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ORIGINAL PAPER

# Influence of Fatty Acid Desaturation on Spontaneous Acyl Migration in 2-Monoacylglycerols

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**Abstract** The effect of desaturation from the C9 to the C15 carbon of 2-monoacylglycerol (2-MAG) fatty acids on spontaneous acyl migration is described. Three 2-MAG species, 2-monooleoylglycerol (C18:1 *cis*-Δ9), 2-monolinoleoylglycerol (C18:2 *cis*-Δ9,12), and 2-monolinolenoylglycerol (C18:3 *cis*-Δ9,12,15) were synthesized by lipase-catalyzed ethanolysis of their respective triacylglycerols and isolated in >60 % yield and at 2-MAG purities of >95 % relative to 1-monoacylglycerol (1-MAG). $^{1}$H-NMR spectroscopy was used to monitor the spontaneous acyl migration of the 2-MAG species over a temperature range from 20 to 80 °C. The relative energies of activation calculated from the Arrhenius relationships of the 2-MAG acyl migration rate constants were 73.3, 68.0, and 72.9 kJ mol$^{-1}$ for the three 2-MAG species, respectively. Density functional calculations performed using the B3LYP functional at the 6-31+G* basis set on the three ketal ring intermediate of the three 2-MAG species followed a similar trend with a lack of relative energetic preference associated with the degree of desaturation. The kinetically determined relative activation energies were approximately twofold higher than the theoretical relative Gibbs free energies of the intermediates, suggesting that other factors influence acyl migration. In general, increasing desaturation after the C9 carbon of 2-MAG fatty acids had no appreciable effect on acyl migration rates.

**Keywords** Activation energy · Acyl migration · Kinetics · 2-Monoacylglycerol · Structured lipids

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## Introduction

Since the rigorous investigation of the chemical composition and physical properties of oils and fats began in the late eighteenth century and M. E. Chevreul's subsequent elucidation of the triacylglycerol structure in the early nineteenth century [1], scientists have endeavored to manipulate the chemical and physical properties of fats and oils for specific purposes. The properties of lipids can be manipulated through blending, additives, and random redistribution of fatty acids on the glycerol backbone through glycerolysis and transesterification. However, the ultimate control of chemical and physical properties of lipids is achieved through the synthesis of structured lipids [2]. Structured lipids differ from simple blends and random

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fatty acid redistribution in that the fatty acid moieties of triacylglycerols (TAG) are biologically (genetically engineered), chemically or enzymatically removed and exchanged with precise regiospecific positioning of specific fatty acids on the glycerol backbone [3]. Today, the synthesis of structured lipids is an integral part of research conducted to understand human physiological effects such as the preferential absorption and metabolism of lipids by the gut and liver [4] and the influence of lipids on a host of brain disorders [5].

Of recent interest has been the synthesis of ABA-type structured TAG with polyunsaturated fatty acids (PUFA, e.g. eicosapentaenoic, docosahexaenoic acids) in the middle, *sn*-2, glycerol position flanked by saturated medium chain fatty acids at the *sn*-1 and -3 glycerol positions [6–8]. These lipids are typically synthesized through either a two-step chemoenzymatic route or a two-step enzymatic route. The chemoenzymatic method involves the *sn*-1,3 regiospecific enzymatic esterification of glycerol with medium or short chain saturated fatty acids to form 1,3-diacylglycerols (1,3-DAG) followed by the chemical esterification of a PUFA to the *sn*-2 position [7]. The two-step enzymatic route involves deacylation of the *sn*-1,3 fatty acids of s*n*-2 PUFA enriched TAG to obtain PUFA enriched 2-monoacylglycerols (2-MAG) followed by enzymatic esterification of the PUFA enriched 2-MAG at the *sn*-1,3 positions with medium chain length, saturated fatty acids [4, 9].

