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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 *Candida antarctica* within 2h with yields ranging from 36%–83%. Similar reaction conditions with immobilized *Rhizomucor miehei* yielded exclusively the 2-MAG after 24h with yields ranging from 37% to 88%. Yields vary on the acyl group at the *sn*-2 position and choice of enzyme involved.

Keywords

Cannabinoid; Receptor; Endogenous ligand; 2-Monoacylglycerol; Biocatalysis

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 STGs 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.^{5,6,7} 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,

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Supporting Information

¹H and ¹³C NMR spectra for all new compounds reported.

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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 24h 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 ester 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

Application of lipase in the syntheses of 1,3-diacylglycerol, and 1(3)-*rac*-monoacyl glycerol have 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 have 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 *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 *Candida 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 by product that requires exhaustive purification (Figure 1).

Encouraged by this literature, we recently reported a method for the synthesis 2-AG which utilizes a structured glyceride, 1,3-dibutyl-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.

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 *Candida antarctica* (Novozym 435) to glycerol and vinyl butyrate in anhydrous CH₂Cl₂ 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:CH₂Cl₂ along with a catalytic amount of 4-dimethylaminopyridine (DMAP) at 0 °C for four hours. 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 one hour 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 (1h), 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 towards to over hydrolysis compare 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 Nonimpregnated 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-diacetyl glycerol-protected compound as compared to the 1,3-dibutyl glycerol-protected compound.

We also screened other 1,3-specific lipases to investigate whether the transformation can be performed in better yield and selectivity towards the range of substrates and found that lipase from *Rhizomucor miehei* showed excellent selectivity towards hydrolyzing “ABA” type triglycerides. The reaction proceeded in a similar fashion where the triglyceride was consumed quickly, but hydrolysis of diglycerides took 24h to 48h. Even though the reaction proceeded very slowly compared to *Candida antarctica* lipase, the *R. miehei* lipase offered a remarkable improvement in selectivity, 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 *Rhizomucor miehei* lipase the maximal yield once again reached 80% 2-MAG formation. Surprisingly, in contrast to *Candida Antartica* lipase, *Rhizomucor miehei* lipase showed less reactivity towards aryl esters (**13** and **14**).

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.

Experimental

General Methods

Lipase acrylic resin from *Candida antarctica* and Lipozyme[®], immobilized from *Rhizomucor 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 by products, ethyl arachidonate, ethyl butyrate, and all diglycerides were removed during column chromatography. All glycerols were purified on a Biotage Isolera One using Luknova prepackaged 12g 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**.

2-hydroxypropane-1,3-diyl dibutyrate (1)—Immobilized *Candida 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 CH₂Cl₂ (10 mL) at 0°C. The resulting mixture was stirred for 3h 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). ¹H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 4.20 (dd, *J*=11.72, 4.39 Hz, 2 H) 4.14 (dd, *J*=11.72, 5.86 Hz, 2 H) 4.04 – 4.12 (m, 1 H) 2.41 – 2.58 (m, 1 H) 2.33 (t, *J*=7.32 Hz, 4 H) 1.67 (sxt, *J*=7.32 Hz, 4 H) 0.96 (t, *J*=7.57 Hz, 4 H). The ¹³C NMR spectral data (100 MHz, CDCl₃) are in agreement with literature values.³²

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/CH₂Cl₂ (10 mL) at 0 °C. The reaction was allowed to stir for 4h. The reaction was then diluted with CH₂Cl₂ (15 mL) and H₂O (15 mL). The organic layer was separated, dried over MgSO₄, and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (0% to 15% ethyl acetate/hexanes) to yield **2a** (221 mg, 67%) as a colorless oil. R_f = 0.50 (15% ethyl acetate/hexanes). ¹H NMR (400 MHz, CHLOROFORM-*d*) δ = 5.24 – 5.31 (m, 1 H) 4.30 (dd, *J*=12.09, 4.03 Hz, 2 H) 4.16 (dd, *J*=12.46, 5.86 Hz, 2 H) 2.27 – 2.35 (m, 6 H) 1.57 – 1.70 (m, 6 H) 1.21 – 1.36 (m, 16 H) 0.92 – 0.98 (m, 6 H) 0.88 (t, *J*=6.60 Hz, 3 H). ¹³C NMR (100MHz, 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 C₂₃H₄₂O₆Na (MNa⁺) 437.2881. Calcd. 437.2879.

