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Application of chemoenzymatic hydrolysis in the synthesis of 2-monoacylglycerols

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ABSTRACT

The selective biocatalyzed synthesis of 2-monoacylglycerols (2-MAGs) through the use of commercially available immobilized *Candida antarctica* (Novozym435) and *Rhizomucor miehei* is explored. Reactions at room temperature result in the formation of a 2-MAG and a corresponding ethyl ester of the fatty acid with immobilized *C. antarctica* within 2 h with yields ranging from 36% to 83%. Similar reaction conditions with immobilized *R. miehei* yielded exclusively the 2-MAG after 24 h with yields ranging from 37% to 88%. Yields vary on the acyl group at the *sn*-2 position and choice of enzyme involved.

1. Introduction

2-Monoacylglycerols (2-MAGs) exhibit beneficial emulsifying properties that are utilized in the food industry,[1,2] and in the administration of pharmaceuticals.[3] The polyunsaturated fatty acid (PUFA) occupying the *sn*-2 position is important in the influence of structured triglycerides absorption and digestion.[4] The biological effects of fatty acids released from the metabolism of glycerols and amides, or through ingestion, have also been studied.[5–7] One of the more intensively studied 2-MAGs, 2-arachidonoylglycerol (2-AG, **10b**), is a physiologically important lipid signaling molecule acting as a receptor ligand in the endocannabinoid system. Pharmacological properties of 2-AG include hypotension, neuroprotection, and appetite stimulation.[8]

2-AG and other 2-MAGs in biological systems are usually inactivated/catabolized by the hydrolyzing enzyme monoacylglycerol lipase (MAGL) to produce a fatty acid and glycerol.[9]

The synthesis and study of 2-MAGs is made difficult due to acyl migration from the *sn*-2 to the *sn*-1 or -3 position (Scheme 1).[10,11] This migration is facile and occurs in the presence of acid, base, heat, and protic solvents.[12,13] In the case of 2-AG, acyl migration renders 1-AG, which is incapable of binding to the endocannabinoid receptors.[14] Many reported syntheses of 2-MAGs involve multiple laborious steps with unfavorable reaction conditions and work ups that may promote the unwanted acyl migration. An earlier 2-MAG synthesis began with the coupling of the fatty acid to a 1,3-triisopropylsilyl (TIPS) glycerol. The removal of the silyl protecting groups required 24 h with the addition of acetic acid and tetrabutylammonium fluoride.[15] Another procedure involved coupling of fatty acid with 1,3-benzylideneglycerol and removal of the benzylidene with phenylboronic acid. The reaction resulted in the formation of the mixture of the 1,3- and 1,2-phenylboronate esters, which was separated and cleaved with methanol and water.[16] A third technique utilizes the ring opening of a glycidal ester with trifluoroacetic acid to produce a triacylglycerol. The 2-MAG was then formed after treatment of the triacylglycerol methanol and pyridine.[17]

Scheme 1.

Application of lipase in the syntheses of 1,3-diacylglycerol and 1(3)-*rac*-monoacyl glycerol has been extensively studied and reviewed.[18–26] The selectivity and yield are determined by various factors, which include amount of enzyme, solvent, temperature, and the type of lipase used.[27,28] Even though, the preparation of selective 1,3-diacylglycerols has been achieved successfully, it has been a challenging task for the synthesis of 2-acylglycerols mainly due to over hydrolysis and the acyl migration from *sn*-2 to *sn*-1 or

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sn-3 position. Lipase-mediated selective hydrolysis of triglyceride using 1,3-regiospecific lipases, esterification of fatty acids or transesterification of fatty esters with glycerol, and the glycerolysis of triglycerides have been documented in the literature.[29,30] Irimescu et al. reported a successful synthesis of various 2-acylglycerols of fatty acids using regiospecific ethanolysis of symmetrical triglycerides with immobilized *Candida antarctica* lipase (Novozym 435).[22,31] Even though *C. antarctica* is not considered as a 1,3-regiospecific enzyme, it has been consistently used for the preparation of 1,3-acylglycerols and ethanolysis of triglycerides.[31,32] All existing methods utilize symmetrical ('AAA' type) triglycerides resulting in the formation of corresponding ester as the byproduct that requires exhaustive purification (Fig. 1).

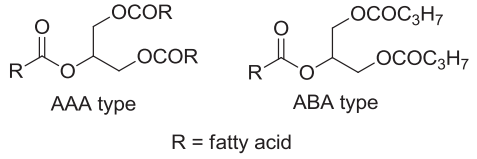


Fig. 1. Types of triglycerides.

Encouraged by this literature, we recently reported a method for the synthesis 2-AG, which utilizes a structured glyceride, 1,3-dibutyryl-2-arachidonate ('ABA' type), as a substrate, for we reasoned that the anticipated byproduct, ethyl butyrate, can be easily removed.[33] Benefits of this procedure include reactions at ambient temperature, neutral pH, and conservative reaction time. The method is simple and green, as the lipase can be recycled. Nevertheless, a significant amount of ethyl arachidonate formed due to over hydrolysis. Since the reaction is selective and proceeded quickly, it has become a valuable tool for the radiolabelled synthesis of 2-AG.[34] The following work extends our method to the synthesis of 2-acylglycerols starting from saturated and unsaturated fatty acids, and alkyl and aryl carboxylic acids.

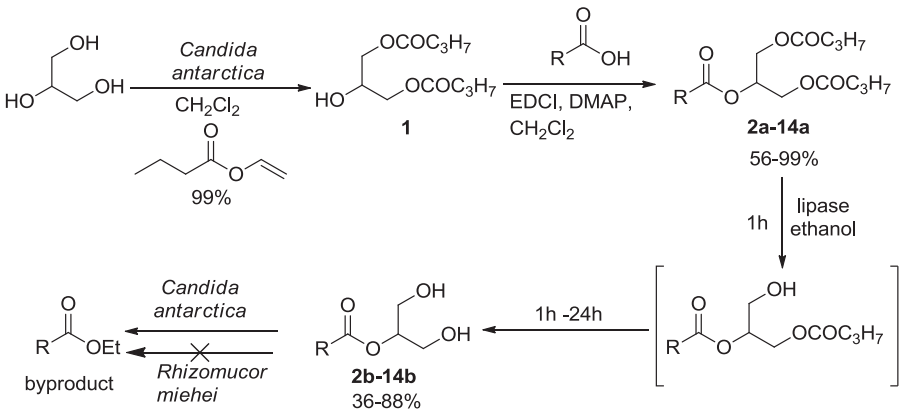
2. Results and discussion

To test the general practicality of our method (Scheme 2), we have synthesized 2-MAGs from various commercially available long-chain carboxylic acids, including those of biological importance. The synthesis began with the enzymatic 1,3-diacyl protection of glycerol by the addition of immobilized *C. antarctica* (Novozym 435) to glycerol and vinyl butyrate in anhydrous CH_2Cl_2 at 0 °C, resulting in the protected glycerol in quantitative yield.[32,35] The 1,3-diacylglycerol was then coupled to various medium and long-chain acids through 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) coupling in a 1:1 mixture of anhydrous THF/CH_2Cl_2 along with a catalytic amount of 4-dimethylaminopyridine (DMAP) at 0 °C for 4 h. This generated the structured triglyceride ('ABA' type) in 67–99% yield.