A foible of the two-step enzymatic route, which may be preferred to avoid the use of chemical reagents and solvents is the stability of the 2-MAG intermediate during isolation, purification, and subsequent use in structured TAG synthesis. Since the 1920s it has been known that the fatty acid moieties of MAG and DAG spontaneously migrate between the three regiospecific *sn*-positions of the glycerol backbone [10, 11]. Careful consideration must be given to the method of 2-MAG purification to avoid spontaneous acyl migration to form 1(3)-MAG [4, 12]. The likely mechanism of spontaneous acyl migration of fatty acids from the *sn*-2 to the 1(3) position involves a five-member, intramolecular ketal ring intermediate formed from a nucleophilic attack of a primary hydroxyl oxygen on the secondary acyl carbonyl group of the *sn*-2 fatty acid [10, 11, 13, 14]. The spontaneous acyl migration of fatty acids in 2-MAG results in a thermodynamic equilibrium of ~1:9 2-MAG:1-MAG. This equilibrium is reached at a rate influenced by a host of variables including temperature, polarity, water activity, fatty acid chain length, acid/base impurities, and time [13, 15–18].

In addition to acyl chain length, it has been proposed that desaturation of the acyl chain may increase 2-MAG to 1-MAG acyl migration rates [19]. However, comparative acyl migration kinetics of 2-monopalmitoylglycerol versus 2-monooleoylglycerol determined in model chylomicra solutions (buffered lipid-water emulsions) showed no appreciable difference between the unsaturated and monounsaturated acyl migration rates [20]. In the context of the ABA-type structured lipids containing high degrees of *sn*-2 fatty acid desaturation used as structured lipid intermediates (discussed above), we thought it valuable to determine the effect of fatty acid desaturation on acyl migration rates of isolated 2-MAG absent the influence of water, organic solvents, and polar buffering agents. Varying degrees of fatty acid desaturation affects the bulk physical properties of the MAG. Increased fatty acid desaturation in the form of *cis*-bonds decreases the linearity of the fatty acid chains, lowering the TAG's melting point and decreasing the MAG's viscosity. We hypothesized that while desaturation at the C9 carbon and beyond may be too far removed from the glycerol backbone to mechanistically influence acyl migration, the increasing non-linearity of the more highly desaturated MAG may affect bulk oil properties (i.e. lower the viscosity) enough to influence spontaneous acyl migration rates. The current work describes the use of $^{1}$H-NMR spectroscopy to determine the acyl migration rates [17, 18] of the steric family of unsaturated MAG, specifically 2-monooleoylglycerol (C18:*cis*-Δ9), 2-monolinoleoylglycerol (C18:*cis*-Δ9,12), and 2-monolinolenoylglycerol (C18:*cis*-Δ9,12,15).

## Materials and Methods

### Materials

Glyceryl trioleate (triolein) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Glyceryl trilinoleate (trilinolein), glyceryl trilinolenate (trilinolenin), 2-MAG standards of olein, linolein, linolenin, and fatty acid ethyl ester standards of olein, linolein, linolenin were purchased from Nu-Chek-Prep, Inc. (Elysian, MN, USA). The acylglycerols were stored at 0 °C and used as purchased. Ethanol (200 proof) was purchased from Decon Laboratories, Inc. (King of Prussia, PA, USA) and used fresh. Novozym 435, *Candida antartica* lipase B immobilized on a macroporous acrylic resin was purchased from Brenntag Great Lakes (Chicago, IL, USA). Ethylene glycol, glycerol, and all other solvents were purchased from Sigma-Aldrich and used as received.