1,3-dihydroxypropan-2-yl dodecanoate (2b, utilizing *Candida antarctica*)—Immobilized *Candida 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** (1h, TLC monitoring), additional lipase (100 mg) was added until reaction completion was observed (1h). The reaction mixture was diluted with CH₂Cl₂ (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% to 50% acetone/hexanes) to yield **2b** (31mg, 47%) as a white solid. R_f = 0.26 (30% acetone/hexanes). MP = 56–57 °C. ^1H NMR (400 MHz, CHLOROFORM-*d*) δ = 4.93 (quin, J =4.76 Hz, 1 H) 3.84 (br. s., 4 H) 2.38 (t, J =7.69 Hz, 2 H) 2.08 (br. s., 2 H) 1.58 – 1.69 (m, 2 H) 1.20 – 1.37 (m, 16 H) 0.88 (t, J =6.60 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{15}\text{H}_{30}\text{O}_4\text{Na}$ (MNa^+) 297.2041. Calcd. 297.2042.

1,3-dihydroxypropan-2-yl dodecanoate (2b, utilizing *Rhizomucor miehei*)—

Lipozyme[®], immobilized from *Rhizomucor miehei* (100mg) was added to a solution of **2a** (100 mg, 0.24 mmol) stirred in anhydrous EtOH (1 mL). The reaction was stirred for 24h, 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% to 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*.

2-(tetradecanoyloxy)propane-1,3-diyl dibutyrate (3a)—347 mg, 99%, colorless oil.

R_f = 0.47 (15% ethyl acetate/hexanes). ^1H NMR (399MHz, CHLOROFORM-*d*) δ = 5.32 - 5.22 (m, 1 H), 4.29 (dd, J = 4.4, 11.7 Hz, 2 H), 4.15 (dd, J = 5.9, 11.7 Hz, 2 H), 2.35 - 2.25 (m, 6 H), 1.70 - 1.56 (m, 6 H), 1.36 - 1.19 (m, 20 H), 0.94 (t, J = 7.3 Hz, 6 H), 0.87 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{25}\text{H}_{46}\text{O}_6\text{Na}$ (MNa^+) 465.3195. Calcd. 465.3192.

1,3-dihydroxypropan-2-yl tetradecanoate (3b)—*C. antarctica*: 34mg, 49%; *R.*

miehei: 57 mg, 82%; white solid. R_f = 0.22 (30% acetone/hexanes). MP = 57–58 °C. ^1H NMR (399MHz, CHLOROFORM-*d*) δ = 4.93 (quin, J = 4.8 Hz, 1 H), 3.89 - 3.78 (m, 3 H), 2.38 (t, J = 7.3 Hz, 2 H), 2.17 - 2.10 (m, 2 H), 1.70 - 1.58 (m, 2 H), 1.38 - 1.19 (m, 20 H), 0.88 (t, J = 7.3 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{17}\text{H}_{34}\text{O}_4\text{Na}$ (MNa^+) 325.2354. Calcd. 325.2355.

2-(palmitoyloxy)propane-1,3-diyl dibutyrate (4a)—309 mg, 84%, colorless oil. R_f =

0.39 (15% ethyl acetate/hexanes). ^1H NMR (500MHz, CHLOROFORM-*d*) δ = 5.30 - 5.25 (m, 1 H), 4.30 (dd, J = 4.2, 12.0 Hz, 2 H), 4.16 (dd, J = 5.9, 11.7 Hz, 2 H), 2.35 - 2.27 (m, 6 H), 1.70 - 1.58 (m, 6 H), 1.34 - 1.21 (m, 24 H), 0.98 - 0.92 (m, 6 H), 0.88 (t, J = 6.8 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{27}\text{H}_{50}\text{O}_6\text{Na}$ (MNa^+) 493.3503. Calcd. 493.3505.

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. ^1H NMR (500MHz, CHLOROFORM-*d*) δ = 4.93 (quin, J = 4.8 Hz, 1 H), 3.84 (t, J = 4.9 Hz, 4 H), 2.38 (t, J = 7.6 Hz, 2 H), 2.13 - 2.05 (m, 2 H), 1.69 - 1.59 (m, 2 H), 1.38 - 1.20 (m, 24 H), 0.88 (t, J = 7.3 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{19}\text{H}_{38}\text{O}_4\text{Na}$ (MNa^+) 353.2668. Calcd. 353.2668.

