**9a**	PMB	OH	12:0	>99%	-1.28
(*R*)-**9b**	PMB	OH	14:0	91%	-1.93
(*R*)-**9c**	PMB	OH	16:0	94%	-1.18
(*R*)-**14**	PMB	OH	18:1	87%	-0.72

The structures of the PMB protected MAGs were confirmed by the characteristic pattern for the glycerol region (δ 5.40-3.60 ppm) of their ^{1}H-NMR spectra. Figure *S1* in the *Supplementary Materials* shows a comparison of the glycerol region of the PMB-protected solketal (*R*)-**7**, the PMB-protected glycerol (*S*)-**8** and the PMB-protected *sn*-3-MAG (*R*)-**9c**. No sign of acyl migration was observed in

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the case of the PMB-protected *sn*-3-MAG derivatives. Acyl migration side reactions would distort the peak pattern and give additional peaks into their glycerol proton region.

The *sn*-2 mid position of the PMB-protected *sn*-3-MAGs (*R*)-**9a**-**c** was acylated by use of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) as a coupling agent in the presence of 4-dimethylaminopyridine (DMAP) as a base and a catalyst. The reactions were performed under conditions identical to those previously described in our syntheses of structured and enantiostructured TAGs in dichloromethane at room temperature under which no acyl migration was taking place [12,13,22,23]. The participating fatty acids included the unsaturated oleic acid (18:1 n-9) and linoleic acid (18:2 n-6) in the case of the SUU' subclass category TAGs.

All the PMB-protected diacylglycerol (DAG) products (*R*)-**10a**-**e** were obtained as colourless oils in very high to excellent yields (88-99%), in the same range as previously reported for the benzyl protected acylglycerols [13]. All these PMB-protected *sn*-2,3-DAG derivatives showed specific rotation values between -6.0 and -7.0. Table 2 shows a summary of the identity, the yields obtained, and the specific optical activity of these intermediates. The corresponding (*R*)-**15a**,**b** from the USU' subclass category TAG synthesis (see section 2.2 below) have also been included in the table.

Again, the structures of the PMB-protected DAGs were confirmed by the characteristic pattern for the protons belonging to the glycerol region of their ^{1}H-NMR spectra. Figure *S2* in the *Supplementary Materials* shows a comparison of the glycerol region of the PMB-protected *sn*-3-MAG (*R*)-**9b** and the PMB-protected *sn*-2,3-DAG (*R*)-**10c**. It is of interest to notice the dramatic changes taking place in the spectrum upon acylation of the *sn*-2 position confirming a successful reaction.

Table 2. Summary of the yields and specific rotation of the intermediates (*R*)-**10a**-**c** and (*R*)-**15a**,**b**.

Compound	*sn*-1	*sn*-2	*sn*-3	Yields	$[\alpha]^{20}_{D}$
(*R*)-**10a**	PMB	18:1	12:0	99%	-6.77
(*R*)-**10b**	PMB	18:1	14:0	99%	-6.61
(*R*)-**10c**	PMB	18:1	16:0	88%	-6.24
(*R*)-**10d**	PMB	18:2	12:0	88%	-6.05
(*R*)-**10e**	PMB	18:2	16:0	89%	-6.80
(*R*)-**15a**	PMB	14:0	18:1	94%	-6.86
(*R*)-**15b**	PMB	16:0	18:1	91%	-6.75

2.1.1. DDQ Deprotection with an Incorporated MUFA Present

The most challenging key step in the proposed synthetic approach was the removal of the PMB protecting group by use of DDQ in a water-dichloromethane medium. DDQ is a mild oxidant capable of removing the *p*-methoxybenzyl protective group from alcohols and other derivatives under neutral conditions [15,16,26]. The first important question as to whether a monounsaturated fatty acid would survive the oxidative treatment would already be an important achievement in the synthesis of the SUU' and USU' subclass categories of the enantiostructured ABC type TAGs. A more critical question related to whether a more unsaturated fatty acid such as a diene fatty acid would survive such oxidation or perhaps the longer chain PUFAs.

It is believed that the PMB group and the oxidizing benzoquinone form a charge-transfer complex before reacting in an oxidation-reduction type reaction involving a transfer of single electrons [16]. The reduced hydroquinone (DDQ-H_2) is insoluble in both water and organic solvents and is therefore easily removed in the work-up. Finally, the now oxidized PMB group is vulnerable to a nucleophilic attack by water at the benzyl position and ends up cleaving off as a *p*-methoxybenzaldehyde.

First the compounds listed in Table 2 containing the monounsaturated oleic acid along with a saturated fatty acid were introduced to the deprotection, that is (*R*)-**10a**-**c**, but also (*R*)-**15a**,**b** (see section 2.2 below). Those PMB-protected DAGs possessing linoleic acid (*R*)-**10d**,**e** were looked at separately. This was intended to test the compatibility of a single double bond in oleic acid to the reaction conditions. The reaction was highly successful with all compounds being deprotected in very high to excellent yields with no indication of any deterioration of the double bond present in the

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monounsaturated fatty acid. The identity, obtained yields, along with the specific optical rotation of each of the *sn*-2,3-DAG products (*R*)-**11a**-**c** and (*R*)-**16a**,**b** (section 2.2) is revealed in Table 3.

Table 3. Summary of yields and specific rotation of intermediates (*R*)-**11a**-**c** and (*R*)-**16a**,**b**.

Compound	*sn*-1	*sn*-2	*sn*-3	Yields	$[\alpha]^{20}_D$
(*R*)-**11a**	OH	18:1	12:0	91%	+2.43
(*R*)-**11b**	OH	18:1	14:0	92%	+2.09
(*R*)-**11c**	OH	18:1	16:0	85%	+2.10
(*R*)-**16a**	OH	14:0	18:1	95%	+2.41
(*R*)-**16b**	OH	16:0	18:1	91%	+2.39

The formation of a charge-transfer complex turned the solution a dark colour, usually dark green or brown, which slowly faded until the reaction solution had become colourless with a bright red aqueous phase. At that time the reaction had proceeded to completion as indicated by TLC monitoring. By some reason care had to be taken during the work-up of the reaction. The reaction product mixture appeared to be quite vulnerable even after extraction into dichloromethane. It could not be kept in solution for more than a couple of hours or be exposed to temperatures above 20°C without a clear deterioration of the product as indicated by ^{1}H NMR spectroscopy. Treatment on a rotary evaporator to remove the solvent had to be performed at room temperature without heating. Once the product had been isolated and purified it remained stable and there were no signs of any acyl migration taking place as is evident from Figure *S3* in the *Supplementary Materials* displaying the glyceryl proton region of the ^{1}H NMR spectrum of the (*R*)-**16a** being typical for 1,2-DAGs [12,13].

2.1.2. DDQ Deprotection with an Incorporated PUFA Present

Having confirmed that a single double bond present in oleic acid was unaffected by the oxidative cleavage conditions for the PMB-protective group we wanted to investigate further whether fatty acids of higher unsaturation would survive. Two of the PMB-protected diacylglycerols in Table 2 constitute linoleic acid possessing two methylene interrupted double bonds, namely (*R*)-**10d** and (*R*)-**10e**. Both adducts contain the linoleic acid (C18:2 n-6) in their *sn*-2 position with saturated lauric (C12:0) and palmitic (C16:0) acids in the sn-3 position. They serve as intermediates in the intended synthesis of (*S*)-**3** and (*S*)-**4**, respectively, and belong to the SUU' subclass category TAGs. They were prepared by the chemoenzymatic approach shown in Figure 2. We were particularly interested in finding out whether dienes of that type might possibly survive the deprotection conditions involving the DDQ oxidation. They are far more prone to undergo oxidation as compared to an isolated double bond present in monounsaturated fatty acids [27–31]. For that investigation the (*R*)-**10e** constituting palmitic acid was chosen to experiment on.

First the DDQ reaction was performed under the exact conditions as when deprotecting the monounsaturated (*R*)-**10a**-**c** described above, using 1.3 equivalents of the DDQ oxidant stirred with a solution of the PMB-protected diacylglycerols in water-dichloromethane for 6h at room temperature. The results were disappointing since the desired deprotected (*R*)-**11e** was isolated in only 26% yield along with some unreacted starting material and a highly polar fraction. The polar fraction was comprised of oxidized breakdown products of linoleic acid as indicated by ^{1}H NMR spectroscopy.

Most likely the oxidizer DDQ was destroying the double bond system. Skipped polyenes are much more sensitive to oxidation than the single double bond of MUFAs [27–31]. To confirm this breakdown, free linoleic acid was stirred with DDQ, and the solution monitored with TLC. As time passed, the single spot of linoleic acid started to break down into more polar spots, indicating that indeed the diene system was being destroyed. A ^{1}H NMR of linoleic acid before and after exposure to DDQ supported the postulate with the olefin peaks getting diminished after the experiment.