### 2-MAG Syntheses

#### *General Synthesis*

The three 2-MAG species were synthesized using the same procedure [17]. TAG (10.0 g, ~28.0 mmol) and ethanol (40 g, 868 mmol) were combined with Novozym 435 (5.0 g) and stirred vigorously for 4 h. The enzyme was

removed by filtration through a spectra/mesh (Cole-Parmer Instrument Co., Vernon Hills, IL, USA) 20 μm nylon mesh. Excess ethanol was removed from the filtrate by rotoevaporation, and the resultant oily residue was dissolved in 5 % aqueous acetonitrile. The solution was extracted with three 40 ml portions of hexanes and the extracts discarded. The aqueous acetonitrile phase was analyzed by HPLC to confirm 2-MAG purity versus fatty acid ethyl esters and glycerol, and the 2-MAG products were determined to be >98 % pure. The acetonitrile was removed by rotoevaporation, and the resultant pale yellow oils were freeze dried for 24 h. The NMR data for the 2-MAG species are consistent with previously reported data [17, 21].

*2-Monooleoylglycerol,* ***1a***

Yield: 2.54 g, 63.0 % based on triolein. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 5.36 (m, 2 H, –C*H*=C*H*–), 4.94 (p, 1 H, –$CH_2$–C*H*–$CH_2$–), 3.84 (d, 4 H, $J = 4.73$ Hz, –$CH_2$–CH–$CH_2$–), 2.39 (t, 2 H, –O–C(O)–$CH_2$–), 2.02 (m, 4 H, –$CH_2$–CH=CH–), 1.66 (m, 2 H, –O–C(O)–$CH_2$– $CH_2$–), 1.30 (m, 20 H, –$CH_2$–$CH_2$–$CH_2$–), and 0.89 (t, 3 H, –$CH_3$) ppm.

*2-Monolinoleoylglycerol,* ***1b***

Yield: 2.54 g, 65.7 % based on trilinolein. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 5.39 (m, 4 H, –C*H*=C*H*–), 4.95 (p, 1 H, –$CH_2$–C*H*–$CH_2$–), 3.85 (d, 4 H, $J = 4.36$ Hz, –$CH_2$–CH–$CH_2$–), 2.79 (t, 2 H, =CH–$CH_2$–CH=), 2.39 (t, 2 H, –O–C(O)–$CH_2$–), 2.06 (broad m, 4 H, –$CH_2$–CH=CH–), 1.66 (m, 2 H, –O–C(O)–$CH_2$– $CH_2$–), 1.33 (m, 14 H, –$CH_2$–$CH_2$–$CH_2$–), and 0.91 (t, 3 H, –$CH_3$) ppm.

*2-Monolinolenoylglycerol,* ***1c***

Yield: 2.44 g, 60.4 % based on trilinolenin. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 5.38 (m, 6 H, –C*H*=C*H*–), 4.94 (p, 1 H, –$CH_2$–C*H*–$CH_2$–), 3.84 (d, 4 H, $J = 4.65$ Hz, –$CH_2$–CH–$CH_2$–), 2.82 (t, 4 H, =CH–$CH_2$–CH=), 2.39 (t, 2 H, –O–C(O)–$CH_2$–), 2.07 (m, 4 H, –$CH_2$–CH=CH–), 1.66 (m, 2 H, –O–C(O)–$CH_2$–$CH_2$–), 1.33 (broad s, 8 H, –$CH_2$–$CH_2$–$CH_2$–), and 0.99 (t, 3 H, –$CH_3$) ppm.

## Methods

### *2-MAG Acyl Migration Kinetics*

Kinetic studies were conducted using standard, air-sensitive, Schlenk line techniques [22]. Four samples of 0.5 ml each of **1a**, **b**, **c** directly from dry freezing were placed in open HPLC vials that were overpacked into 5 ml Shlenck tubes for a total of 12 samples. The samples were evacuated and flushed with nitrogen. One of each of the 2-MAG samples was kept at ambient temperature 20 °C and heated in incubators at 40, 60, and 80 °C. Aliquots (~20 μl) were taken from each of the 12 samples at 24 h time intervals and dispensed into NMR tubes. The tubes were sealed and stored at 0 °C for NMR analyses. The kinetic studies were performed in triplicate at each temperature.