(Z)-2-(hexadec-9-enoyloxy)propane-1,3-diyl dibutyrate (5a)—291 mg, 79%, colorless oil. R_f = 0.50 (15% ethyl acetate/hexanes). ^1H NMR (399MHz, CHLOROFORM- d) δ = 5.38 - 5.32 (m, 2 H), 5.31 - 5.24 (m, 1 H), 4.30 (dd, J = 4.4, 11.7 Hz, 2 H), 4.16 (dd, J = 5.9, 12.5 Hz, 2 H), 2.38 - 2.26 (m, 6 H), 2.01 (q, J = 6.6 Hz, 4 H), 1.72 - 1.56 (m, 6 H), 1.39 - 1.20 (m, 16 H), 0.95 (t, J = 7.3 Hz, 6 H), 0.88 (t, J = 7.0 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{27}\text{H}_{48}\text{O}_6\text{Na}$ (MNa^+) 491.3347. Calcd. 491.3349.

(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). ^1H NMR (399MHz, CHLOROFORM- d) δ = 5.40 - 5.31 (m, 2 H), 4.92 (quin, J = 4.8 Hz, 1 H), 3.86 - 3.79 (m, 4 H), 2.37 (t, J = 7.7 Hz, 2 H), 2.33 (br. s., 2 H), 2.05 - 1.97 (m, 4 H), 1.63 (quin, J = 7.3 Hz, 2 H), 1.39 - 1.23 (m, 16 H), 0.88 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{19}\text{H}_{36}\text{O}_4\text{Na}$ (MNa^+) 351.2512. Calcd. 351.2511.

2-(stearoyloxy)propane-1,3-diyl dibutyrate (6a)—300 mg, 85%, colorless oil. R_f = 0.34 (15% ethyl acetate/hexanes). ^1H NMR (500MHz, CHLOROFORM- d) δ = 5.30 - 5.24 (m, 1 H), 4.30 (dd, J = 4.4, 11.7 Hz, 2 H), 4.16 (dd, J = 6.1, 12.0 Hz, 2 H), 2.34 - 2.27 (m, 6 H), 1.70 - 1.59 (m, 6 H), 1.35 - 1.20 (m, 28 H), 0.98 - 0.93 (m, 6 H), 0.88 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{29}\text{H}_{54}\text{O}_6\text{Na}$ (MNa^+) 521.3813. Calcd. 521.3818.

1,3-dihydroxypropan-2-yl stearate (6b)—*C. antarctica*: 32mg, 44%; *R. miehei*: 56 mg, 78%; white solid. R_f = 0.23 (30% acetone/hexanes). MP = 68–69 °C. ^1H NMR (399MHz, CHLOROFORM- d) δ = 4.93 (quin, J = 4.6 Hz, 1 H), 3.88 - 3.82 (m, 4 H), 2.38 (t, J = 7.7 Hz, 2 H), 2.04 (t, J = 5.9 Hz, 2 H), 1.65 (quin, J = 7.3 Hz, 2 H), 1.38 - 1.19 (m, 28 H), 0.88 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{21}\text{H}_{42}\text{O}_4\text{Na}$ (MNa^+) 381.2982. Calcd. 381.2981.

(Z)-2-(oleoyloxy)propane-1,3-diyl dibutyrate (7a)—290 mg, 82%, colorless oil. R_f = 0.43 (15% ethyl acetate/hexanes). ^1H NMR (500MHz, CHLOROFORM- d) δ = 5.39 - 5.30 (m, 2 H), 5.30 - 5.24 (m, 1 H), 4.30 (dd, J = 4.4, 11.7 Hz, 2 H), 4.15 (dd, J = 6.1, 12.0 Hz, 2 H), 2.36 - 2.25 (m, 6 H), 2.01 (q, J = 6.2 Hz, 4 H), 1.71 - 1.55 (m, 6 H), 1.38 - 1.19 (m, 20 H), 0.95 (t, J = 7.3 Hz, 3 H), 0.88 (t, J = 6.8 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{29}\text{H}_{52}\text{O}_6\text{Na}$ (MNa^+) 519.3658. Calcd. 519.3662.