For the hydrolysis step, Novozym 435 was added to the triglyceride in a minimal amount of anhydrous ethanol at room temperature. By TLC analysis, it was observed that within 1 h the triglyceride had been completely consumed, and a mixture of 2-MAG, mono-protected 2-MAG, and ethyl butyrate was generated. There is no formation of ethyl ester of fatty acid observed during this period. At this point, additional lipase was added to the mixture, which was allowed to stir until all the mono-protected 2-MAG was consumed (1 h), affording the 2-MAG. Some significant amount of ethyl ester was observed during this period, and the formation of ethyl ester largely depended on the type of carboxylic acid used. Aryl and unsaturated carboxylic acids showed more resistant toward over hydrolysis compared to saturated fatty acids.

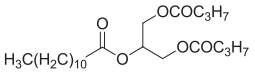
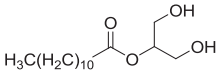
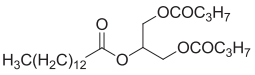
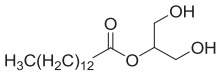
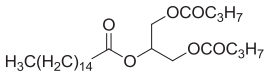
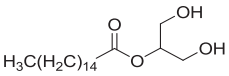
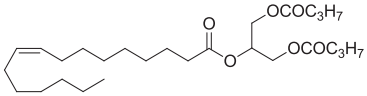
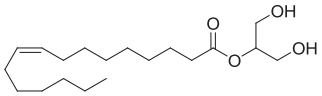
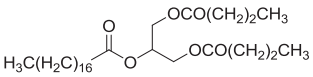
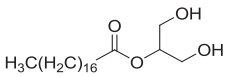
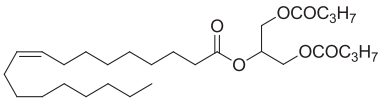
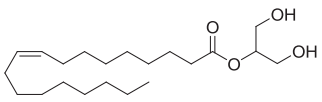
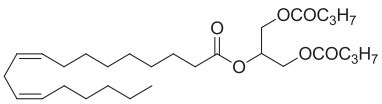
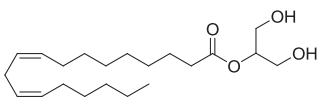
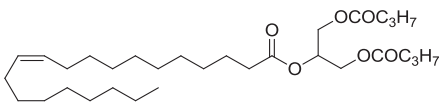
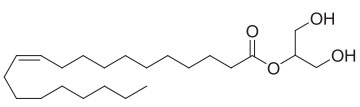
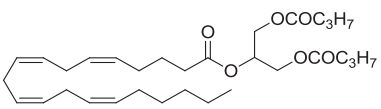
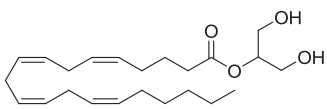
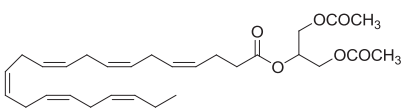
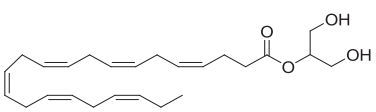
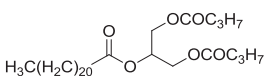
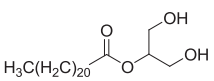
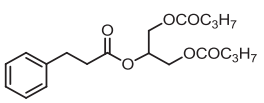
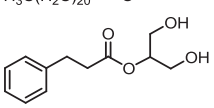
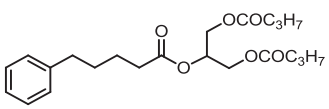
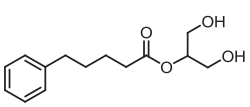
The separation of 1-MAG and 2-MAG is generally performed on boric acid impregnated TLC plates and silica gel columns.[36] Non-impregnated silica TLC plates do not resolve 1- and 2-MAG. This separation is a necessary step for most 2-MAG syntheses due to the unfavorable synthetic conditions used, which result in formation of considerable 1-MAG as well. In contrast, the highly regiospecific and neutral reaction conditions when using the lipase result in minimal or no 1-MAG formation. Although silica gel purification has been reported to be an inevitable cause of acyl migration in 2-MAG to 1-MAG,[37] we did not observe any migration during column chromatography with untreated silica gel. The only required step prior to purification was equilibration of silica gel with hexanes. During chromatography, the highly non-polar ethyl ester byproduct eluted with ethyl butyrate, and the 2-MAG was collected without any acyl migration. It was observed that the lipase-catalyzed hydrolysis reactions involving saturated triglycerides had isolated yields <50%, with the ethyl ester byproduct being the major product, whereas the unsaturated triglycerides had yields in the range of 55–75%, and triglycerides containing phenylalkyl groups had yields >80% (Table 1). It should also be noted that there was no observable difference in rate of reaction or isolated yield from the hydrolysis of a 1,3-diacetylglycerol-protected compound as compared to the 1,3-dibutrylglycerol-protected compound.

We also screened other 1,3-specific lipases to investigate whether the transformation can be performed in better yield and selectivity toward the range of substrates and found that lipase from *Rhizomucor miehei* showed excellent selectivity toward hydrolyzing 'ABA' type triglycerides. The reaction proceeded in a similar fashion where the triglyceride was consumed quickly, but hydrolysis of diglycerides took 24–48 h. Even though the reaction proceeded very slowly compared to *C. antarctica* lipase, the *R. miehei* lipase offered a remarkable improvement in selectivity,



Scheme 2.

Table 1
Structures and yields of lipase-catalyzed 2-MAGs

Compound no.	Triglyceride (a)	2-MAG (b)	*C. antarctica*[a] yield (%)	*R. miehei*[b] yield (%)
2			47	84
3			49	82
4			36	80
5			75	83
6			44	78
7			72	83
8			63	77
9			55	79
10			67	75
11			63	76
12			40	88
13			83	40
14			83	37

[a] Remaining yield consisted of the ethyl ester of *sn-2* acyl group.
[b] Remaining yield consisted of intermediate diglyceride.

providing exclusively 2-acylglycerols in excellent yields without formation of ethyl ester byproduct. The saturated and unsaturated fatty acid triglycerides were hydrolyzed in good yields (75–88%) after 24 h. However, in most of the reactions, some unreacted diglyceride intermediate remained. Allowing the reaction to proceed for an additional 24 h or adding more enzyme did not improve the yield. When unreacted diglyceride that was separated from the 2-MAG after 24 h was subjected to an additional treatment of *R. miehei* lipase the maximal yield once again reached 80% 2-MAG formation. Surprisingly, in contrast to *C. antarctica* lipase, *R. miehei* lipase showed less reactivity toward aryl esters (**13** and **14**).