Several attempts were made to try to adjust the reaction conditions to the more vulnerable diene system. The amount of DDQ was reduced to one equivalent in hopes that less free oxidizer in the solution would yield more product. Additionally, DDQ was dissolved in dichloromethane and

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added slowly to the solution via a dropping funnel, instead of all at once, to further minimizing the free DDQ and the reaction time was decreased. These adjustments had some limited success resulting in increased yields from 26% to 36%, and more unreacted starting material (43%) was obtained than oxidized side products. However, it was clear that the reaction was far from ideal. The product (*R*)-**11e** obtained from these experiments was acylated with lauric acid to accomplish the TAG product (*R*)-**12f** that has been included in Table 4 (see Section 2.2).

Table 4. Summary of yields and specific rotation of TAGs (*S*)-**1**, **2**, **5** and **6** and (*R*)-**12a**-**d**, (*S*)-**12e** and (*R*)-**12f**.

Compound	*sn*-1	*sn*-2	*sn*-3	Yields	$[\alpha]^{20}_{D}$
(*S*)-**1**	18:2	18:1	12:0	85%	+0.03
(*S*)-**2**	18:2	18:1	16:0	92%	-0.01
(*S*)-**5**	18:2	14:0	18:1	94%	+0.05
(*S*)-**6**	18:2	16:0	18:1	95%	-0.02
(*R*)-**12a**	12:0	18:1	14:0	90%	+0.04
(*R*)-**12b**	10:0	18:1	16:0	98%	+0.05
(*R*)-**12c**	12:0	18:1	16:0	99%	+0.02
(*R*)-**12d**	14:0	18:1	16:0	96%	-0.02
(*S*)-**12e**	20:0	18:1	16:0	97%	+0.01
(*R*)-**12f**	12:0	18:2	16:0	86%	+0.09

2.1.3. Chemical Coupling of the Final Fatty Acid

To complete the synthesis of the intended enantiostructured TAGs (*S*)-**1** and (*S*)-**2**, the final step involved chemical coupling of the third and final fatty acid into the remaining *sn*-1 position of the *sn*-2,3-DAG precursors. In accordance with the scheme in Figure 2 this was brought about by EDCI and DMAP as described in previous steps using free fatty acids. The final TAGs were obtained as colourless oils in very good to excellent yields. Table 4 outlines the identity, yields and optical activity of the final products. The corresponding (*S*)-**5** and (*S*)-**6** from the USU' subclass category TAG synthesis (see section 2.2 below) have also been included in the table.

As can be further noticed from Table 4, six enantiostructured ABC type TAGs belonging to the SUS' subclass category have also been included there. Earlier, we had reported the synthesis of TAGs of that subclass by use of the benzyl protective group that involved two separate lipase steps of vinyl esters of different saturated fatty acids [13]. Alternatively, the additional TAGs included were prepared using the PMB-protective group approach described herein, and thus involving only one lipase step of the saturated fatty acid vinyl esters. Besides that, the TAGs included are the enantiomers of those previously prepared, i.e. (*R*)-**12a**, **b**, **d** and **f**, whereas the (*R*)-**12c** and (*S*)-**12e** TAGs had not been synthesised before. The TAG (*R*)-**12f** was prepared by acylation of diacylglycerol (*R*)-**11e** as obtained in the previous section.

Figure *S4* in the *Supplementary Materials* depicts the glyceryl proton region of the ^{1}H NMR spectrum of the (*S*)-**5** being characteristic of TAGs [22,23]. The optical activity of all the TAGs was extremely low, such that it was rather difficult to measure the exact value. Frequently when a sample was measured the rotation angle of the polarized light would bounce up and down around the zero value and a high concentration sample (40-60 mg as per 1 mL) was needed to obtain a good stable measurement. This observation can be explained by a phenomenon known as cryptoactivity or cryptochirality [32–34]. Compounds that show cryptoactivity are chiral but the stereogenic center possesses two moieties that are so similar that optical rotation becomes non-measurable. The carbon chains of the fatty acids in the *sn*-1 and *sn*-3 position in TAGs, that give rise to the molecule's chirality, are essentially too alike such that the TAGs are functionally optically inactive. Although the TAGs showed very low optical rotation, it is without a doubt that they are indeed chiral since all the intermediates in the synthesis retained their enantiopurity throughout and the final reaction would not cause racemization.

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2.2. Chemoenzymatic Synthesis of the USU' Subclass Category TAGs (S)-5 and 6

Like the SUU' subclass category TAGs synthesis a six-step chemoenzymatic approach was designed for the two intended USU' subclass category TAGs (*S*)-**5** and (*S*)-**6** with both syntheses sharing the (*S*)-**8** PMB-protected glycerol intermediate. The proposed approach is depicted in the scheme in Figure 3. It involves a lipase promoted regioselective acylation of oleic acid into the *sn*-3 position of the protected glycerol. This is followed by introduction of saturated fatty acids into the *sn*-2 position by chemical coupling, deprotection of the PMB-group, and finally, an introduction of linoleic acid to the *sn*-1 position by the second chemical coupling to complete the synthesis.

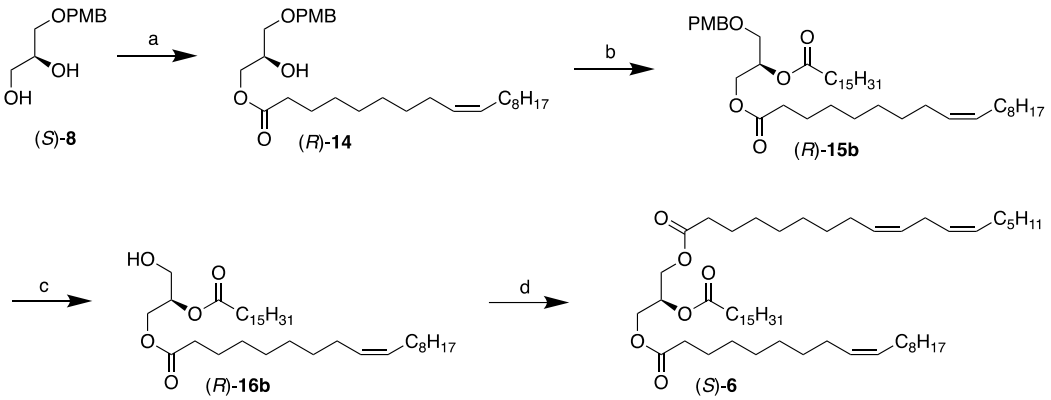

Figure 3. Chemoenzymatic synthesis of the USU' subclass category TAGs (*S*)-**5** and (*S*)-**6** (shown for (*S*)-**6**). *Reagents and conditions*: (a) Oleic acid acetoxime ester **13**, CAL-B, vacuum, r.t. (87%); (b) Hexadecanoic acid, EDCI, DMAP, CH_2Cl_2, r.t. (91%); (c) DDQ, CH_2Cl_2, H_2O, 0 °C to r.t. (91%); (d) Linoleic acid, EDCI, DMAP, CH_2Cl_2, r.t. (95%).

Since vinyl esters of unsaturated fatty acids including oleic acid are not readily available, we were dependent on a different activated ester form when dealing with such fatty acids in regioselective lipase biotransformations. Previously, we have successfully activated polyunsaturated fatty acids as acetoxime (acetone oxime) esters, including EPA (20:5 n-3) and DHA (22:6 n-3) [35], to obtain excellent regioselectivity in their lipase reactions involving the CAL-B with glycerol to prepare symmetrically structured TAGs [35,36]. However, the oximes are clearly less reactive than the vinyl esters and the irreversibility is not as explicit as when using the enol esters so that we have depended on the use of vacuum to retard the reversibility of the system [35].

Oleic acid in its reaction with acetone oxime was converted into its acetoxime ester **13** in a quantitative yield by use of the EDCI coupling agent in the presence of DMAP in dichloromethane at room temperature. The reaction of the acetoxime ester **13** with the PMB-protected glycerol (*S*)-**8** promoted by the CAL-B at first only gave about 70% yield of the (*R*)-**14** when performed under the same conditions as the vinyl esters, this time in the presence of activated molecular sieves. However, when the reaction was performed without a solvent, under vacuum to rid the reaction environment of the acetone oxime co-product, the yield jumped up to 87%, with the product (*R*)-**14** obtained as a colourless oil (see Table 1).

The saturated myristic (14:0) and palmitic (16:0) acids were introduced to the *sn*-2 position of (*R*)-**14** by use of the EDCI coupling reaction described above to accomplish the PMB-protected DAG products (*R*)-**15a** and (*R*)-**15b** as colourless oils in excellent yields (94 and 91%, respectively; Table 2). Their PMB-protective group was successfully removed under the DDQ reaction conditions identical to those described above to accomplish the *sn*-2,3-DAG intermediates (*R*)-**16a** and (*R*)-**16b** in excellent yields (95 and 91%, respectively; Table 3). As before, they were obtained in excellent regiopurity with no signs of acyl migration taking place.