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). ^1H NMR (399MHz, CHLOROFORM- d) δ = 5.41 - 5.31 (m, 2 H), 4.92 (quin, J = 4.8 Hz, 1 H), 3.88 - 3.77 (m, 4 H), 2.49 (br. s., 2 H), 2.37 (t, J = 7.7 Hz, 2 H), 2.01 (q, J = 6.4 Hz, 4 H), 1.63 (quin, J = 7.3 Hz, 2 H), 1.40 - 1.19 (m, 20 H), 0.88 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{21}\text{H}_{40}\text{O}_4\text{Na}$ (MNa^+) 379.2827. Calcd. 379.2824.

2-((9Z,12Z)-octadeca-9,12-dienoyloxy)propane-1,3-diyl dibutyrate (8a)—344 mg, 98%, colorless oil. R_f = 0.38 (15% ethyl acetate/hexanes). ^1H NMR (399MHz, CHLOROFORM- d) δ = 5.43 - 5.30 (m, 4 H), 5.29 - 5.24 (m, 1 H), 4.30 (dd, J = 4.4, 11.7 Hz, 2 H), 4.15 (dd, J = 5.9, 11.7 Hz, 2 H), 2.77 (t, J = 6.6 Hz, 2 H), 2.36 - 2.26 (m, 6 H), 2.05 (q, J = 6.6 Hz, 4 H), 1.72 - 1.56 (m, 6H), 1.41 - 1.22 (m, 14 H), 0.95 (t, J = 7.7 Hz, 6 H), 0.89 (t, J = 7.0 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{29}\text{H}_{50}\text{O}_6\text{Na}$ (MNa^+) 517.3506. Calcd. 517.3505.

(9Z,12Z)-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). ^1H NMR (399MHz, CHLOROFORM- d) δ = 5.44 - 5.30 (m, 4 H), 4.93 (quin, J = 4.8 Hz, 1 H), 3.89 - 3.76 (m, 4 H), 2.77 (t, J = 6.6 Hz, 2 H), 2.38 (t, J = 7.3 Hz, 2 H), 2.13 (t, J = 6.2 Hz, 2 H), 2.05 (q, J = 6.8 Hz, 4 H), 1.69 - 1.59 (m, 2 H), 1.41 - 1.23 (m, 14 H), 0.89 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{21}\text{H}_{38}\text{O}_4\text{Na}$ (MNa^+) 377.2667. Calcd. 377.2668.

(Z)-2-(icos-11-enoyloxy)propane-1,3-diyl dibutyrate (9a)—328 mg, 88%, colorless oil. R_f = 0.42 (15% ethyl acetate/hexanes). ^1H NMR (399MHz, CHLOROFORM- d) δ = 5.38 - 5.32 (m, 2 H), 5.30 - 5.24 (m, 1 H), 4.30 (dd, J = 4.4, 11.7 Hz, 2 H), 4.16 (dd, J = 5.9, 12.5 Hz, 2 H), 2.36 - 2.26 (m, 6 H), 2.05 - 1.97 (m, 4 H), 1.71 - 1.57 (m, 6 H), 1.27 (br. s., 24 H), 0.95 (t, J = 7.3 Hz, 6 H), 0.88 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{31}\text{H}_{56}\text{O}_6\text{Na}$ (MNa^+) 547.3978. Calcd. 547.3975.

(Z)-1,3-dihydroxypropan-2-yl icos-11-enoate (9b)—*C. antarctica*: 40mg, 55%; *R. miehei*: 58 mg, 79%; white solid. R_f = 0.24 (30% acetone/hexanes). MP = 32–33 °C. ^1H NMR (399MHz, CHLOROFORM- d) δ = 5.38 - 5.32 (m, 2 H), 4.93 (quin, J = 4.8 Hz, 1 H), 3.89 - 3.78 (m, 4 H), 2.38 (t, J = 7.3 Hz, 2 H), 2.20 - 2.12 (m, 2 H), 2.01 (q, J = 6.6 Hz, 4 H), 1.71 - 1.57 (m, 2 H), 1.40 - 1.18 (m, 24 H), 0.88 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (100MHz, 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 $\text{C}_{23}\text{H}_{44}\text{O}_4\text{Na}$ (MNa^+) 407.3142. Calcd. 407.3137.