3. Conclusion

Synthesis of 2-MAGs is complicated by the propensity of the acyl group to shift from the *sn*-2 to the more stable *sn*-1 or -3 positions, the acyl migration being promoted by heretofore standard reaction conditions. We demonstrate herein that chemoenzymatic hydrolysis of structured triglycerides is a mild and efficient means to synthesize 2-MAGs. The ambient temperature, neutral pH, and lack of caustic work up are conditions, which markedly limit 2-MAG acyl migration. The ability to synthesize 2-MAGs from 'ABA' type triglycerides is an important aspect of this current methodology in

comparison to utilizing 'AAA' type triglycerides. An excess of fatty acid is not required for this method, which is important when the preparation of the modified fatty acid involves laborious multistep-synthesis.[38–40] This will further enhance the study of structure–activity relationships of these biologically important lipid signaling molecules.

4. Experimental

4.1. General methods

Lipase acrylic resin from *C. antarctica* and Lipozyme®, immobilized from *R. miehei* were purchased from Sigma Aldrich (USA). All other reagents were used without prior purification. All reactions were performed under an atmosphere of argon.

All byproducts, ethyl arachidonate, ethyl butyrate, and all diglycerides were removed during column chromatography. All glycerols were purified on a Biotage Isolera One using Luknova prepackaged 12 g columns equilibrated with hexanes.

Compounds **3a**–**14a** were synthesized following the procedure described for **2a**; while compounds **3b**–**14b** were synthesized following the *C. antarctica* and *R. miehei* procedures described for **2b**.

4.2. 2-Hydroxypropane-1,3-diyl dibutyrate (1)

Immobilized *C. antarctica* (750 mg) was added to a solution of glycerol (2.0 g, 21.6 mmol) and vinyl butyrate (6.2 g, 54.0 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C. The resulting mixture was stirred for 3 h under argon atmosphere. Then, additional lipase (400 mg) was added to the reaction mixture, which was stirred for an additional 2 h at 0 °C. The lipase was filtered off, the solvent was evaporated off under reduced pressure, and the residue was chromatographed on silica to yield **1** (5.0 g, 99%) as a colorless oil. R_f=0.55 (40% ethyl acetate/hexanes). ^{1}H NMR (500 MHz, chloroform-*d*) δ=4.20 (dd, *J*=11.72, 4.39 Hz, 2H), 4.14 (dd, *J*=11.72, 5.86 Hz, 2H), 4.04–4.12 (m, 1H), 2.41–2.58 (m, 1H), 2.33 (t, *J*=7.32 Hz, 4H), 1.67 (sxt, *J*=7.32 Hz, 4H), 0.96 (t, *J*=7.57 Hz, 4H). The ^{13}C NMR spectral data (100 MHz, $CDCl_3$) are in agreement with literature values.[32]

4.3. 2-(Dodecanoyloxy)propane-1,3-diyl dibutyrate (2a)

EDCI (383 mg, 2.0 mmol), DMAP (19 mg, 0.16 mmol), and **1** (204 mg, 0.88 mmol) were added to a solution of lauric acid (160 mg, 0.80 mmol) in a 1:1 mixture of anhydrous THF/CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was allowed to stir for 4 h. The reaction mixture was then diluted with CH_2Cl_2 (15 mL) and H_2O (15 mL). The organic layer was separated, dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (0–15% ethyl acetate/hexanes) to yield **2a** (221 mg, 67%) as a colorless oil. R_f=0.50 (15% ethyl acetate/hexanes). ^{1}H NMR (400 MHz, chloroform-*d*) δ=5.24–5.31 (m, 1H), 4.30 (dd, *J*=12.09, 4.03 Hz, 2H), 4.16 (dd, *J*=12.46, 5.86 Hz, 2H), 2.27–2.35 (m, 6H), 1.57–1.70 (m, 6H), 1.21–1.36 (m, 16H), 0.92–0.98 (m, 6H), 0.88 (t, *J*=6.60 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-*d*) δ=173.4 (2C), 173.2, 77.4, 69.1, 62.3 (2C), 36.1 (2C), 34.4, 32.1, 29.8, 29.7, 29.6, 29.5, 29.3, 25.1, 22.9, 18.6 (2C), 14.4, 13.9 (2C). IR (neat, cm^{-1}) 2926, 2855, 1742, 1460. HRMS for $C_{23}H_{42}O_6Na$ (MNa^+) 437.2881. Calcd 437.2879.

4.4. 1,3-Dihydroxypropan-2-yl dodecanoate (2b, utilizing *C. antarctica*)

Immobilized *C. antarctica* (Novozym 435, 100 mg) was added to a solution of **2a** (100 mg, 0.24 mmol), stirred in anhydrous EtOH (1 mL). After the full consumption of **2a** (1 h, TLC monitoring), additional lipase (100 mg) was added until reaction completion was observed (1 h). The reaction mixture was diluted with CH_2Cl_2 (3 mL), and the lipase was filtered off. The solvent was removed under reduced pressure, and the resulting residue was chromatographed on silica gel (10–50% acetone/hexanes) to yield **2b** (31 mg, 47%) as a white solid. R_f=0.26 (30% acetone/hexanes). Mp=56–57 °C. ^{1}H NMR (400 MHz, chloroform-*d*) δ=4.93 (quin, *J*=4.76 Hz, 1H), 3.84 (br s, 4H), 2.38 (t, *J*=7.69 Hz, 2H), 2.08 (br s, 2H), 1.58–1.69 (m, 2H), 1.20–1.37 (m, 16H), 0.88 (t, *J*=6.60 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-*d*) δ=174.3, 75.3, 62.8 (2C), 34.6, 32.1, 29.8, 29.7, 29.6, 29.5, 29.3, 25.2 (2C), 22.9, 14.4. IR (neat, cm^{-1}) 3352, 2922, 2856, 1730, 1464. HRMS for $C_{15}H_{30}O_4Na$ (MNa^+) 297.2041. Calcd 297.2042.

4.5. 1,3-Dihydroxypropan-2-yl dodecanoate (2b, utilizing *R. miehei*)

Lipozyme®, immobilized from *R. miehei* (100 mg) was added to a solution of **2a** (100 mg, 0.24 mmol) stirred in anhydrous EtOH (1 mL). The reaction mixture was stirred for 24 h, diluted with CH_2Cl_2 (3 mL), and the lipase was filtered off. The solvent was removed under reduced pressure, and the resulting residue was chromatographed on silica gel (10–50% acetone/hexanes) to yield **2b** (55 mg, 84%) as an oil. All spectral data was consistent with that obtained using the procedure with *C. antarctica*.

