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Chemoenzymatic synthesis of structured triacylglycerols

Arnar Halldorsson, Carlos D. Magnusson and Gudmundur G. Haraldsson*

Science Institute, University of Iceland, Dunhaga 3, 107 Reykjavik, Iceland

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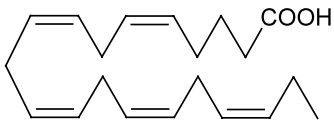
Abstract—Six regioisomerically pure structured triacylglycerols possessing a medium-chain fatty acid (C_8, C_{10} or C_{12}) at the primary positions and pure eicosapentaenoic acid or docosahexaenoic acid at the secondary position of the glycerol moiety were prepared in two steps by a chemoenzymatic approach using lipase.

Because of their high regioselectivity, lipases are effective catalysts for the synthesis of structured lipids[1,2] that have a predetermined composition and distribution of fatty acids at the glycerol backbone. Structured triacylglycerols (TAG) possessing long-chain polyunsaturated fatty acids (PUFA) located at the mid position with medium-chain fatty acids (MCFA) at the end positions have gained considerable attention recently as nutritional and health supplements.[3,4] The beneficial health effects of the n-3 PUFA are well established and have been attributed to *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA, **1**) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA, **2**)[5,6] (See Fig. 1). Therefore, structured triacylglycerols containing EPA or DHA located at the mid position with MCFA at the end postitions should be of particular interest.[7,8]

The main objective of the work described in this report was to synthesize structured TAG comprising pure EPA or DHA at the mid position with a pure MCFA (C_8, C_{10} or C_{12}) located at the end positions. The synthesis of such positionally labeled structured lipids requires a full regioselectivity control and can hardly be undertaken by traditional synthetic organic chemistry methods without multi-step protection–deprotection processes.

Lipases due to their 1,3-regioselectivity are ideally suited as biocatalysts for producing such structured lipids by acting preferably or exclusively at the primary positions of the glycerol moiety.[7,8] The mild conditions offered by lipases also help in retarding or preventing intramolecular acyl-migration side reactions which create problems in association with partially acylated diols or polyols.[9,10] Such processes must be avoided to ensure the required regioselectivity. On top of that the mildness offered by lipases may also be of benefit when the highly labile n-3 type PUFA are involved.[7,11]

A two-step chemoenzymatic route shown in Scheme 1 was designed to accomplish this goal. In the first step an immobilized *Rhizomucor miehei* lipase (Lipozyme™ provided by Novo Nordisk in Denmark) was exploited to esterify glycerol at the end positions with stoichiometric amount of caprylic, capric and lauric acids to afford the 1,3-diacylglycerols **3a**–**c**, respectively, in moderate yields (55–70%) after recrystallization from methanol. This step was based on procedures of Schneider and coworkers[12,13] with glycerol adsorbed on silica gel in diethyl ether at room temperature in the presence of molecular sieves.



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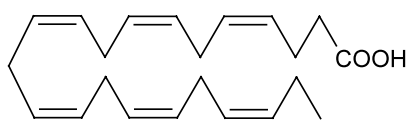

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Figure 1. Chemical structures of EPA (**1**) and DHA (**2**).

Keywords: DHA; 1,3-diacylglycerol; EPA; esterification; lipase; medium-chain fatty acids; n-3 fatty acids; structured triacylglycerol.
* Corresponding author. Tel.: +354-525-4818; fax: +354-552-8911; e-mail: gghar@raunvis.hi.is

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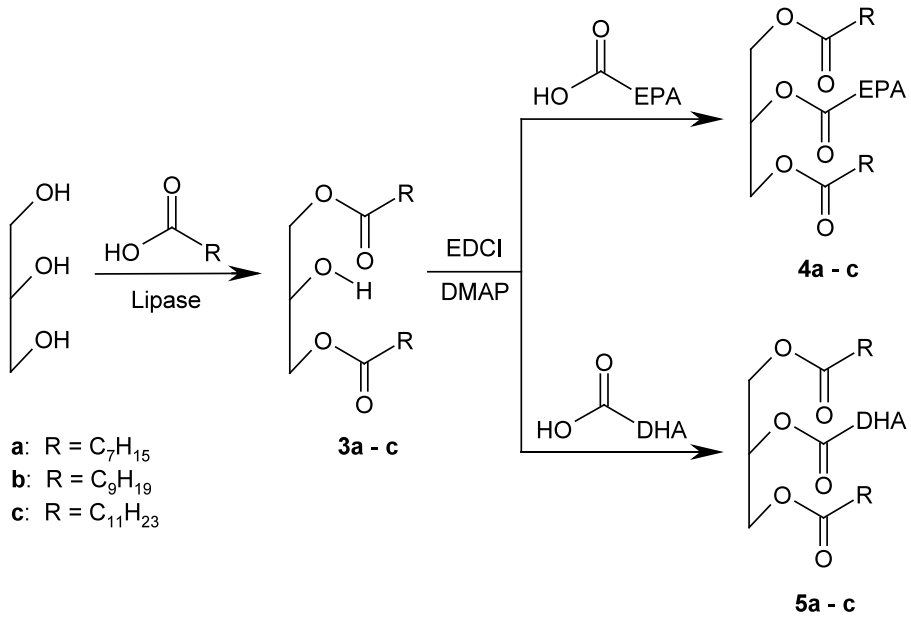

Scheme 1.