To complete the synthesis of the intended enantiostructured TAGs (*S*)-**5** and (*S*)-**6** the final step like before involved chemical coupling of the third and final fatty acid, linoleic acid, into the remaining *sn*-1 position of the *sn*-2,3-DAGs precursors. This was brought about by EDCI and DMAP

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under identical conditions to those described in previous steps using free linoleic acid. The final TAGs (*S*)-**5** and (*S*)-**6** were obtained as colourless oils in excellent yields (94 and 95%, respectively; Table 4).

Tables 1–4 outline the yields and optical activity of all acylated intermediates and the final products in the synthesis of the intended SUU' and USU' subclass category TAGs obtained so far. The total yields from the (*R*)-solketal starting material to complete the TAGs over the six steps were 48-56%.

2.3. Chemoenzymatic Synthesis of the Remaining SUU' Subclass Category TAGs (S)-3 and 4

Returning to the problem of the polyenes of PUFAs being slowly destroyed by DDQ in the deprotection of the PMB-ethers. For the remaining two SUU' subclass category TAGs, (*S*)-**3** and (*S*)-**4**, that were to be synthesized by the approach based on the PMB-protective group, the linoleic acid is attached to the mid position. Since the DDQ reaction could not be adequately adapted to include linoleic acid, an adjustment was clearly needed. Instead of coupling in the first two fatty acids, a saturated fatty acid followed by linoleic acid, and then deprotecting the PMB ether, it was opted to deprotect with only the saturated fatty acid present in the end position, leaving the mid position open. Then, after deprotection, CAL-B might be used a second time to regioselectively acylate the now free *sn*-1 position, and finally, the linoleic acid could be chemically coupled into the *sn*-2 position.

2.3.1. Deprotection with an Open sn-2 Position

To investigate this option further the previously described DDQ reaction conditions were applied to the (*R*)-**9a** derivative possessing the saturated lauric acid (C12:0) in the *sn*-3 position (see Figure 4). This reaction yielded quite interesting results. Four compounds were isolated from the reaction mixture and their structure elucidated by ^{1}H NMR spectroscopy and accurate mass spectrometry studies. The desired monoacylglycerol (MAG) product (*R*)-**17** was obtained in 42% yield, a cyclic acetal (*R*)-**18** with the protective group still attached in 26% yield, a small fraction of *p*-anisaldehyde and, finally, an unknown compound in 30% yield [37].

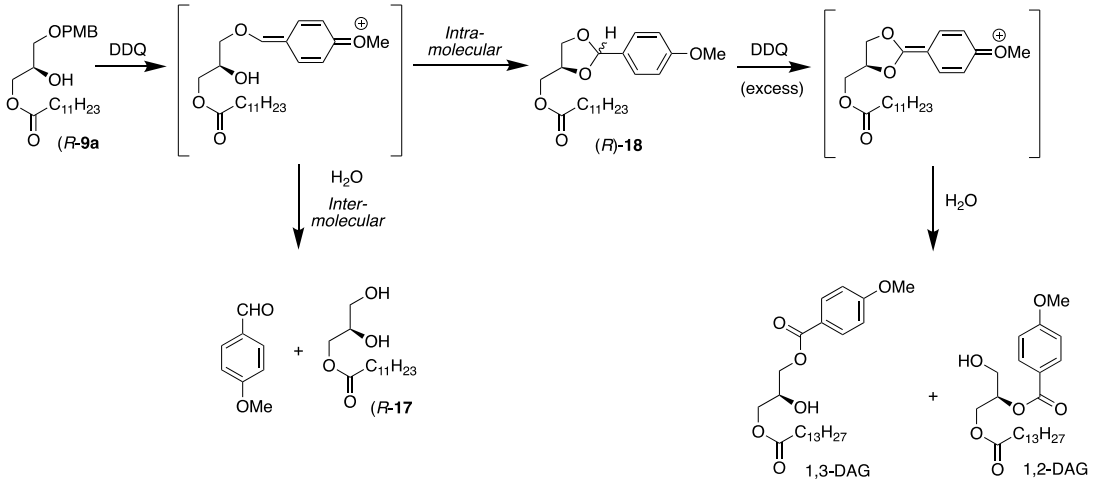

Figure 4. Intermediates and products obtained when the (*R*)-**9a** was treated with DDQ to deprotect the PMB-ether moiety in dichloromethane with water at room temperature.

The unknown product turned out to be a mixture of two *sn*-2,3- and *sn*-1,3-DAG regioisomers in a ratio of 70% and 30%, respectively, with a *p*-methoxybenzoate ester present in the molecule. The acetal (*R*)-**18** was contaminated with the *p*-anisaldehyde, and they were not completely separable on a flash column despite several attempts. Figure 4 outlines the reaction, all products that had formed, and a rationalization of what happened in the reaction.

As indicated in Figure 4 the reaction began as anticipated by DDQ oxidizing the PMB group to transform it into a quinone-like moiety that is vulnerable to a nucleophilic attack. This time, however, unlike the previous cases where two fatty acyl groups were present, there were two options for the

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reaction to proceed further. The reaction can continue as wanted by external water attacking in an intermolecular reaction mode. Secondly, the free hydroxyl group on the glycerol can also perform an intramolecular nucleophilic attack to form a cyclic acetal. The second option is usually considered a more likely scenario due to the proximity of the hydroxyl group to the reactive benzyl position.

Like in the previous reactions 30% excess of DDQ was used and therefore, there was plenty of DDQ present to continue oxidation of the cyclic acetal. This resulted in a second quinone-like moiety that underwent an intermolecular nucleophilic attack of water to form a benzoate attached to the glycerol backbone. It was of interest that the resulting diacylglycerols were formed in a ratio of 70% 1,2-DAG and 30% 1,3-DAG, which is opposite to the thermodynamic composition of 70% 1,3-DAG at an equilibrium involving an acyl migration [17–19], indicating that the overall DAG formation was somewhat under a kinetic control. It should be emphasized that under the DDQ deprotection conditions no acyl migration was observed to take place.

Armed with the newfound knowledge of the process, the reaction was adjusted to minimize the undesired side products. First, the amount of DDQ was lowered to exactly one equivalent to retard the formation of the double-oxidized benzoate DAG regioisomers. Secondly, to enhance the intermolecular reaction with water over the intramolecular reaction of the free hydroxyl group, the ratio of water to dichloromethane in the reaction medium was increased from 1:10 to 1:3. Additionally, the reaction was performed with the magnetic stirrer on a full spin to increase the accessibility of water in the reaction.

When the reaction was repeated with the new adjustments the deprotected (*R*)-**17** was isolated in a 72% yield with a minor fraction of the acetal (*R*)-**18**, which is quite acceptable. Moreover, it was demonstrated that the acetal (*R*)-**18** could be converted into the desired (*R*)-**17** in a 91% yield by using elemental iodine along with water in acetonitrile, to further improve the yield of the modified reaction. This is the same reaction as was applied to remove the isopropylidene group from solketal in the second step of the total synthesis.

2.3.2. TAG Synthesis via a Double Lipase-Step Method

Now when the MAG (*R*)-**17** had been obtained, the next step involved acylation of oleic acid as an oxime ester **13** into the *sn*-1 end position utilizing the CAL-B to form the intended 1,3-DAG intermediate. However, the results from that reaction were disappointing. Since oxime esters are less reactive than vinyl esters, the enzymatic reaction was slow, and a stirring overnight was needed for the reaction to proceed to completion. The problem is that the lipase, unlike the previous case, was in a dynamic system with the acylated glycerol. And, given enough time, the CAL-B started acting on the saturated ester already present in the monoacylglycerol starting material, thus interfering with the intended reaction. The consequences were losses in the regioselectivity of the reaction, lower yields of the desired product, and unreacted starting material present. In other words, the increased reaction time that was needed to introduce the oxime, allowed the lipase to slowly start destroying the desired compound and the regiocontrol of the reaction.

The double lipase-step method has been successfully used before to synthesize ABC-type TAGs belonging to the SUS' subclass categories [13]. However, in that case the lipase reactions involved two saturated fatty acids to be introduced into the end positions as the much faster vinyl esters in two lipase steps each taking no more than four hours. Therefore, it became evident that if the double lipase-step method was to be utilized, involving a saturated and a monounsaturated fatty acid to be incorporated into the end positions, the monounsaturated one would need to be introduced first and then, the saturated one in the second lipase step. Consequently, the synthesis would need to be initiated from the opposite (*S*)-solketal enantiomer starting material to secure the intended stereochemistry of the resulting TAGs. The accordingly modified approach is outlined in Figure 5.