4.6. 2-(Tetradecanoyloxy)propane-1,3-diyl dibutyrate (3a)

Yield 347 mg, 99%; colorless oil. R_f=0.47 (15% ethyl acetate/hexanes). ^{1}H NMR (399 MHz, chloroform-*d*) δ=5.32–5.22 (m, 1H), 4.29 (dd, *J*=4.4, 11.7 Hz, 2H), 4.15 (dd, *J*=5.9, 11.7 Hz, 2H), 2.35–2.25 (m, 6H), 1.70–1.56 (m, 6H), 1.36–1.19 (m, 20H), 0.94 (t, *J*=7.3 Hz, 6H), 0.87 (t, *J*=6.6 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-*d*) δ=173.4 (2C), 173.2, 69.1, 62.3 (2C), 36.1 (2C), 34.4, 32.2, 29.91, 29.89 (2C), 29.86, 29.7, 29.6, 29.5, 29.3, 25.1, 22.9, 18.6 (2C), 14.4, 13.9 (2C). IR (neat, cm^{-1}) 2925, 2854, 1741, 1460. HRMS for $C_{25}H_{46}O_6Na$ (MNa^+) 465.3195. Calcd 465.3192.

4.7. 1,3-Dihydroxypropan-2-yl tetradecanoate (3b)

C. antarctica: 34 mg, 49%; *R. miehei*: 57 mg, 82%; white solid. R_f=0.22 (30% acetone/hexanes). Mp=57–58 °C. ^{1}H NMR (399 MHz, chloroform-*d*) δ=4.93 (quin, *J*=4.8 Hz, 1H), 3.89–3.78 (m, 3H), 2.38 (t, *J*=7.3 Hz, 2H), 2.17–2.10 (m, 2H), 1.70–1.58 (m, 2H), 1.38–1.19 (m, 20H), 0.88 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-*d*) δ=174.3, 75.2, 62.8 (2C), 34.6, 32.2, 29.91, 29.87 (2C), 29.8, 29.7, 29.6, 29.5, 29.3, 25.2, 22.9, 14.4. IR (neat, cm^{-1}) 3418, 2926, 2855, 1729, 1466. HRMS for $C_{17}H_{34}O_4Na$ (MNa^+) 325.2354. Calcd 325.2355.

4.8. 2-(Palmitoyloxy)propane-1,3-diyl dibutyrate (4a)

Yield 309 mg, 84%; colorless oil. R_f=0.39 (15% ethyl acetate/hexanes). ^{1}H NMR (500 MHz, chloroform-*d*) δ=5.30–5.25 (m, 1H), 4.30 (dd, *J*=4.2, 12.0 Hz, 2H), 4.16 (dd, *J*=5.9, 11.7 Hz, 2H), 2.35–2.27 (m, 6H), 1.70–1.58 (m, 6H), 1.34–1.21 (m, 24H), 0.98–0.92 (m, 6H), 0.88 (t, *J*=6.8 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-*d*) δ=173.4, 173.2 (2C), 69.1, 62.3 (2C), 36.1, 34.4, 32.2, 29.9 (6C), 29.7, 29.6, 29.5, 29.3, 25.1 (2C), 18.6 (3C), 14.4, 13.9 (2C). IR (neat, cm^{-1}) 2924, 1742, 1460. HRMS for $C_{27}H_{50}O_6Na$ (MNa^+) 493.3503. Calcd 493.3505.

4.9. 1,3-Dihydroxypropan-2-yl palmitate (4b)

C. antarctica: 25 mg, 36%; *R. miehei*: 56 mg, 80%; white solid. R_f=0.27 (30% acetone/hexanes). Mp=64–65 °C. ^{1}H NMR (500 MHz, chloroform-*d*) δ=4.93 (quin, *J*=4.8 Hz, 1H), 3.84 (t, *J*=4.9 Hz, 4H),

2.38 (t, J=7.6 Hz, 2H), 2.13–2.05 (m, 2H), 1.69–1.59 (m, 2H), 1.38–1.20 (m, 24H), 0.88 (t, J=7.3 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-d) δ=174.3, 75.2, 62.8 (2C), 34.6, 32.2, 29.93, 29.92, 29.89, 29.84, 29.7, 29.6, 29.5, 29.3, 25.2 (2C), 23.3, 22.9, 14.4. IR (neat, cm^{-1}) 3320, 2917, 2850, 1730, 1471. HRMS for $C_{19}H_{38}O_4Na$ (MNa^+) 353.2668. Calcd 353.2668.

4.10. (*Z*)-2-(hexadec-9-enoyloxy)propane-1,3-diyl dibutyrate (5a)

Yield 291 mg, 79%; colorless oil. R_f=0.50 (15% ethyl acetate/hexanes). ^{1}H NMR (399 MHz, chloroform-d) δ=5.38–5.32 (m, 2H), 5.31–5.24 (m, 1H), 4.30 (dd, J=4.4, 11.7 Hz, 2H), 4.16 (dd, J=5.9, 12.5 Hz, 2H), 2.38–2.26 (m, 6H), 2.01 (q, J=6.6 Hz, 4H), 1.72–1.56 (m, 6H), 1.39–1.20 (m, 16H), 0.95 (t, J=7.3 Hz, 6H), 0.88 (t, J=7.0 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-d) δ=173.3 (2C), 173.1, 130.2, 129.9, 69.1, 62.3 (2C), 36.1 (2C), 34.4, 32.0, 30.0, 29.9, 29.4, 29.3, 29.25, 29.21, 27.5, 27.4, 25.1, 22.9, 18.6 (2C), 14.3, 13.8 (2C). IR (neat, cm^{-1}) 3007, 2928, 2856, 1742, 1459. HRMS for $C_{27}H_{48}O_6Na$ (MNa^+) 491.3347. Calcd 491.3349.

4.11. (*Z*)-1,3-Dihydroxypropan-2-yl hexadec-9-enoate (5b)

C. antarctica: 46 mg, 66%; *R. miehei*: 58 mg, 83%; colorless oil. R_f=0.25 (30% acetone/hexanes). ^{1}H NMR (399 MHz, chloroform-d) δ=5.40–5.31 (m, 2H), 4.92 (quin, J=4.8 Hz, 1H), 3.86–3.79 (m, 4H), 2.37 (t, J=7.7 Hz, 2H), 2.33 (br s, 2H), 2.05–1.97 (m, 4H), 1.63 (quin, J=7.3 Hz, 2H), 1.39–1.23 (m, 16H), 0.88 (t, J=6.6 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-d) δ=174.3, 130.3, 129.9, 75.2, 62.6 (2C), 34.6, 32.0, 30.0, 29.9, 29.4, 29.32, 29.30, 29.2, 27.4, 27.4, 25.2, 22.9, 14.3. IR (neat, cm^{-1}) 3405, 3008, 2924, 2855, 1736, 1462. HRMS for $C_{19}H_{36}O_4Na$ (MNa^+) 351.2512. Calcd 351.2511.