### *$^1$H-NMR Analyses*

Spectra were obtained on a Bruker (Bruker-Biospin Corp., Billerica, MA, USA) Avance 500 spectrometer (500 MHz $^1$H) using a 5 mm BBI probe (Bruker-Biospin Corp.). All samples were dissolved in $CDCl_3$, and all spectra were acquired at 27 °C within 2 min of dilution in $CDCl_3$. Chemical shifts are reported as ppm from tetramethylsilane calculated from the lock signal ($\Xi_D = 15.350609$ %).

### *HPLC Analysis for 2-MAG*

2-MAG samples were analyzed using a Thermo Separations Product (Thermo Fischer Scientific, Rockville, MD, USA) HPLC system consisting of a Spectra System AS3000 autosampler, a Spectra System P4000 pump, a Spectra System UV6000LP detector, and an All-Tech (Alltech Associates Inc., Deerfield, IL, USA) 500 ELS detector. HPLC solvents were filtered through a Whatman 0.45 μm nylon membrane filter and degassed using a thermo separation products SCM 1000 membrane degasser. Injected (10 μl) samples were eluted from a Prodigy (Phenomenex, Torrance, CA, USA) C8 column (5 mm, 250 × 4.6 mm) with a 1.5 ml/min. isocratic flow of 40:60 (v:v) acetone (containing 1 vol % glacial acetic acid): acetonitrile mobile phase. The eluate was monitored by ELSD (nitrogen; 20 slpm, 70 °C).

### *HPLC Analysis for Glycerol*

Residual glycerol in the 2-MAG samples was determined using a Spectra System (Thermo Fisher Scientific) SCM1000 HPLC comprised of a Spectra System P4000 pump, Thermo Separation Products AS3000 autosampler and a Thermo Finnegan RI Plus detector. 2-MAG samples (~100 μl) were placed in tared eppendorf tubes and 20 ml of ethylene glycol was added as an internal standard. The samples were washed with 480 μl of 500 mM $H_2SO_4$ (mobile phase) and the washed samples were spun at 3,000 rpm in a microfuge for 5 min. The aqueous phase was transferred to an HPLC vial and washed again with 450 μl of the mobile phase. The sample was then filtered through a Pall Life Sciences (Pall Corp., Port Washington, NY, USA) 0.45 mm PVDF membrane syringe filter. Injected samples (8 μl) were developed on a Biorad (Bio-Rad Laboratories Inc., Hercules, CA, USA) Aminex HPX-87H Ion Exclusion

column (300 × 7.8 mm) with an isocratic mobile phase of 0.8 ml/min and an RI detector temperature of 37 °C. Quantification of glycerol was determined using the internal standard and a glycerol standard calibration curve [23].

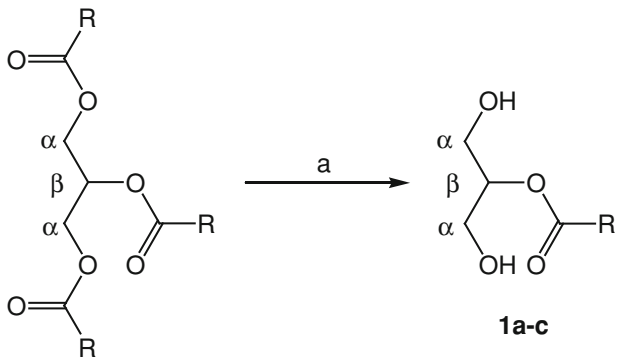
*Density Functional Calculations*

All density functional calculations were carried out as implemented in Spartan' 10 v1.1.0 (Wavefunction Inc., Irvine, CA, USA). Thermodynamic properties ($\Delta G$, $\Delta H$, and $\Delta S$) were calculated based on frequency calculations on geometry optimized structures in vacuo using the B3LYP functional at the 6-31+G* basis set. Thermodynamic properties are reported at 298.15 K. Initial structures were built using semiempirical PM3 methods and results are displayed using HyperChem v8.0.9 (Hypercube Inc., Gainesville, FL, USA).