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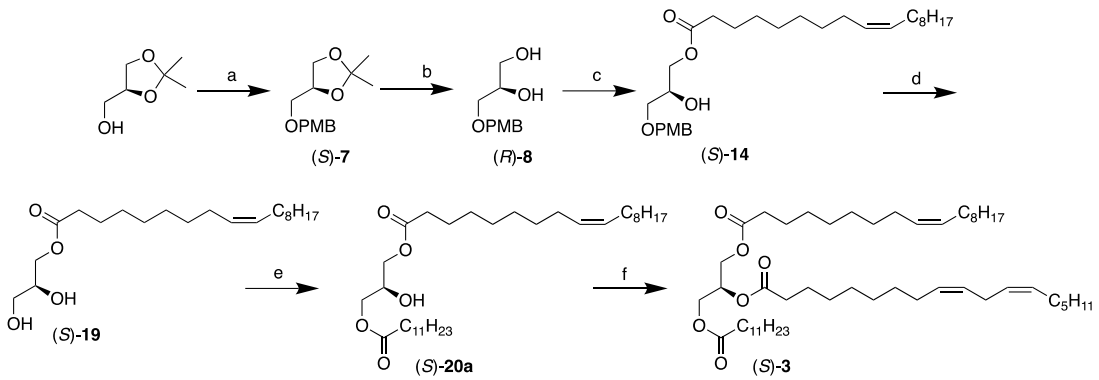

Figure 5. Chemoenzymatic synthesis of the SUU' subclass category TAGs (*S*)-**3** and **4** (shown for (*S*)-**3**). *Reagents and conditions*: (a) NaH, THF, then PMB-Cl, reflux (76%); (b) I_2, H_2O, acetonitrile r.t. (92%); (c) Oleic acid acetoxime ester **13**, CAL-B, vacuum, r.t. (74%); (d) DDQ, CH_2Cl_2, H_2O, 0 °C to r.t. (70%); (e) Vinyl dodecanoate, CAL-B, CH_2Cl_2, r.t. (83%); (f) Linoleic acid, EDCI, DMAP, CH_2Cl_2, r.t. (97%).

The first three reactions of the synthesis were identical to those of the opposite enantiomers shown in Figures 2 and 3. Once oleic acid had been introduced into the *sn*-1 position of (*S*)-**8** in 74% yield, the PMB group of the resulting (*S*)-**14** was deprotected to accomplish the MAG (*S*)-**19** in 70% yield, following the modifications described above. The second lipase step was performed with vinyl esters of lauric acid and palmitic acid to accomplish the resulting 1,3-DAGs (*S*)-**20a** and (*S*)-**20b** in 83 and 87% yields, respectively. The reactions only required 4h to proceed to completion with no observed acyl migration taking place as was to be expected. Finally, linoleic acid was incorporated into the *sn*-2 position by the usual chemical coupling involving EDCI and DMAP to complete the desired target molecules (*S*)-**3** and (*S*)-**4**, that were obtained as colourless oils in 97 and 96% yields, respectively. Table 5 shows the identity, yields and specific rotation for the final two TAGs and all their intermediates, starting from (*S*)-solketal via the double lipase-step approach.

The double lipase-step method, based on an introduction of a PUFA into the *sn*-2 position with a monounsaturated and a saturated fatty acid present in each of the end positions, afforded the two TAGs belonging to the SUU' subclass category in 30% overall yields from (*S*)-solketal over six steps. This is significantly lower than the average of 52% overall yields obtained from the PMB-based strategy described above in Figures 2 and 4. The lower total yields can mainly be attributed to the oleic acid coupling reaction, and the less efficient deprotection with the *sn*-2 position free. Nevertheless, the target compounds were obtained in excellent regiopurity with no acyl migration occurring during the synthesis. This was established through studies of the quite characteristic glyceryl proton region from the ^{1}H NMR spectra of all individual 1-MAGs, 1,3- and 1,2-DAGs, and TAGs featured in the synthesis. This is demonstrated in Figure *S4* of the *Supporting Information*.

Table 5. Summary of yields and specific optical rotation for all intermediates and SUU' subclass category TAGs (*R*)-**3** and (*R*)-**4** synthesized via the two-lipase step method.

Compound	*sn*-1	*sn*-2	*sn*-3	Yields	$[\alpha]^{20}_D$
(*S*)-**7**	isopropylidene		12:0	85%	+0.03
(*R*)-**8**	OH	OH	PMB	92%	-1.84
(*S*)-**14**	18:1	OH	PMB	74%	+0.79
(*S*)-**19**	18:1	OH	OH	70%	+1.05
(*S*)-**20a**	18:1	OH	12:0	83%	-1.28
(*S*)-**20b**	18:1	OH	16:0	87%	-0.76
(*S*)-**3**	18:1	18:2	12:0	97%	+0.08
(*S*)-**4**	18:1	18:2	16:0	96%	+0.09

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3. Materials and Methods

3.1. General Information

The ^{1}H- and ^{13}C-NMR spectra were recorded on a 400 MHz Bruker Avance spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) from tetramethylsilane with the solvent resonance used as an internal standard. In all cases the solvent was deuterochloroform which had been filtered through aluminium oxide to get rid of acid contamination. The coupling constants (J) are given in Hertz (Hz). The following abbreviations are used to describe the multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; dd, doublet of doublets; dt, doublet of triplets; ABq, AB-quartet; m, multiplet. For ^{13}C-NMR, the number of carbon nuclei contributing to each signal is indicated in parentheses after the chemical shift value. Infrared spectra were recorded on a Thermo Nicolet iS50 FT-IR spectrometer using either sodium chloride windows (NaCl) for liquid compounds, in potassium bromide pellets (KBr) for solids or a diamond ATR crystal that was used for both liquids and solids. The following abbreviations are used to describe the peaks: s, strong; vs, very strong; m, medium; w, weak; br, broad. The high-resolution mass spectra (HMRS) were recorded on a Bruker micrOTOF-Q mass spectrometer. Optical activity was measured on an Autopol 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 length and 0.7 mL volume with c (concentration) referring to g sample/100mL. Melting points were determined using a Büchi m-560 melting point apparatus. TLC monitoring was done on silica plates from SiliCycle and the plates were developed in 4% PMA solution in methanol. Boric acid impregnated silica gel was prepared by dissolving 4g of boric acid in 100 mL methanol and then adding 55g of silica and swirling the resulting slurry for a few minutes. The methanol was then evaporated off and the silica dried *in vacuo* for 6h at 40°C.

All chemicals and solvents were used without further purification unless otherwise stated. Most solvents used, deuterated chloroform (99.8% D), diethyl ether (99.8%), chloroform (≥99.5%), dichloromethane (99.8%), ethanol (≥99.8%), hexane (>97%), methanol (99.9%) and tetrahydrofuran (99.9%), were from Sigma-Aldrich. Tetrahydrofuran and dichloromethane were dried over molecular sieves and stored under nitrogen. Ethyl acetate and petroleum ether (special boiling point 60-95°C) were purchased from Brenntag in barrels. The petroleum ether was purified by distillation on rotary evaporator. All the following chemicals: p-anisaldehyde (98%), boric acid (≥99.5%), hydrochloric acid (37%), magnesium sulfate (≥99.5%), phosphomolybdic acid, sodium bicarbonate (≥99.0%), sodium hydride (60% dispersion in mineral oil), sodium sulfate (≥99%), sodium thiosulfate (≥98.5%), (R)-solketal (98%, 98% ee), (S)-solketal (98%, 99% ee), stearic acid (≥99%), vinyl caprate (>95%) and vinyl laurate (≥99%) were obtained from Sigma-Aldrich. Capric acid (>99%), lauric acid (>99.5%), myristic acid (>99.5%), oleic alcohol and tetrabutylammonium bromide (99%) were from Acros Organics. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (>97%), 1-Ethyl-3-(3-dimethylamino propyl) carbodiimide (>98%), p-methoxybenzyl chloride (>98%), vinyl myristate (>99%), vinyl palmitate (>96%) and vinyl stearate (>95%) were purchased from TCI. Arachidic acid, linoleic acid, oleic acid and palmitic acid were all from Larodan. Acetoxime (98%), 4-Dimethylaminopyridine (≥99%), elemental iodine, potassium hydroxide and sodium chloride (≥99.8%) were obtained from Merck. The immobilized *Candida antarctica* lipase B (CAL-B, Novozym 435) was obtained as a gift from Novozymes.