4.12. 2-(Stearoyloxy)propane-1,3-diyl dibutyrate (6a)

Yield 300 mg, 85%; colorless oil. R_f=0.34 (15% ethyl acetate/hexanes). ^{1}H NMR (500 MHz, chloroform-d) δ=5.30–5.24 (m, 1H), 4.30 (dd, J=4.4, 11.7 Hz, 2H), 4.16 (dd, J=6.1, 12.0 Hz, 2H), 2.34–2.27 (m, 6H), 1.70–1.59 (m, 6H), 1.35–1.20 (m, 28H), 0.98–0.93 (m, 6H), 0.88 (t, J=6.6 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-d) δ=173.4 (2C), 173.2, 69.1, 62.3 (2C), 36.2 (2C), 34.4, 32.2, 29.9 (4C), 29.89 (3C), 29.86, 29.7, 29.6, 29.5, 29.3, 25.1, 22.9, 18.6 (2C), 14.4, 13.9 (2C). IR (neat, cm^{-1}) 2924, 1742, 1460. HRMS for $C_{29}H_{54}O_6Na$ (MNa^+) 521.3813. Calcd 521.3818.

4.13. 1,3-Dihydroxypropan-2-yl stearate (6b)

C. antarctica: 32 mg, 44%; *R. miehei*: 56 mg, 78%; white solid. R_f=0.23 (30% acetone/hexanes). Mp=68–69 °C. ^{1}H NMR (399 MHz, chloroform-d) δ=4.93 (quin, J=4.6 Hz, 1H), 3.88–3.82 (m, 4H), 2.38 (t, J=7.7 Hz, 2H), 2.04 (t, J=5.9 Hz, 2H), 1.65 (quin, J=7.3 Hz, 2H), 1.38–1.19 (m, 28H), 0.88 (t, J=6.6 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-d) δ=174.3, 75.3, 62.8 (2C), 34.6, 32.2, 29.95 (5C), 29.91 (2C), 29.8, 29.7, 29.6, 29.5, 29.3, 25.2, 23.0, 13.8. IR (neat, cm^{-1}) 3313, 2916, 2849, 1730, 1472. HRMS for $C_{21}H_{42}O_4Na$ (MNa^+) 381.2982. Calcd 381.2981.

4.14. (*Z*)-2-(Oleoyloxy)propane-1,3-diyl dibutyrate (7a)

Yield 290 mg, 82%; colorless oil. R_f=0.43 (15% ethyl acetate/hexanes). ^{1}H NMR (500 MHz, chloroform-d) δ=5.39–5.30 (m, 2H), 5.30–5.24 (m, 1H), 4.30 (dd, J=4.4, 11.7 Hz, 2H), 4.15 (dd, J=6.1, 12.0 Hz, 2H), 2.36–2.25 (m, 6H), 2.01 (q, J=6.2 Hz, 4H), 1.71–1.55 (m, 6H), 1.38–1.19 (m, 20H), 0.95 (t, J=7.3 Hz, 3H), 0.88 (t, J=6.8 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-d) δ=173.4 (2C), 173.1, 130.3, 129.9, 69.1, 62.3, 36.1 (2C), 34.4, 32.1, 30.0, 29.9, 29.8, 29.6 (2C), 29.4, 29.3, 29.2, 27.5, 27.4, 25.1, 22.9, 18.6 (3C), 14.4, 13.9 (2C). IR (neat, cm^{-1}) 3007, 2925, 1742, 1460. HRMS for $C_{29}H_{52}O_6Na$ (MNa^+) 519.3658. Calcd 519.3662.

4.15. 1,3-Dihydroxypropan-2-yl oleate (7b)

C. antarctica: 48 mg, 67%; *R. miehei*: 60 mg, 83%; colorless oil. R_f=0.30 (30% acetone/hexanes). ^{1}H NMR (399 MHz, chloroform-d) δ=5.41–5.31 (m, 2H), 4.92 (quin, J=4.8 Hz, 1H), 3.88–3.77 (m, 4H), 2.49 (br s, 2H), 2.37 (t, J=7.7 Hz, 2H), 2.01 (q, J=6.4 Hz, 4H), 1.63 (quin, J=7.3 Hz, 2H), 1.40–1.19 (m, 20H), 0.88 (t, J=6.6 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-d) δ=174.4, 130.3, 129.9, 75.1, 62.5 (2C), 34.6, 32.1, 30.0, 29.9, 29.8, 29.6, 29.4, 29.33, 29.31, 27.45, 27.38, 25.2 (2C), 22.9, 14.4. IR (neat, cm^{-1}) 3415, 3008, 2923, 2854, 1735, 1464. HRMS for $C_{21}H_{40}O_4Na$ (MNa^+) 379.2827. Calcd 379.2824.

4.16. 2-((9*Z*,12*Z*)-Octadeca-9,12-dienoyloxy)propane-1,3-diyl dibutyrate (8a)

Yield 344 mg, 98%; colorless oil. R_f=0.38 (15% ethyl acetate/hexanes). ^{1}H NMR (399 MHz, chloroform-d) δ=5.43–5.30 (m, 4H), 5.29–5.24 (m, 1H), 4.30 (dd, J=4.4, 11.7 Hz, 2H), 4.15 (dd, J=5.9, 11.7 Hz, 2H), 2.77 (t, J=6.6 Hz, 2H), 2.36–2.26 (m, 6H), 2.05 (q, J=6.6 Hz, 4H), 1.72–1.56 (m, 6H), 1.41–1.22 (m, 14H), 0.95 (t, J=7.7 Hz, 6H), 0.89 (t, J=7.0 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-d) δ=173.3 (2C), 173.1, 130.5, 130.2, 128.3, 128.1, 69.1, 62.3 (2C), 36.1 (2C), 34.4, 31.8, 29.8, 29.6, 29.4 (2C), 29.3, 29.2, 27.4, 25.8, 25.1, 22.8, 18.6 (2C), 14.3, 13.9 (2C). IR (neat, cm^{-1}) 3008, 2929, 2856, 1741, 1459. HRMS for $C_{29}H_{50}O_6Na$ (MNa^+) 517.3506. Calcd 517.3505.