2-((5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoyloxy)propane-1,3-diyl dibutyrate (10a)—168mg, 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) 3012, 2931, 1741, 1456. HRMS for $\text{C}_{31}\text{H}_{50}\text{O}_6\text{Na}$ (MNa^+) 541.3502. Calcd. 541.3505.

(5Z,8Z,11Z,14Z)-1,3-dihydroxypropan-2-yl icos-5,8,11,14-tetraenoate (10b)—*C. antarctica*: 48mg, 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) 3420, 2013, 2927, 1736, 1456. HRMS for C₂₃H₃₈O₄Na (MNa⁺) 401.2677. Calcd. 401.2668.

2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoxyloxy)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 CH₂Cl₂ (5 mL) at 0 °C. The reaction was allowed to stir for 4h. Upon completion, the reaction mixture was diluted with CH₂Cl₂ and H₂O. The organic layer was separated, dried over MgSO₄, and removed under reduced pressure. The resulting residue was chromatographed on silica gel (0% to 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 (500MHz, CHLOROFORM-d) δ = 5.47 - 5.32 (m, 12 H), 5.30 - 5.21 (m, 1 H), 4.29 (dd, *J* = 4.4, 11.7 Hz, 2 H), 4.16 (dd, *J* = 5.9, 12.2 Hz, 2 H), 2.93 - 2.77 (m, 10 H), 2.40 (d, *J* = 2.9 Hz, 4 H), 2.14 - 2.02 (m, 6 H), 0.98 (t, *J* = 7.6 Hz, 3 H). The ¹³C spectral data (100 MHz, CDCl₃) and IR data are in agreement with literature values.³² HRMS for C₂₉H₄₂O₆Na (MNa⁺) 509.2880. Calcd. 509.2879.

(4Z,7Z,10Z,13Z,16Z,19Z)-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 (399MHz, CHLOROFORM-d) δ = 5.52 - 5.22 (m, 12 H), 4.92 (quin, *J* = 4.6 Hz, 1 H), 3.82 (t, *J* = 5.1 Hz, 4 H), 2.91 - 2.77 (m, 10 H), 2.49 - 2.37 (m, 4 H), 2.22 (t, *J* = 6.2 Hz, 2 H), 2.07 (quin, *J* = 7.5 Hz, 2 H), 0.97 (t, *J* = 7.3 Hz, 3 H). ¹³C NMR (100MHz, 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 C₂₅H₃₈O₄Na (MNa⁺) 425.2666. Calcd. 425.2668.

2-(docosanoyloxy)propane-1,3-diyl dibutyrate (12a)—201 mg, 56%, colorless oil. R_f = 0.48 (15% ethyl acetate/hexanes). MP = 27–28 °C. ¹H NMR (399MHz, CHLOROFORM-d) δ = 5.32 - 5.23 (m, 1 H), 4.30 (dd, *J* = 4.4, 11.7 Hz, 2 H), 4.16 (dd, *J* = 5.9, 11.7 Hz, 2 H), 2.36 - 2.26 (m, 6 H), 1.71 - 1.57 (m, 6 H), 1.25 (s, 36 H), 0.95 (t, *J* = 7.3 Hz, 6 H), 0.88 (t, *J* = 6.6 Hz, 3 H). ¹³C NMR (100MHz, 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 C₃₃H₆₂O₆Na (MNa⁺) 577.4446. Calcd. 577.4444.

1,3-dihydroxypropan-2-yl docosanoate (12b)—*C. antarctica*: 32mg, 40%; *R. miehei*: 70 mg, 88%, white solid. R_f = 0.27 (30% acetone/hexanes). MP = 79–80 °C. ¹H NMR (399MHz, CHLOROFORM-d) δ = 4.93 (quin, *J* = 4.6 Hz, 1 H), 3.88 - 3.81 (m, 4 H), 2.38 (t, *J* = 7.7 Hz, 2 H), 2.08 (s, 2 H), 1.69 - 1.59 (m, 2 H), 1.38 - 1.19 (m, 36 H), 0.88 (t, *J* = 6.2 Hz, 3 H). ¹³C NMR (100MHz, 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 C₂₅H₅₀O₄Na (MNa⁺) 437.3610. Calcd. 437.3607.