3.2. Synthesis of the SUU' Subclass Category TAGs (S)-1 and 2

3.2.1. Synthesis of 2,3-O-Isopropylidene-1-O-(p-methoxybenzyl)-sn-glycerol, (R)-7

Sodium hydride (60% mineral oil dispersion, 490mg, 20.43 mmol) was added to a 250 mL flame-dried two-necked round bottom flask with a magnetic stirrer and rinsed three times with dry THF (10 mL portions) under nitrogen atmosphere. After that, a fresh portion of dry THF (15 mL) was added and the solution cooled to 0°C. (*R*)-Solketal (900mg, 6.81 mmol) was added dropwise to the solution in dry THF, the mixture was then allowed to reach room temperature and stirred for 1.5 h. After that time, the solution was again cooled to 0°C and *p*-methoxybenzyl chloride (1226mg, 7.83 mmol) was added. Finally, the solution was refluxed at for 22h after which the mixture had a deep

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orange colour. The reaction was carefully quenched with water and extracted three times with dichloromethane. The combined organic extracts were washed with water and brine, then dried over $MgSO_4$ and concentrated *in vacuo*. The crude concentrate was then purified by flash column chromatography using ethyl acetate:petroleum ether (2:8) as eluent affording the product (*R*)-**7** as a slightly yellow liquid (1303mg, 76% yield). TLC (Silica, ethyl acetate:petroleum ether, 2:8): Rf = 0.39. $[\alpha]^{20}_D$ = -1.24 (c. 2.16, CH_2Cl_2). IR (NaCl, ν_{max} / cm^{-1}): 2935 (s), 2865 (s), 1613 (m), 1248 (vs), 1037 (s). ^1H NMR (400 MHz, $CDCl_3$) δ_H: 7.26 (d, *J*=8.8 Hz, 2H, Ph-H), 6.88 (d, *J*=8.8 Hz, 2H, Ph-H), 4.49 (m, 2H, $PhCH_2$), 4.27 (m, 1H, CH *sn*-2), 4.04 (dd, *J*=8.3, 6.4 Hz, 1H, CH_2 *sn*-3), 3.80 (s, 3H, OCH_3), 3.72 (dd, *J*=8.3, 6.3 Hz, 1H, CH_2 *sn*-3), 3.52 (dd, *J*=9.8, 5.7 Hz, 1H, CH_2 *sn*-1), 3.44 (dd, *J*=9.8, 5.6 Hz, 1H, CH_2 *sn*-1), 1.42 (s, 3H, $C(CH_3)_2$), 1.36 (s, 3H, $C(CH_3)_2$) ppm. $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ_C: 159.3, 130.0, 129.4 (2), 113.8 (2), 109.4, 74.7, 73.2, 70.8, 66.9, 55.3, 26.8, 25.4 ppm. HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{14}H_{20}O_4Na$ 275.1254; found, 275.1255.

3.2.2. Synthesis of 1-O-(p-methoxybenzyl)-sn-glycerol, (S)-8

PMB-solketal (*R*)-**7** (1300mg, 5.15 mmol) in acetonitrile (25 mL) was added to a 50 mL round bottom flask equipped with a magnetic stirrer. Subsequently, elemental iodine (392mg, 1.54 mmol) and water (1 mL) were added to the solution, and it allowed to stir for 22h at room temperature under nitrogen atmosphere. After that time, the solution was quenched with 50 mL $Na_2S_2O_3$ (20% w/w aqueous solution) and extracted three times with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The crude concentrate was then purified by flash column chromatography, first using ethyl acetate/petroleum ether (3:7) as eluent, and then gradually increasing the proportion of ethyl acetate until the eluent was pure ethyl acetate. That afforded the product (*S*)-**8**, which solidified upon drying under vacuum into a slightly yellow solid. It was then recrystallized in hexane which afforded colourless fine needles (1092mg, 97% yield). TLC (Silica, ethyl acetate:petroleum ether, 30:70): Rf = 0.11. Mp. 43.1-43.6°C. $[\alpha]^{20}_D$ = +2.48 (c. 1.73, CH_2Cl_2). IR (NaCl, ν_{max} / cm^{-1}): 3389 (br), 2935 (s), 2837 (vs), 1612 (m), 1463 (m), 1247 (s), 1033 (vs). ^1H NMR (400 MHz, $CDCl_3$) δ_H: 7.25 (d, *J*=8.7 Hz, 2H, Ph-H), 6.89 (d, *J*=8.7 Hz, 2H, Ph-H), 4.49 (s, 2H, $PhCH_2$), 4.88 (m, 1H, CH *sn*-2), 3.81 (s, 3H, OCH_3), 3.71 (dd, *J*=11.5, 3.8 Hz, 1H, CH_2 *sn*-3), 3.63 (dd, *J*=11.5, 5.6 Hz, 1H, CH_2 *sn*-3), 3.57 (dd, *J*=9.6, 3.6 Hz, 1H, CH_2 *sn*-1), 3.52 (dd, *J*=9.6, 3.5 Hz, 1H, CH_2 *sn*-1), 2.59 (br s, 1H, OH), 2.10 (br s, 1H, OH) ppm. $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ_C: 159.4, 129.8, 129.5 (2), 113.9 (2), 73.4, 71.5, 70. 6, 63.9, 55.3 ppm. HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{11}H_{16}O_4Na$ 235.0941; found, 235.0943.

3.2.3. Synthesis of 3-Dodecanoyl-1-O-(p-methoxybenzyl)-sn-glycerol, (R)-9a

PMB-protected glycerol (*S*)-**8** (130mg, 0.55 mmol) and vinyl laurate (160mg, 0.71 mmol) dissolved in dichloromethane (3 mL) were added under slow magnetic stirring to a 10 mL round bottom flask. Subsequently, an immobilized *Candida antarctica* lipase (CAL-B) (28mg, 10% w/w) was added, and the atmosphere replaced with nitrogen gas. The mixture was allowed to stir for 4h while being monitored by TLC. After that time, the reaction was complete, and the lipase was filtered off. The solvent was removed *in vacuo*, the crude concentrate washed with a 15mg/mL $NaHCO_3$ / methanol (1:1) solution and then extracted twice with hexane. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was then purified by flash column chromatography with 4% boric acid impregnated silica gel using ethyl acetate:hexane (2:8) as eluent affording the product (I)-**9a** as a colourless liquid (231mg, quantitative yield). TLC (Silica, ethyl acetate:petroleum ether, 20:80): Rf = 0.27. $[\alpha]^{20}_D$ = -1.28 (c. 2.50, CH_2Cl_2). IR (NaCl, ν_{max} / cm^{-1}): 3449 (br), 2925 (s), 2854 (vs), 1736 (vs), 1612 (m), 1466 (m), 1377 (m), 1248 (s), 1037 (m). ^1H NMR (400 MHz, $CDCl_3$) δ_H: 7.25 (d, *J*=9.0 Hz, 2H, Ph-H), 6.88 (d, *J*=8.7 Hz, 2H, Ph-H), 4.49 (s, 2H, $PhCH_2$), 4.17 (dd, *J*=11.5, 4.4 Hz, 1H, CH_2 *sn*-3), 4.12 (dd, *J*=11.5, 6.0 Hz, 1H, CH_2 *sn*-3), 4.01 (m, 1H, CH *sn*-2), 3.81 (s, 3H, OCH_3), 3.52 (dd, *J*=9.6, 4.3 Hz, 1H, CH_2 *sn*-1), 3.46 (dd, *J*=9.6, 6.2 Hz, 1H, CH_2 *sn*-1), 2.49 (br s, 1H, OH), 2.32 (t, *J*=7.6 Hz, 2H, CH_2COO), 1.61 (m, 2H, CH_2CH_2COO), 1.29-1.24 (m, 16H, CH_2), 0.88 (t, *J*=6.8 Hz, 3H, CH_2CH_3) ppm. $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ_C: 174.1, 159.5, 129.9, 129.6 (2), 114.0 (2), 73.3, 70.7, 69.1, 65.5, 55.4, 34.3, 32.1, 29.7, 29.6, 29.45, 29.4 (2), 29.3, 25.1, 22.8, 14.3 ppm. HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{23}H_{38}O_5Na$ 417.2611; found, 417.2610.

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3.2.4-5. Synthesis of (R)-9b and (R)-9c

See full experimental details in *Supplementary Materials*

3.2.6. Synthesis of 3-Dodecanoyl-1-O-(p-methoxybenzyl)-2-[(9Z)-octadec-9-enoyl)]-sn-glycerol, (R)-10a

Monoacylglycerol (*R*)-**9a** (200mg, 0.51 mmol) and oleic acid (165mg, 0.58 mmol) in dry dichloromethane (5mL) were added to a flame-dried 10 mL round bottom flask equipped with a magnetic stirrer. Then 4-dimethylaminopyridine (DMAP) (50 mg, 0.41 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (117mg, 0.61 mmol) were added to the solution and stirred at room temperature, under nitrogen for 20h. After that time, the reaction mixture was passed through a short column packed with silica gel by using ethyl acetate. The solvent was removed *in vacuo,* and the crude concentrate was then purified by flash column chromatography using ethyl acetate/hexane (1:9) as eluent affording the product (*R*)-**10a** as a colourless liquid (188mg, 99% yield). TLC (Silica, ethyl acetate:petroleum ether, 20:80): Rf = 0.60. $[\alpha]^{20}_{D}$ = -6.77 (c. 1.92, CH_2Cl_2). IR (NaCl, ν_{max} / cm^{-1}): 3003 (m) ,2925 (s), 2854 (s), 1743 (s), 1613 (m), 1464 (m), 1248 (s), 1172 (s), 1038 (m). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 7.23 (d, *J*=8.8 Hz, 2H, Ph-H), 6.87 (d, *J*=8.8 Hz, 2H, Ph-H), 5.34 (m, 2H, =CH), 5.23 (m, 1H, CH *sn*-2), 4.45 (ABq, $\Delta\delta_{AB}$=0.04, *J*=11.8, 2H, $PhCH_2$), 4.33 (dd, *J*=11.9, 3.9 Hz, 1H, CH_2 *sn*-3), 4.17 (dd, *J*=11.9, 6.5 Hz, 1H, CH_2 *sn*-3), 3.80 (s, 3H, OCH_3), 3.55 (dd, *J*=5.2, 1.3 Hz, 2H, CH_2 *sn*-1), 2.31 (t, *J*=7.6 Hz, 2H, CH_2COO SFA), 2.27 (t, *J*=7.6 Hz, 2H, CH_2COO MUFA), 2.01 (m, 4H, CH_2CH=), 1.65-1.56 (m, 4H, CH_2CH_2COO), 1.36-1.22 (m, 36H, CH_2), 0.88 (t, *J*=6.7 Hz, 6H, CH_2CH_3) ppm. ^{13}C{H} NMR (101 MHz, $CDCl_3$) δ_C: 173.6, 173.2, 159.5, 130.2, 129.92, 129.87, 129.5 (2), 114.0 (2), 73.1, 70.2, 68.1, 62.8, 55.4, 34.5, 34.3, 32.1 (2), 29.92, 29.87, 29.8, 29.7, 29.6 (2), 29.49 (2), 29.47 (2), 29.44, 29.36, 29.3 (2), 29.2, 27.4, 27.3, 25.1, 25.0, 22.8, 14.3 (2) ppm. $[M + Na]^+$ calcd for $C_{41}H_{70}O_6Na$ 681.5065; found, 681.5050.