## Results and Discussion

2-MAG Synthesis and Characterization

Three species of 2-MAG, 2-monooleoylglycerol (**1a**), 2-monolinoleoylglycerol (**1b**), and 2-monolinolenoylglycerol (**1c**) were synthesized by the Novozym 435 catalyzed ethanolysis of their respective TAG (Scheme 1) [9, 17]. The




**a**: R = $(CH_2)_7CH{=}CH(CH_2)_7CH_3$
**b**: R = $(CH_2)_7CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$
**c**: R = $(CH_2)_7CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH_3$

**Scheme 1** Synthesis of 2-MAG, reaction conditions: **a** excess ethanol, Novozym 435, 25 °C, and 4 h. $\alpha$ and $\beta$ denote carbon labels for the glycerol backbone

2-MAG were isolated by liquid–liquid solvent extraction and characterized by $^1$H-NMR spectroscopy and HPLC chromatography [17, 21]. It is well established that 2-MAG can undergo spontaneous acyl migration of the fatty acid to the *sn*-1(3) position to form 1-MAG (Scheme 2). The 2-MAG (**1a–c**) were isolated in 60–65 % yields with purities >95 mol% relative to their respective 1-MAG (**2a–c**) indicating that very little acyl migration occurred during purification. While the lipase-catalyzed, *sn*-1,3 specific ethanolysis was nearly quantitative in yielding 2-MAG, the 2-MAG products did contain <5 mol% DAG, resulting from incomplete ethanolysis. Due to similar solubilities the small quantities of DAG were not removed during the liquid–liquid extraction step. Purification, however did remove all fatty acid ethyl ester byproduct and most of the residual glycerol (<2 wt% detected by HPLC).

2-MAG Acyl Migration Density Functional Modeling

Molecular modeling was used to determine if the degree of desaturation of a fatty acid had a theoretical effect on energetic stability of the intermediate (Scheme 2) in 2-MAG acyl migration. A density functional study was carried out to investigate the influence of energies and thermodynamic properties on the intermediates of **1a** to **2a** (Oleic), **1b** to **2b** (Linoleic), and **1c** to **2c** (Linolenic). Similar density functional studies have provided information on transition states for aldol reactions and intermediates in rearrangement reactions [24, 25]. Geometry optimization calculations were carried out with Becke's three parameter hybrid functional with the Lee-Yang-Paar correlation functional (B3LYP) and the 6-31+G* basis set.

As seen in Table 1, the relative electronic energy ($\Delta E$), Gibbs free energy ($\Delta G$), enthalpy ($\Delta H$) and entropy ($\Delta S$) calculated at 25 °C (298.15 K) are similar for all the intermediates for oleic acid, linoleic acid, and linolenic acid. The reduced entropy in the intermediates ($\Delta S$) is associated with a favorable intermolecular hydrogen bond between the two hydroxyls of the ketal moiety (see Scheme 2), and this interaction is shared by all MAG in this study. The hydrogen bond length is 2.00 Å for oleic acid. The intermediate confirmations without this hydrogen bond were 41.8–44.7 kJ mol$^{-1}$ in free energy ($\Delta G$) over the free energy of the reactants. The reaction coordinate for the acyl migration of MAG of oleic acid is provided in

**Scheme 2** Spontaneous acyl migration of 2-MAG (**1a–c**) fatty acid moieties to form 1-MAG (**2a–c**). R-groups **a–c** are defined in Scheme 1