In the subsequent step 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) was used as a chemical coupling agent in the presence of 30–50% 4-dimethylaminopyridine (DMAP) as based on the number of mol in dichloromethane at room temperature for 24 h to introduce EPA and DHA into the mid-position of the 1,3-DAG adducts. Pure 2-EPA-TAG **4a–c** and 2-DHA-TAG **5a–c** were afforded as colorless oils and slightly yellowish oils, respectively for the EPA and DHA adducts, in yields of 90–95% after treatment on silica gel to get rid of a slight excess of EPA and DHA. Besides much higher yields EDCI offers the advantage over other coupling agents such as DCC[14] of possessing a polar amino group and being water soluble, which is of benefit when working-up the reaction and purifying the products. Presumably, DMAP serves both as a catalyst and a base for the acylation process, since far more than a catalytic amount was required for obtaining adequate reaction rate and optimal yields.

All intermediates and products were fully characterized by high-field ^{1}H and ^{13}C NMR and IR spectroscopy analysis. Elementary analysis (solid intermediates) and accurate mass spectrometry (oil products) data are under way. In the ^{1}H NMR spectra the protons belonging to the glyceryl backbone of the acylglycerols were highly useful for characterizing the products and evaluating their purity. These protons resonate quite characteristically in individual acylglycerols (1- and 2-monoacylglycerols, 1,2- and 1,3-DAG and TAG).[3,9] The ^{13}C NMR spectroscopy warrants a special comment as a powerful means to monitor the regiocontrol of the reactions. This is based on two distinctive resonance signals being present in the ^{13}C NMR spectra for the carbonyl group carbon of the fatty acids depending on their location at the end-positions (α) or the mid-position (β) of the glyceryl backbone.[3,9] In all products **3a–c** and **4a–c** the MCFA carbonyl carbons resonated at identical chemical shift, but there was a distinct chemical shift between the EPA and DHA carbonyl groups. This is illustrated in Fig. 2. The spectra displayed only two carbonyl peaks in the 2:1 ratio and there were no indications of any contamination of other type triacylglycerols implicating a full regiocontrol of the reactions. There are several reports in the recent literature on the synthesis of the caprylic acid adducts **4a** and **5a**. Yamane and co-workers[15,16] based their synthesis on a two-enzymatic step process. In the first step trieicosapentaenoin and tridocosahexaenoin were synthesized by a procedure of Haraldsson et al.[9] They were subsequently transesterified with caprylic acid or

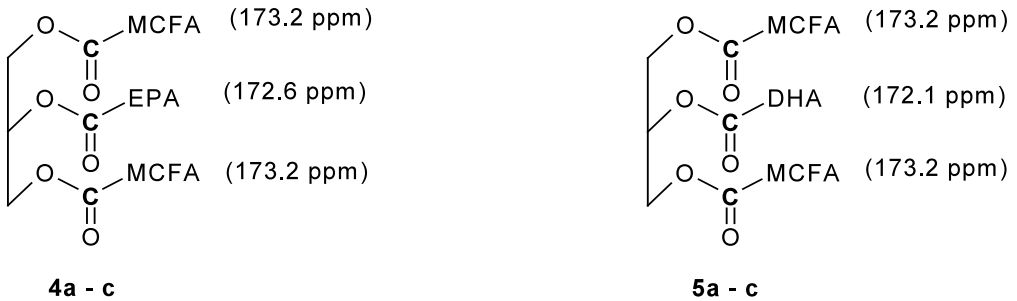

Figure 2. Assignment of fatty acids into TAG positions by ^{13}C NMR on carbonyl carbons.

its ethyl ester into the end positions by a 1,3-regioselective lipase. This approach has various drawbacks related to the three equivalents of pure highly valuable EPA or DHA required as well as a waste excess of the MCFA or its ester. Besides that, rather complicated mixtures were obtained requiring tedious separation and purification procedures. Shimada and co-workers[17] have managed to simplify that. Finally, Yamane and co-workers[18] have reported on a modification of their approach based on a highly 1,3-regioselective lipase catalyzed ethanolysis of the homogeneous EPA and DHA triacylglycerol intermediates and a subsequent lipase promoted esterification of the resulting 2-monoacylglycerols with a different lipase. Currently, we are highly successfully working on improving the yields of the 1,3-regioselective enzymatic reaction to make the reported approach even more feasible and efficient for the synthesis of the structured triacylglycerols described.

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