3.2.7-10. Synthesis of (R)-10b, (R)-10c, (R)-10d and (R)-10e

See full experimental details in *Supplementary Materials*

3.2.11. Synthesis of 3-Dodecanoyl-2-[(9Z)-octadec-9-enoyl)]-sn-glycerol, (R)-11a

Diacylglycerol (*R*)-**10a** (306mg, 0.465 mmol) was added to a 25mL round bottom flask in dichloromethane (6 mL) equipped with a magnetic stirrer. Water (1 mL) was pipetted to the solution, and it was cooled down to 0°C. Subsequently 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (137mg, 0.60 mmol) was added, which turned the solution to a dark green colour. The mixture was stirred under nitrogen for an hour and then the cooling bath was removed. It was allowed to stir for additional three hours at room temperature, during which the colour slowly changed to colourless with a bright red aqueous phase. When all the dark colour had vanished, the reaction was complete as indicated by TLC monitoring. The reaction mixture was extracted three times with dichloromethane and the combined organic layers washed with $NaHCO_3$, water and brine. Then they were dried over $MgSO_4$ and concentrated *in vacuo*, and the crude concentrate then purified by flash column chromatography with 4% boric acid impregnated silica gel using ethyl acetate:hexane (1:9) as eluent affording the product (*R*)-**11a** as a colourless liquid (227mg, 91% yield). TLC (Silica, ethyl acetate:petroleum ether, 20:80): Rf = 0.41. $[\alpha]^{20}_{D}$ = +2.43 (c. 4.24, CH_2Cl_2). IR (NaCl, ν_{max} / cm^{-1}): 3480 (br), 3004 (m), 2925 (vs), 2854 (vs), 1744 (s), 1466 (m), 1352 (m), 1167 (s). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 5.34 (m, 2H, =CH), 5.08 (p, *J*=4.9 Hz, 1H, CH *sn*-2), 4.32 (dd, *J* = 12.0, 4.6 Hz, 1H, CH_2 *sn*-3), 4.23 (dd, *J* = 12.0, 5.7 Hz, 1H, CH_2 *sn*-3), 3.72 (br s, 2H, CH_2 *sn*-1), 2.34 (t, *J*=7.5 Hz, 2H, CH_2COO SFA), 2.32 (t, *J*=7.6 Hz, 2H, CH_2COO MUFA), 2.09 (br s, 1H, OH), 2.01 (q, *J*=6.6 Hz, 4H, CH_2CH=), 1.62 (m, 4H, CH_2CH_2COO), 1.39– 1.22 (m, 36H, CH_2), 0.88 (t, *J*=6.7, 6H, CH_2CH_3) ppm. ^{13}C{H} NMR (101 MHz, $CDCl_3$) δ_C: 173.9, 173.5, 130.2, 129.8, 72.3, 62.1, 61.7, 34.4, 34.2, 32.0 (2), 29.9, 29.84, 29.75 (2), 29.7, 29.6, 29.5 (2), 29.4, 29.32, 29.29, 29.25 (2), 29.2, 27.4, 27.3, 25.1, 25.0, 22.8 (2), 14.3 (2) ppm. $[M + Na]^+$ calcd for $C_{33}H_{62}O_5Na$ 561.4489; found, 561.4470.

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3.2.12-13. Synthesis of 2-[(9Z)-Octadec-9-enoyl)]-3-tetradecanoyl-sn-glycerol, (R)-11b and (R)-11c

See full experimental details in *Supplementary Materials*

3.2.14. Synthesis of 3-Hexadecanoyl-2-[(9Z,12Z)-octadeca-9,12-dienoyl)]-sn-glycerol, (R)-11e

Diacylglycerol (*R*)-**10e** (1297mg, 1.82 mmol) was added to a 50mL round bottom flask in dichloromethane (5mL) equipped with a magnetic stirrer. Then water (3mL) was pipetted to the solution. Subsequently 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (412mg, 1.82 mmol) was dissolved in dichloromethane (10mL) and slowly added dropwise to the solution over a 30min period. The mixture was stirred vigorously for additional 1.5h until the characteristic dark colour had vanished. The reaction mixture was extracted three times with dichloromethane and the combined organic layers washed with $NaHCO_3$, water and brine. Then they were dried over Na_2SO_4 and concentrated *in vacuo*, and the crude concentrate then purified by flash column chromatography with 4% boric acid impregnated silica gel using ethyl acetate:hexane (1:9) as eluent affording the product (*R*)-**11e** as a colourless liquid (390mg, 36% yield) along with unreacted (*R*)-**10e** (562mg, 43% recovery). TLC (Silica, ethyl acetate:petroleum ether, 20:80): Rf = 0.40. $[\alpha]^{20}_D$ = +2.45 (c. 2.57, CH_2Cl_2). IR (ATR, ν_{max} / cm^{-1}): 3213 (br), 3009 (m), 2925 (vs), 2853 (vs), 1713 (vs), 1465 (m), 1349 (m), 1162 (s). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 5.36 (m, 4H, =CH), 5.08 (m, 1H, CH *sn*-2), 4.32 (dd, *J*=11.9, 4.6 Hz, 1H, CH_2 *sn*-3), 4.23 (dd, *J*=11.9, 5.6 Hz, 1H, CH_2 *sn*-3), 3.73 (m, 2H, CH_2 *sn*-1), 2.77 (t, *J*=6.6 Hz, 2H, =CHCH_2CH=), 2.32 (t, *J*=7.7 Hz, 2H, CH_2COO PUFA), 2.31 (t, *J*=7.6 Hz, 4H, CH_2COO SFA), 2.05 (q, *J*=6.8 Hz, 4H, CH_2CH=), 1.65–1.58 (m, 4H, CH_2CH_2COO), 1.33–1.25 (m, 38H, CH_2), 0.89 (t, *J*=6.9, 3H, CH_2CH_3 PUFA), 0.88 (t, *J*=7.0, 6H, CH_2CH_3) ppm. ^{13}C{H} NMR (101 MHz, $CDCl_3$) δ_C: 173.9, 173.5, 130.4, 130.1, 128.2, 128.0, 72.3, 62.1, 61.7, 34.4, 34.3, 32.1, 31.7, 29.9 (3), 29.81 (2), 29.76, 29.6, 29.51, 29.50, 29.4, 29.33, 29.27 (2), 29.2, 27.4 (2), 25.8 (2), 25.1, 25.0, 22.8, 22.7, 14.3, 14.2 ppm. $[M + Na]^+$ calcd for $C_{37}H_{68}O_5Na$ 615.4964; found, 615.5067.