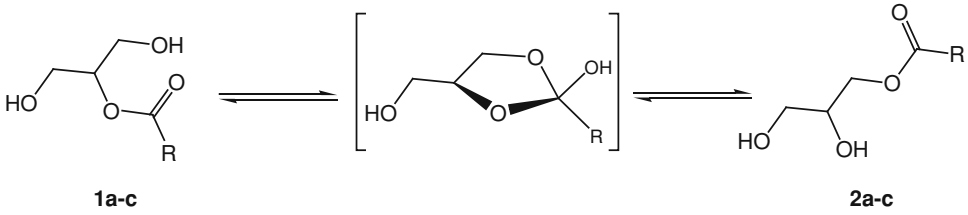


**Table 1** Density function calculated energies and thermodynamic parameters for the oleic, linoleic, and α-linolenic acid MAG intermediates at the 6-31+G* level

| Glycerol ester intermediate | $\Delta E$ (kJ mol$^{-1}$) | $\Delta G_{298.15}$ (kJ mol$^{-1}$) | $\Delta H_{298.15}$ (kJ mol$^{-1}$) | $\Delta S_{298.15}$ (kJ mol$^{-1}$) |
|---|---|---|---|---|
| Oleic | 28.88 | 36.28 | 32.06 | −14.23 |
| Linoleic | 30.27 | 35.65 | 31.30 | −14.55 |
| Linolenic | 28.51 | 37.18 | 32.85 | −14.49 |

Fig. 1. This general reaction coordinate profile was shared by the MAG of linoleic acid and linolenic acid. The slight discrepancy between the acyl migration parameters of oleic acid, linoleic acid and linolenic acid MAG may be associated with the packing of the neat (i.e. absent solvent) MAG molecules and the kink associated with the fatty ester tail (see Fig. 2). The density function calculations on the intermediates show that increasing desaturation past the C9 carbon did not have a significant theoretical influence on 2-MAG acyl migration.

## 2-MAG Acyl Migration Kinetics

$^1$H NMR has been shown to be an expedient method for directly measuring the rate of spontaneous acyl migration while minimizing the effects of solvent, temperature, and time during analysis [17, 18]. The technique relies on determining the 2-MAG:1-MAG molar ratio using the ratios of the integrated peaks of the center glycerol protons. The peripheral α-protons and center β-proton of the glycerol backbone of the 2-MAG (Scheme 1) were attributed to the 3.84 and 4.94 ppm signals, respectively [17, 21]. The β-protons of the 1,2 and 1,3-DAG species were assigned to the peaks at 5.08 and 4.1 ppm, respectively [17, 21]. The β-protons of the acylglycerol species were unique in that they were equimolar to the respective acylglycerol species, thus, the integrated peak ratios were used to directly determine MAG and DAG mole ratios [17, 18]. The 2-MAG:1-MAG mole ratios determined over time at temperatures ranging from 20 to 80 °C were used to resolve the acyl migration kinetics of **1a–c** converting to **2a–c** [17].

Neat samples of **1a–c** were held at constant temperatures over the course of 2 weeks. The samples were kept under nitrogen to inhibit lipid oxidation and degradation. Aliquots were drawn daily for analysis by $^1$H NMR to determine the mol% of 2-MAG. Fig. 3 shows the conversion of 2-MAG to1-MAG for **1a–c** over the course of 2 weeks. All three 2-MAG species started at >95 mol% 2-MAG relative to 1-MAG. At 20 °C after 2 weeks **1b** showed the most conversion to its respective 1-MAG with ~85 mol% of **1b** remaining. The other 2-MAG species, **1a** and **c** showed <10 % 2-MAG acyl migration to 1-MAG after 2 weeks. These data demonstrated that isolated, desaturated 2-MAG can be practically used as intermediates for the synthesis of structured TAG at ambient temperatures on the time scale of hours without significant loss due to acyl migration. Indeed, all three 2-MAG species showed no loss of 2-MAG when stored at 0 °C for over 2 weeks (data not shown) as corroborated by Fureby et al. [13].