2-(3-phenylpropanoyloxy)propane-1,3-diyl dibutyrate (13a)—280mg, 98%, colorless oil. R_f = 0.48 (35% ethyl acetate/hexanes). ¹H NMR (399MHz, CHLOROFORM-d) δ = 7.33 - 7.25 (m, 2 H), 7.24 - 7.15 (m, 3 H), 5.31 - 5.23 (m, 1 H), 4.28 (dd, *J* = 4.4, 11.7 Hz, 2 H), 4.13 (dd, *J* = 5.9, 11.7 Hz, 2 H), 2.96 (t, *J* = 7.7 Hz, 2 H), 2.66 (t, *J* = 8.1 Hz, 2 H), 2.34 - 2.24 (m, 6 H), 1.64 (sxt, *J* = 7.3 Hz, 4 H), 0.94 (t, *J* = 7.3 Hz, 6 H). ¹³C NMR (100MHz, 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 $\text{C}_{20}\text{H}_{28}\text{O}_6\text{Na}$ (MNa^+) 387.1787. Calcd. 387.1784.

1,3-dihydroxypropan-2-yl 3-phenylpropanoate (13b)—*C. antarctica*: 38mg, 83%; *R. miehei*: 8 mg, 40%; white foam. R_f = 0.18 (40% acetone/hexanes). ^1H NMR (399MHz, CHLOROFORM-d) δ = 7.36 - 7.28 (m, 2 H), 7.26 - 7.18 (m, 3 H), 4.89 (td, J = 4.5, 9.3 Hz, 1 H), 3.79 - 3.71 (m, 4 H), 3.02 - 2.96 (m, 2 H), 2.77 - 2.70 (m, 2 H), 1.91 - 1.83 (m, 2 H). ^{13}C NMR (100MHz, 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 $\text{C}_{12}\text{H}_{16}\text{O}_4\text{Na}$ (MNa^+) 247.0945. Calcd. 247.0946.

2-(5-phenylpentanoyloxy)propane-1,3-diyl dibutyrate (14a)—298mg, 99%, colorless oil. R_f = 0.63 (35% ethyl acetate/hexanes). ^1H NMR (500MHz, CHLOROFORM-d) δ = 7.31 - 7.24 (m, 2 H), 7.21 - 7.14 (m, 3 H), 5.31 - 5.23 (m, 1 H), 4.30 (dd, J = 4.4, 11.7 Hz, 2 H), 4.14 (dd, J = 5.9, 11.7 Hz, 2 H), 2.63 (t, J = 7.1 Hz, 2 H), 2.35 (t, J = 6.8 Hz, 2 H), 2.29 (t, J = 6.8 Hz, 4 H), 1.71 - 1.58 (m, 8 H), 0.94 (t, J = 7.3 Hz, 6 H). ^{13}C NMR (100MHz, 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 $\text{C}_{22}\text{H}_{32}\text{O}_6\text{Na}$ (MNa^+) 415.2094. Calcd. 415.2097.

1,3-dihydroxypropan-2-yl 5-phenylpentanoate (14b)—*C. antarctica*: 50mg, 83%; *R. miehei*: 24mg, 37%; white foam. R_f = 0.26 (40% acetone/hexanes). ^1H NMR (399MHz, CHLOROFORM-d) δ = 7.31 - 7.25 (m, 2 H), 7.22 - 7.13 (m, 3 H), 4.92 (td, J = 4.8, 9.5 Hz, 1 H), 3.86 - 3.76 (m, 4 H), 2.64 (t, J = 7.0 Hz, 2 H), 2.41 (t, J = 7.0 Hz, 2 H), 2.17 - 2.11 (m, 2 H), 1.74 - 1.60 (m, 4 H). ^{13}C NMR (100MHz, 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 $\text{C}_{14}\text{H}_{20}\text{O}_4\text{Na}$ (MNa^+) 275.1257. Calcd. 275.1259.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Scheme 1.

Scheme 2.

TABLE 1

Structures and Yields of Lipase catalyzed 2-MAGs

Compound No.	Triglyceride (a)	2-MAG (b)	<i>C. antarctica</i> ^a Yield (%)	<i>R. miehei</i> ^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.