3.2.15. Synthesis of 3-Dodecanoyl-1-[(9Z,12Z)-octadeca-9,12-dienoyl)]-2-[(9Z)-octadec-9-enoyl)]-sn-glycerol, (S)-1

Diacylglycerol (*R*)-**11a** (72mg, 0.13 mmol) and linoleic acid (43mg, 0.15 mmol) in dry dichloromethane (3 mL) were added to a 10 mL flame-dried round bottom flask equipped with a magnetic stirrer. Then 4-dimethylaminopyridine (DMAP) (13mg, 0.11 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (31mg, 0.16 mmol) were added to the solution and stirred at room temperature, under nitrogen for 20 h. After that time, the reaction mixture was passed through a short column packed with silica gel by using ethyl acetate. The solvent was removed *in vacuo*, and the crude concentrate then purified by flash column chromatography using ethyl acetate:hexane (1:19) as eluent affording the product (*S*)-**1** as a colourless liquid (91mg, 85% yield). TLC (Silica, ethyl acetate:petroleum ether, 20:80): Rf = 0.75. $[\alpha]^{20}_D$ = +0.03 (c. 2.96, CH_2Cl_2). IR (NaCl, ν_{max} / cm^{-1}): 3008 (s), 2926 (vs), 2855 (s), 1747 (s), 1464 (m), 1378 (m), 1161 (s). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 5.35 (m, 6H, =CH), 5.26 (m, 1H, CH *sn*-2), 4.29 (dd, *J*=11.9, 4.3 Hz, 2H, CH_2 *sn*-1/*sn*-3), 4.14 (dd, *J*=11.9, 6.0 Hz, 2H, CH_2 *sn*-1/*sn*-3), 2.77 (t, *J*=6.6 Hz, 2H, =CHCH_2CH=), 2.32 (t, *J*=7.5 Hz, 2H, CH_2COO), 2.31 (t, *J*=7.6 Hz, 4H, CH_2COO), 2.04 (q, *J*=6.8 Hz, 4H, CH_2CH= MUFA), 2.01 (q, *J*=6.6 Hz, 4H, CH_2CH= PUFA), 1.65-1.57 (m, 6H, CH_2CH_2COO), 1.38–1.21 (m, 50H, CH_2), 0.89 (t, *J*=6.9, 3H, CH_2CH_3 PUFA), 0.88 (t, *J*=6.7, 6H, CH_2CH_3) ppm. ^{13}C{H} NMR (101 MHz, $CDCl_3$) δ_C: 173.43, 173.38, 173.0, 130.4, 130.17, 130.15, 129.8, 128.2, 128.0, 69.0, 62.2 (2), 34.3, 34.20, 34.17, 32.1 (2), 31.7, 29.91, 29.87, 29.8 (3), 29.7, 29.6, 29.49 (2), 29.47 (2), 29.41, 29.35, 29.32, 29.28, 29.27 (2), 29.23, 29.20, 27.4, 27.34 (2), 27.32, 25.8, 25.03, 25.01, 24.98, 22.8 (2), 22.7, 14.3 (2), 14.2 ppm. $[M + Na]^+$ calcd for $C_{51}H_{92}O_6Na$ 823.6786; found, 823.6766.

3.2.16-22. Synthesis of (S)-2, (R)-12a, (R)-12b, (R)-12c, (R)-12d, (R)-12e and (R)-12f

See full experimental details in *Supplementary Materials*

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3.3. Synthesis of the USU' Subclass Category TAGs (S)-5 and 6

3.3.1. Synthesis of Oleic Acid Acetoxime Ester, 13

Oleic acid (500mg, 1.77 mmol) was added to a 100 mL flame-dried two-necked round bottom flask equipped with a magnetic stirrer in dry dichloromethane (8mL). Acetone oxime (130mg, 1.77 mmol), DMAP (43mg, 0.35 mmol) and EDCI (407mg, 2.12 mmol) were added to the solution and it stirred at room temperature, under nitrogen for 20h. After that time, the reaction mixture was flushed through a short column packed with silica gel with ethyl acetate. The solvent was removed *in vacuo,* and the crude concentrate then purified by flash column chromatography using ethyl acetate:hexane (1:9) as eluent affording the product **13** as a colourless liquid (596mg, quantitative yields). TLC (Silica, ethyl acetate:petroleum ether, 30:70): Rf = 0.59. IR (NaCl, ν_{max} / cm^{-1}): 2925 (s), 2854 (vs), 1765 (vs), 1654 (w), 1460 (m), 1377 (m), 1271 (m), 1136(s). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 5.34 (m, 2H, =CH), 2.40 (t, *J*=7.6 Hz, 2H, CH_2COO), 2.05 (s, 3H, $(CH_3)_2C=N$), 2.01 (m, 4H, $CH_2CH=$), 1.99 (s, 3H, $(CH_3)_2C=N$), 1.69 (p, *J*=7.5 Hz, 2H, CH_2CH_2COO), 1.38-1.23 (m, 20H, CH_2), 0.87 (t, *J*=6.7 Hz, 3H, CH_2CH_3) ppm. $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ_C: 171.4, 163.8, 130.2, 129.9, 33.2, 32.0, 29.9, 29.8, 29.7, 29.5 (2), 29.3, 29.2 (2), 27.4, 27.3, 25.1, 22.8, 22.2, 17.1, 14.3 ppm. $[M + Na]^+$ calcd for $C_{21}H_{39}O_2NNa$ 360.2873; found, 360.2879.

3.3.2. Synthesis of 1-O-(p-Methoxybenzyl)-3-[(9Z)-octadec-9-enoyl)]-sn-glycerol, (R)-14

PMB-protected glycerol (*S*)-**8** (300mg, 1.41 mmol) and oleic acid acetoxime ester **13** (573mg, 1.70 mmol) were added to a flame dried 5mL round bottom flask. Subsequently, immobilized *Candida antarctica* lipase B (CAL-B) (70mg, 8% w/w) was added, the flask connected to a vacuum pump system (10^{-2} mmHg) and the resulting mixture stirred at room temperature for 6h. After that time, the vacuum was disconnected and additional CAL-B (10mg) along with dried dichloromethane (1.5mL) were added to the flask. The mixture was allowed to stir under nitrogen atmosphere overnight. Then the reaction was complete, and the lipase was filtered off. The solvent was removed *in vacuo,* and the crude concentrate then purified by flash column chromatography with 4% boric acid impregnated silica gel using ethyl acetate:hexane (2:8) as eluent affording the product (*R*)-**14** as a colourless liquid (585mg, 87% yield). TLC (Silica, ethyl acetate:petroleum ether, 30:70): Rf = 0.50. $[\alpha]^{20}_D$ = -0.72 (c. 1.67, CH_2Cl_2). IR (NaCl, ν_{max} / cm^{-1}): 3449 (br), 2925 (s), 2854 (vs), 1739 (vs), 1612 (m), 1463 (m), 1377 (m), 1248 (s). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 7.25 (d, *J*=9.1 Hz, 2H, Ph-H), 6.88 (d, *J*=8.8 Hz, 2H, Ph-H), 5.34 (m, 2H, =CH), 4.49 (s, 2H, $PhCH_2$), 4.17 (dd, *J*=11.5, 4.5 Hz, 1H, CH_2 *sn*-3), 4.12 (dd, *J*=11.5, 6.1 Hz, 1H, CH_2 *sn*-3), 4.01 (m, 1H, CH *sn*-2), 3.81 (s, 3H, OCH_3), 3.52 (dd, *J*=9.6, 4.3 Hz, 1H, CH_2 *sn*-1), 3.46 (dd, *J*=9.6, 6.1 Hz, 1H, CH_2 *sn*-1), 2.48 (d, *J*=4.9 Hz, 1H, OH), 2.32 (t, *J*=7.6 Hz, 2H, CH_2COO), 2.00 (m, 4H, $CH_2CH=$), 1.61 (m, 2H, CH_2CH_2COO), 1.38-1.23 (m, 20H, CH_2), 0.88 (t, *J*=6.7 Hz, 3H, CH_2CH_3) ppm. $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ_C: 174.1, 159.5, 130.2, 129.89, 129.87, 129.6 (2), 114.0 (2), 73.3, 70.7, 69.1, 65.5, 55.4, 34.3, 32.0, 29.9, 29.8, 29.7, 29.5 (2), 29.31, 29.25 (2), 27.4, 27.3, 25.0, 22.8, 14.3 ppm. $[M + Na]^+$ calcd for $C_{29}H_{48}O_5Na$ 499.3394; found, 499.3353.

3.3.3-8. Synthesis of (R)-15a, (R)-15b, (R)-16a, (R)-16b, (S)-5 and (S)-6

See full experimental details in *Supplementary Materials*

3.4. Synthesis of the SUU' Subclass Category TAGs (S)-3 and 4

3.4.1. Synthesis of 3-Dodecanoyl-sn-glycerol, (R)-17

Acylglycerol (*R*)-**9a** (200mg, 0.51 mmol) dissolved in dichloromethane (3mL) was added to a 10mL round bottom flask equipped with a magnetic stirrer. Water (1mL) was pipetted to the solution, which was cooled down to 0°C. Subsequently DDQ (115mg, 0.51 mmol) was added, which turned the solution to a dark green colour. The cooling bath was removed after 30min. The solution was stirred under nitrogen atmosphere overnight with the magnetic stirrer on a full speed. After that time the reaction mixture was extracted three times with dichloromethane and the combined organic

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layers washed with water, aqueous $NaHCO_3$ and brine. Then they were dried over Na_2SO_4, concentrated *in vacuo*, and the crude concentrate then purified by flash column chromatography with 4% boric acid impregnated silica gel using a gradient solvent system from ethyl acetate:hexane (1:9) to ethyl acetate:hexane (1:1) as eluent affording the product (*R*)-**17** as a white waxy solid (139mg, 72% yield). TLC (Silica, ethyl acetate:petroleum ether, 50:50): Rf = 0.18. Mp. 53.8-54.3°C. $[\alpha]^{20}_D$ = -1.62 (c. 1.11, CH_2Cl_2). IR (ATR, ν_{max} / cm^{-1}): 3300 (br), 2956 (m), 2918 (vs), 2849 (vs), 1733 (s), 1463 (m), 1175 (s). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: δ 4.21 (dd, *J*=11.8, 4.6 Hz, 1H, CH_2 *sn*-3), 4.15 (dd, *J*=11.6, 6.1 Hz, 1H, CH_2 *sn*-3), 3.93 (m, 1H, CH *sn*-2), 3.70 (ddd, *J*=10.4, 6.4, 4.1 Hz, 1H, CH_2 *sn*-1), 3.60 (dd, *J*=11.4, 5.6 Hz, 1H, CH_2 *sn*-1), 2.51 (d, *J*=5.1 Hz, 1H, CHO*H*), 2.35 (t, *J*=6.1, 2H, C*H*$_2$COO), 2.07 (t, *J*=6.1 Hz, 1H, CH_2O*H*),1.64 (p, *J*=7.6 Hz, 2H, C*H*$_2$$CH_2$COO), 1.32–1.22 (m, 16H, CH_2), 0.88 (t, *J*=6.9, 3H, CH_2C*H*$_3$) ppm. ^{13}C{H} NMR (101 MHz, $CDCl_3$) δ_C: 174.5, 70.4, 65.3, 63.5, 34.3, 32.1, 29.7 (2), 29.6, 29.5, 29.4, 29.3, 25.1, 22.8, 14.3 ppm. $[M + Na]^+$ calcd for $C_{15}H_{30}O_4Na$ 297.2036; found, 297.2023.