4.17. (9*Z*,12*Z*)-1,3-Dihydroxypropan-2-yl octadeca-9,12-dienoate (8b)

C. antarctica: 45 mg, 63%; *R. miehei*: 55 mg, 77%; colorless oil. R_f=0.37 (30% acetone/hexanes). ^{1}H NMR (399 MHz, chloroform-d) δ=5.44–5.30 (m, 4H), 4.93 (quin, J=4.8 Hz, 1H), 3.89–3.76 (m, 4H), 2.77 (t, J=6.6 Hz, 2H), 2.38 (t, J=7.3 Hz, 2H), 2.13 (t, J=6.2 Hz, 2H), 2.05 (q, J=6.8 Hz, 4H), 1.69–1.59 (m, 2H), 1.41–1.23 (m, 14H), 0.89 (t, J=6.6 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-d) δ=174.3, 130.5, 130.2, 128.3, 128.1, 75.2, 62.8 (2C), 34.6, 31.8, 29.8, 29.6, 29.4, 29.33, 29.30, 27.4, 25.9, 25.2 (2C), 22.8, 14.3. IR (neat, cm^{-1}) 3397, 010, 2926, 2855, 1736, 1459. HRMS for $C_{21}H_{38}O_4Na$ (MNa^+) 377.2667. Calcd 377.2668.

4.18. (*Z*)-2-(Icos-11-enoyloxy)propane-1,3-diyl dibutyrate (9a)

Yield 328 mg, 88%; colorless oil. R_f=0.42 (15% ethyl acetate/hexanes). ^{1}H NMR (399 MHz, chloroform-d) δ=5.38–5.32 (m, 2H), 5.30–5.24 (m, 1H), 4.30 (dd, J=4.4, 11.7 Hz, 2H), 4.16 (dd, J=5.9, 12.5 Hz, 2H), 2.36–2.26 (m, 6H), 2.05–1.97 (m, 4H), 1.71–1.57 (m, 6H), 1.27 (br s, 24H), 0.95 (t, J=7.3 Hz, 6H), 0.88 (t, J=6.6 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-d) δ=173.3 (2C), 173.1, 130.2, 130.0, 69.0, 62.3 (2C), 36.1 (2C), 34.4, 32.1, 30.0 (2C), 29.8, 29.7, 29.55 (2C), 29.52, 29.51, 29.3, 27.4 (2C), 25.1 (2C), 22.9, 18.6 (2C), 14.4, 13.9 (2C).IR (neat, cm^{-1}) 3008, 2925, 2855, 1742, 1459. HRMS for $C_{31}H_{56}O_6Na$ (MNa^+) 547.3978. Calcd 547.3975.

4.19. (*Z*)-1,3-Dihydroxypropan-2-yl icos-11-enoate (9b)

C. antarctica: 40 mg, 55%; *R. miehei*, 58 mg, 79%; white solid. R_f=0.24 (30% acetone/hexanes). Mp=32–33 °C. ^{1}H NMR (399 MHz, chloroform-d) δ=5.38–5.32 (m, 2H), 4.93 (quin, J=4.8 Hz, 1H), 3.89–3.78 (m, 4H), 2.38 (t, J=7.3 Hz, 2H), 2.20–2.12 (m, 2H), 2.01 (q, J=6.6 Hz, 4H), 1.71–1.57 (m, 2H), 1.40–1.18 (m, 24H), 0.88 (t, J=6.6 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-d) δ=174.3, 130.2, 130.0, 75.2, 62.8 (2C), 34.6, 32.1, 30.0 (2C), 29.8, 29.7, 29.7, 29.6 (2C),

29.56, 29.51, 29.3, 27.4, 25.2 (2C), 23.0, 14.4. IR (neat, cm^{-1}) 3405, 3008, 2923, 2854, 1737, 1465. HRMS for $C_{23}H_{44}O_4Na$ (MNa^+) 407.3142. Calcd 407.3137.

4.20. 2-((5*Z*,8*Z*,11*Z*,14*Z*)-Icosa-5,8,11,14-tetraenoyloxy)-propane-1,3-diyl dibutyrate (10a)

Yield 168 mg, 98%; colorless oil. R_f=0.36 (30% ethyl acetate/hexanes). The ^1H and ^{13}C spectral data (500 and 100 MHz, $CDCl_3$) are in agreement with literature values.[33] IR (neat, cm^{-1}) 3012, 2931, 1741, 1456. HRMS for $C_{31}H_{50}O_6Na$ (MNa^+) 541.3502. Calcd 541.3505.

4.21. (5*Z*,8*Z*,11*Z*,14*Z*)-1,3-Dihydroxypropan-2-yl icosa-5,8,11,14-tetraenoate (10b)

C. antarctica: 48 mg, 67%; *R. miehei*: 54 mg, 75%; colorless oil. R_f=0.30 (30% acetone/hexanes). The ^1H and ^{13}C spectral data (500 and 100 MHz, $CDCl_3$) are in agreement with literature values.[33] IR (neat, cm^{-1}) 3420, 2013, 2927, 1736, 1456. HRMS for $C_{23}H_{38}O_4Na$ (MNa^+) 401.2677. Calcd 401.2668.

4.22. 2-((4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-Docosa-4,7,10,13,16,19-hexaenoyloxy)propane-1,3-diyl diacetate (11a)

EDCI (111 mg, 0.58 mmol), DMAP (6 mg, 0.06 mmol), and diacetin (44 mg, 0.25 mmol) were added to a solution of docosahexaenoic acid (75 mg, 0.23 mmol) in anhydrous CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was allowed to stir for 4 h. Upon completion, the reaction mixture was diluted with CH_2Cl_2 and H_2O. The organic layer was separated, dried over $MgSO_4$, and removed under reduced pressure. The resulting residue was chromatographed on silica gel (0–30% ethyl acetate/hexanes) to yield to **11a** (111 mg, 99%) as a colorless oil. R_f=0.55 (30% ethyl acetate/hexanes). ^1H NMR (500 MHz, chloroform-*d*) δ=5.47–5.32 (m, 12H), 5.30–5.21 (m, 1H), 4.29 (dd, *J*=4.4, 11.7 Hz, 2H), 4.16 (dd, *J*=5.9, 12.2 Hz, 2H), 2.93–2.77 (m, 10H), 2.40 (d, *J*=2.9 Hz, 4H), 2.14–2.02 (m, 6H), 0.98 (t, *J*=7.6 Hz, 3H). The ^{13}C spectral data (100 MHz, $CDCl_3$) and IR data are in agreement with literature values.[32] HRMS for $C_{29}H_{42}O_6Na$ (MNa^+) 509.2880. Calcd 509.2879.