3.4.2. Synthesis of [2-(p-Methoxyphenyl)-1,3-dioxolan-4-yl]methyl dodecanoate, (R)-18

Acylglycerol (*R*)-**9a** (100mg, 0.25 mmol) was added to a 10mL round bottom flask in dichloromethane (3mL) equipped with a magnetic stirrer. Water (0.5mL) was pipetted to the solution, and it was cooled down to 0°C. Subsequently DDQ (75mg, 0.33 mmol) was added, which turned the solution to a dark green colour. The mixture was stirred under nitrogen for 30 min and then the cooling bath was removed. It was allowed to stir for additional five hours at room temperature, during which the colour slowly changed to bright red. When the dark colour had vanished, the reaction was finished, and it was extracted three times with dichloromethane. The combined organic layers were washed with aqueous $NaHCO_3$, water and brine. Then they were dried over Na_2SO_4, concentrated *in vacuo*, and the crude concentrate then purified by flash column chromatography with 4% boric acid impregnated silica gel using ethyl acetate:petroleum ether (1:9) as eluent affording the product (*R*)-**18** as a colourless liquid (38mg, 54% yield). TLC (Silica, ethyl acetate:petroleum ether, 50:50): Rf = 0.71. $[\alpha]^{20}_D$ = +5.63 (c. 1.92, CH_2Cl_2). IR (ATR, ν_{max} / cm^{-1}): 2923 (s), 2853 (s), 1737 (s), 1614 (w), 1464 (m), 1248 (vs), 1160 (s), 1032 (s). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 7.40 (dd, *J*=8.7, 6.2 Hz, 2H, Ph-H), 6.90 (d, *J*=7.8 Hz, 2H, Ph-H), 5.89 (s, 0.45H, OCH-Ph), 5.78 (s, 0.55H, OCH-Ph), 4.48 (m, 0.45H, CH *sn*-2), 4.43 (m, 0.55H, CH *sn*-2), 4.28 (dd, *J*=6.8, 1.8 Hz, 0.5H, CH_2 *sn*-1), 4.23 (m, 2H, CH_2 *sn*-3), 4.10 (dd, *J*=8.5, 7.1 Hz, 0.5H, CH_2 *sn*-1), 3.95 (dd, *J*=8.4, 5.1 Hz, 0.5H, CH_2 *sn*-1), 3.81 (s, 3H, OCH_3), 3.77 (dd, *J*=8.5, 6.8 Hz, 0.5H, CH_2 *sn*-1), 2.35 (m, 2H, CH_2COO), 1.63 (p, *J*=7.2 Hz, 2H, C*H*$_2$$CH_2$COO), 1.33-1.24 (m, 16H, CH_2), 0.88 (t, *J*=6.9 Hz, 3H, CH_2C*H*$_3$) ppm. ^{13}C{H} NMR (101 MHz, $CDCl_3$) δ_C: 173.82, 173.79, 160.8, 160.6, 129.7, 129.2, 128.3 (2), 128.1 (2), 113.93 (2), 113.91 (2), 104.8, 100.0, 74.2, 74.0, 67.6, 67.4, 64.6, 64.2, 55. 5 (2), 34.3 (2), 32.1 (2), 29.7 (4), 29.6 (2), 29.5 (2), 29.4 (2), 29.3 (2), 25.1 (2), 22.8 (2), 14.3 (2) ppm. $[M + Na]^+$ calcd for $C_{23}H_{36}O_5Na$ 415.2455; found, 415.2445.

3.4.3. Conversion of Acetal (R)-18 into (R)-17

Acetal (*R*)-**18** (32mg, 0.08 mmol) in acetonitrile (1mL) was added to a 10 mL round bottom flask equipped with a magnetic stirrer. Subsequently, elemental iodine (6mg, 0.02 mmol) and water (20µL) were added to the solution, and it allowed to stir for 22h at room temperature under nitrogen atmosphere. After that time, the solution was quenched with $Na_2S_2O_3$ (20% w/w aqueous solution) and extracted three times with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo.* The crude concentrate was then purified by flash column chromatography with 4% boric acid impregnated silica gel using gradient solvent system from ethyl acetate:hexane (1:9) to ethyl acetate:hexane (1:1) as eluent. That afforded the product (*R*)-**17** as a slightly yellow solid (20mg, 91% yield).

3.4.4-11. Synthesis of (R)-8, (S)-14, (S)-19, (S)-20a, (S)-20b, (S)-3 and (S)-4

See full experimental details in *Supplementary Materials*

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4. Conclusions

A lack of enantiopure TAGs as reference standards has been a major barrier in enantiospecific analysis of intact chiral TAGs in natural fats and oils to determine the separation order of TAG enantiomers. In recent years we have contributed various types of enantiostructured TAGs for that purpose including AAB and ABC type TAG enantiomers. In an attempt to expand the existing focused compound library of such standards the current work was undertaken that succeeded in the synthesis of ABC type TAG enantiomers belonging to both subclass categories of TAGs constituting two non-identical unsaturated fatty acids (oleic acid and linoleic acid) along with a saturated fatty acid. Herein we reported the synthesis of six enantiostructured ABC type TAGs. Four of them belong to the SUU' subclass category having the unsaturated fatty acids located in the *sn*-1 and *sn*-2 positions with a saturated fatty acid in the *sn*-3 position of the glycerol skeleton. The two remaining TAGs belong to the USU' subclass category with the different unsaturated fatty acids present in the *sn*-1 and *sn*-3 end positions with the saturated fatty acid in the *sn*-2 position.

As before, the highly regioselective immobilized *Candida antarctica* lipase (CAL-B) played a crucial role in the regiocontrol of the six-step chemoenzymatic synthesis, that was started from enantiopure solketals. The use of the ether PMB-protective group indeed allowed the incorporation of two different unsaturated fatty acids to the glycerol backbone. However, its use was limited to the presence of only a monounsaturated fatty acid in the deprotection step, since a higher unsaturation did not survive the mild oxidative treatment. That created challenges when dealing with the TAGs belonging to the SUU' subclass category that involved the PUFA in the *sn*-2 position, causing the overall yields to drop from the average of 52% over the six steps down to 30% which is still quite acceptable.

This is however, an important achievement since we were unable to prepare TAGs possessing two dissimilar unsaturated fatty acids by the previous strategy based on the use of the benzyl protecting moiety. This highly efficient synthetic strategy should prove of high use to synthesise a variety of similar and related enantiopure ABC type structured TAGs constituting two unsaturated fatty acids with one of them monounsaturated and the other polyunsaturated. It should be emphasized that the PUFA is not limited to linoleic acid and will most certainly suit the bioactive n-6 and n-3 long-chain PUFAs such as arachidonic acid, EPA and DHA that may be incorporated in the final step by aid of chemical coupling as has been demonstrated previously.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Comparison of the glycerol region of the PMB-protected solketal (*R*)-**7**, the PMB-protected glycerol (*S*)-**8** and the PMB-protected *sn*-3-MAG (*R*)-**9c** (p*S1*); Figure S2: Comparison of the glycerol region of the PMB-protected *sn*-3-MAG (*R*)-**9b** and the PMB-protected *sn*-2,3-DAG (*R*)-**10c** (p*S2*); Figure S3: Comparison of the glyceryl proton region of the ^{1}H NMR spectra for the diacylglycerol (*R*)-**16a** and the triacylglycerol (*S*)-**5** (p*S3*); Figure S4: Comparison of the glycerol proton region of the ^{1}H NMR spectra for the 1-MAG (S)-**19**, 1,3-DAG (S)-**20b**, 1,2-DAG (R)-**15a** and TAG (S)-**5** (p*S4*); Experimental Information: p*S5-S20*; NMR spectra: p*S21-S106*.

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