4.23. (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-1,3-Dihydroxypropan-2-yl-docosa-4,7,10,13,16,19-hexaenoate (11b)

C. antarctica: 45 mg, 63%; *R. miehei*: 63 mg, 76%; colorless oil. R_f=0.29 (30% acetone/hexanes). ^1H NMR (399 MHz, chloroform-*d*) δ=5.52–5.22 (m, 12H), 4.92 (quin, *J*=4.6 Hz, 1H), 3.82 (t, *J*=5.1 Hz, 4H), 2.91–2.77 (m, 10H), 2.49–2.37 (m, 4H), 2.22 (t, *J*=6.2 Hz, 2H), 2.07 (quin, *J*=7.5 Hz, 2H), 0.97 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-*d*) δ=173.5, 132.3 (2C), 129.8 (2C), 128.8, 128.6, 128.5 (2C), 128.3 (2C), 128.1, 127.9, 75.4, 62.6 (2C), 34.4, 25.8 (5C), 23.0 (2C), 20.8. IR (neat, cm^{-1}) 3401, 3013, 2663, 1736, 1390. HRMS for $C_{25}H_{38}O_4Na$ (MNa^+) 425.2666. Calcd 425.2668.

4.24. 2-(Docosanoyloxy)propane-1,3-diyl dibutyrate (12a)

Yield 201 mg, 56%; colorless oil. R_f=0.48 (15% ethyl acetate/hexanes). Mp=27–28 °C. ^1H NMR (399 MHz, chloroform-*d*) δ=5.32–5.23 (m, 1H), 4.30 (dd, *J*=4.4, 11.7 Hz, 2H), 4.16 (dd, *J*=5.9, 11.7 Hz, 2H), 2.36–2.26 (m, 6H), 1.71–1.57 (m, 6H), 1.25 (s, 36H), 0.95 (t, *J*=7.3 Hz, 6H), 0.88 (t, *J*=6.6 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-*d*) δ=173.4 (2C), 173.2, 69.1, 62.3 (2C), 36.0 (2C), 34.4, 32.2, 29.95 (9C), 29.91 (2C), 29.88, 29.7, 29.6, 29.5, 29.3, 25.1, 22.9, 18.6 (2C), 14.4, 13.9 (2C). IR (neat, cm^{-1}) 2923. 2853, 1742, 1462. HRMS for $C_{33}H_{62}O_6Na$ (MNa^+) 577.4446. Calcd 577.4444.

4.25. 1,3-Dihydroxypropan-2-yl docosanoate (12b)

C. antarctica: 32 mg, 40%; *R. miehei*: 70 mg, 88%; white solid. R_f=0.27 (30% acetone/hexanes). Mp=79–80 °C. ^1H NMR (399 MHz, chloroform-*d*) δ=4.93 (quin, *J*=4.6 Hz, 1H), 3.88–3.81 (m, 4H), 2.38 (t, *J*=7.7 Hz, 2H), 2.08 (s, 2H), 1.69–1.59 (m, 2H), 1.38–1.19 (m, 36H), 0.88 (t, *J*=6.2 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-*d*) δ=174.3, 75.2, 62.8 (2C), 34.6, 32.2, 31.8, 29.94 (7C), 29.90 (2C), 29.8, 29.7, 29.6, 29.5, 29.3, 25.2, 22.94, 22.89, 14.4. IR (neat, cm^{-1}) 3313, 297, 2850, 1730, 1472. HRMS for $C_{25}H_{50}O_4Na$ (MNa^+) 437.3610. Calcd 437.3607.

4.26. 2-(3-Phenylpropanoyloxy)propane-1,3-diyl dibutyrate (13a)

Yield 280 mg, 98%; colorless oil. R_f=0.48 (35% ethyl acetate/hexanes). ^1H NMR (399 MHz, chloroform-*d*) δ=7.33–7.25 (m, 2H), 7.24–7.15 (m, 3H), 5.31–5.23 (m, 1H), 4.28 (dd, *J*=4.4, 11.7 Hz, 2H), 4.13 (dd, *J*=5.9, 11.7 Hz, 2H), 2.96 (t, *J*=7.7 Hz, 2H), 2.66 (t, *J*=8.1 Hz, 2H), 2.34–2.24 (m, 6H), 1.64 (sxt, *J*=7.3 Hz, 4H), 0.94 (t, *J*=7.3 Hz, 6H). ^{13}C NMR (100 MHz, chloroform-*d*) δ=173.3 (2), 172.2, 140.4, 128.7 (2), 128.5 (2), 126.6, 69.4, 62.2 (2), 36.1 (2), 35.9, 31.0, 18.6 (2), 13.9 (2C). IR (neat, cm^{-1}) 3027, 2966, 2877, 1737, 1455. HRMS for $C_{20}H_{28}O_6Na$ (MNa^+) 387.1787. Calcd 387.1784.

4.27. 1,3-Dihydroxypropan-2-yl 3-phenylpropanoate (13b)

C. antarctica: 38 mg, 83%; *R. miehei*: 8 mg, 40%; white foam. R_f=0.18 (40% acetone/hexanes). ^1H NMR (399 MHz, chloroform-*d*) δ=7.36–7.28 (m, 2H), 7.26–7.18 (m, 3H), 4.89 (td, *J*=4.5, 9.3 Hz, 1H), 3.79–3.71 (m, 4H), 3.02–2.96 (m, 2H), 2.77–2.70 (m, 2H), 1.91–1.83 (m, 2H). ^{13}C NMR (100 MHz, chloroform-*d*) δ=175.2, 140.4, 128.8 (2), 128.5 (2), 126.7, 75.5, 62.6 (2), 36.1, 31.8. IR (neat, cm^{-1}) 3412, 3029, 2935, 2881, 1731, 1454. HRMS for $C_{12}H_{16}O_4Na$ (MNa^+) 247.0945. Calcd 247.0946.

4.28. 2-(5-Phenylpentanoyloxy)propane-1,3-diyl dibutyrate (14a)

Yield 298 mg, 99%; colorless oil. R_f=0.63 (35% ethyl acetate/hexanes). ^1H NMR (500 MHz, chloroform-*d*) δ=7.31–7.24 (m, 2H), 7.21–7.14 (m, 3H), 5.31–5.23 (m, 1H), 4.30 (dd, *J*=4.4, 11.7 Hz, 2H), 4.14 (dd, *J*=5.9, 11.7 Hz, 2H), 2.63 (t, *J*=7.1 Hz, 2H), 2.35 (t, *J*=6.8 Hz, 2H), 2.29 (t, *J*=6.8 Hz, 4H), 1.71–1.58 (m, 8H), 0.94 (t, *J*=7.3 Hz, 6H). ^{13}C NMR (100 MHz, chloroform-*d*) δ=173.4 (2C), 172.9, 142.2, 128.6 (2C), 128.6 (2C), 126.0, 69.2, 62.3 (2C), 36.1 (2C), 35.8, 34.2, 31.0, 24.7, 18.6 (2C), 13.8 (2C). IR (neat, cm^{-1}) 3028, 2965, 2876, 1738, 1454. HRMS for $C_{22}H_{32}O_6Na$ (MNa^+) 415.2094. Calcd 415.2097.

4.29. 1,3-Dihydroxypropan-2-yl 5-phenylpentanoate (14b)

C. antarctica: 50 mg, 83%; *R. miehei*: 24 mg, 37%; white foam. R_f=0.26 (40% acetone/hexanes). ^1H NMR (399 MHz, chloroform-*d*) δ=7.31–7.25 (m, 2H), 7.22–7.13 (m, 3H), 4.92 (td, *J*=4.8, 9.5 Hz, 1H), 3.86–3.76 (m, 4H), 2.64 (t, *J*=7.0 Hz, 2H), 2.41 (t, *J*=7.0 Hz, 2H), 2.17–2.11 (m, 2H), 1.74–1.60 (m, 4H). ^{13}C NMR (100 MHz, chloroform-*d*) δ=174.0, 142.2, 128.6 (4C), 126.1, 75.2, 62.7 (2C), 35.8, 34.4, 31.0, 24.7. IR (neat, cm^{-1}) 3414, 3027, 2936, 2882, 1731, 1454. HRMS for $C_{14}H_{20}O_4Na$ (MNa^+) 275.1257. Calcd 275.1259.

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Supplementary data

^{1}H and ^{13}C NMR spectra for all new compounds reported. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2012.04.101.

References and notes

1. Lauridsen, J. *J. Am. Oil Chem. Soc.* **1976**, *53*, 400.
2. Van Haften, J. *J. Am. Oil Chem. Soc.* **1979**, *56*, 831A.
3. Pouton, C. W. *Eur. J. Pharm. Sci.* **2000**, *11*, S93.
4. Christensen, M.; Hoy, C.; Becker, C.; Redgrave, T. *Am. J. Clin. Nutr.* **1995**, *61*, 56.
5. Aberoumand, A. *World J. Fish Marine Sci.* **2010**, *2*, 226.
6. Czernichow, S.; Thomas, C.; Bruckert, E. *Br. J. Nutr.* **2010**, *104*, 788.
7. Legrand, P.; Rioux, V. *Lipids* **2010**, *45*, 941.
8. Lambert, D. M.; Fowler, C. J. *J. Med. Chem.* **2005**, *48*, 5059.
9. Ortega-Gutiérrez, S.; Viso, A.; Cisneros, J. A. *Curr. Top. Med. Chem.* **2008**, *8*, 231.
10. Boswinkel, G.; Derksen, J.; van't Riet, K.; Cuperus, F. *J. Am. Oil Chem. Soc.* **1996**, *73*, 707.
11. Lyubachevskaya, G.; Boyle-Roden, E. *Lipids* **2000**, *35*, 1353.
12. Martin, J. B. *J. Am. Chem. Soc.* **1953**, *75*, 5483.
13. Kingsley, P. J.; Marnett, L. J. *Anal. Biochem.* **2003**, *314*, 8.
14. Stelt, M. v. d.; Kuik, J. A. v.; Bari, M.; Zadelhoff, G. v.; Leeflang, B. R.; Veldink, G. A.; Finazzi-Agro`, A.; Vliegenthart, J. F. G.; Maccarrone, M. *J. Med. Chem.* **2002**, *45*, 3709.
15. Han, L.; Razdan, R. K. *Tetrahedron Lett.* **1999**, *40*, 1631.
16. Seltzman, H. H.; Fleming, D. N.; Hawkins, G. D.; Carroll, F. I. *Tetrahedron Lett.* **2000**, *41*, 3589.
17. Stamatov, S. D.; Stawinski, J. *Tetrahedron* **2005**, *61*, 3659.
18. Uwe, T. B. *Enzyme Microb. Technol.* **1995**, *17*, 578.
19. Berger, M.; Laumen, K.; Schneider, M. *J. Am. Oil Chem. Soc.* **1992**, *69*, 955.
20. Berger, M.; Schnelder, M. *J. Am. Oil Chem. Soc.* **1992**, *69*, 961.
21. Piyatheerawong, W.; Yamane, T.; Nakano, H.; Iwasaki, Y. *J. Am. Oil Chem. Soc.* **2006**, *83*, 603.
22. Irimescu, R.; Yasui, M.; Iwasaki, Y.; Shimidzu, N.; Yamane, T. *J. Am. Oil Chem. Soc.* **2000**, *77*, 501.
23. Rosu, R.; Yasui, M.; Iwasaki, Y.; Yamane, T. *J. Am. Oil Chem. Soc.* **1999**, *76*, 839.
24. Schmid, U.; Bornscheuer, U. T.; Soumanou, M. M.; McNeill, G. P.; Schmid, R. D. *Biotechnol. Bioeng.* **1999**, *64*, 678.
25. Byun, H.-G.; Eom, T.-K.; Jung, W.-K.; Kim, S.-K. *Biotechnol. Bioprocess Eng.* **2007**, *12*, 491.
26. Waldinger, C.; Schneider, M. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1513.
27. Schmid, U.; Bornscheuer, U.; Soumanou, M.; McNeill, G.; Schmid, R. *J. Am. Oil Chem. Soc.* **1998**, *75*, 1527.
28. Soumanou, M. M.; Bornscheuer, U. T.; Schmid, U.; Schmid, R. D. *Biocatal. Biotransfor.* **1999**, *16*, 443.
29. Soumanou, M.; Bornscheuer, U.; Schmid, R. *J. Am. Oil Chem. Soc.* **1998**, *75*, 703.
30. Wongsakul, S.; Prasertsan, P.; Bornscheuer, U. T.; H-Kittikun, A. *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 68.
31. Irimescu, R.; Iwasaki, Y.; Hou, C. *J. Am. Oil Chem. Soc.* **2002**, *79*, 879.
32. Magnusson, C. D.; Haraldsson, G. G. *Tetrahedron* **2010**, *66*, 2728.
33. Vadivel, S. K.; Whitten, K. M.; Makriyannis, A. *Tetrahedron Lett.* **2011**, *52*, 1149.
34. Duclos, R. I.; Johnston, M.; Vadivel, S. K.; Makriyannis, A.; Glaser, S. T.; Gatley, S. J. *J. Org. Chem.* **2011**, *76*, 2049.
35. Halldorsson, A.; Magnusson, C. D.; Haraldsson, G. G. *Tetrahedron* **2003**, *59*, 9101.
36. Takagi, T.; Ando, Y. *Lipids* **1991**, *26*, 542.
37. Irimescu, R.; Furihata, K.; Hata, K.; Iwasaki, Y.; Yamane, T. *J. Am. Oil Chem. Soc.* **2001**, *78*, 743.
38. Li, C.; Xu, W.; Vadivel, S. K.; Fan, P.; Makriyannis, A. *J. Med. Chem.* **2005**, *48*, 6423.
39. Papahatjis, D. P.; Nahmias, V. R.; Nikas, S. P.; Schimpgen, M.; Makriyannis, A. *Chem.—Eur. J.* **2010**, *16*, 4091.
40. Yao, F.; Li, C.; Vadivel, S. K.; Bowman, A. L.; Makriyannis, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5912.