While relatively stable against acyl migration at 20 °C, as expected, all three 2-MAG species demonstrated accelerated migration rates at higher temperatures (Fig. 3) [15, 17]. Allowed to proceed to equilibrium, the conversion

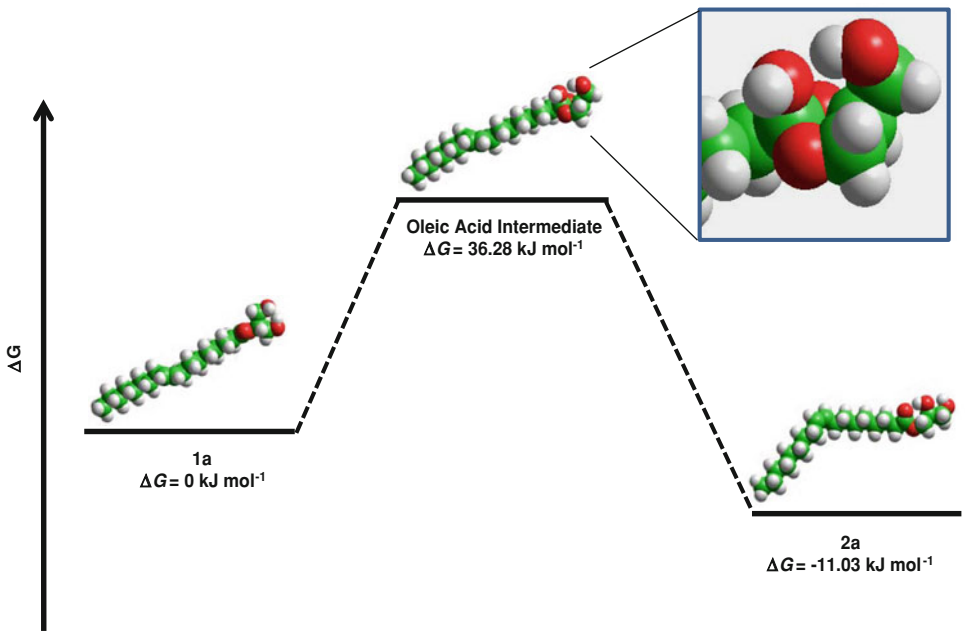



**Fig. 1** Reaction coordinate for the acyl migration of **1a** to **2a** at the B3LYP/6-31+G* level

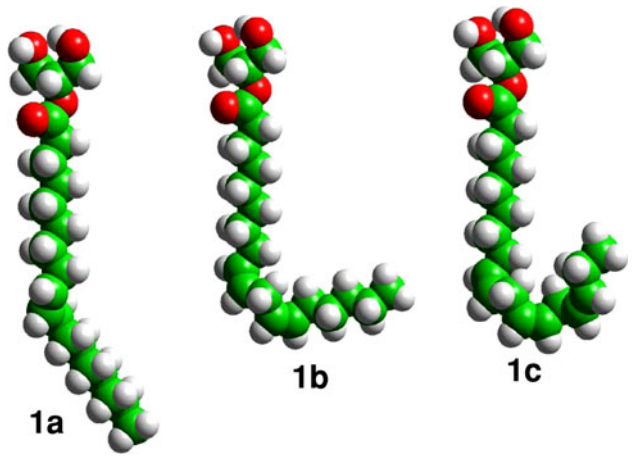



**Fig. 2** Geometry optimized structures of **1a–c** at the B3LYP/6-31+G* level

of 2-MAG to 1-MAG was expected to reach a mole ratio of ~1:9 2-MAG:1-MAG [16, 19]. As seen in Fig. 3, the neat 2-MAG samples only reached the expected equilibrium at 60 and 80 °C. All three 2-MAG species reached ~11 mol% 2-MAG after 96 h at 80 °C, but required 2 weeks to reach equilibrium at 60 °C. None of the 2-MAG species reached migration equilibrium at 20 or 40 °C within the time course of this study. The rate of migration for **1a–c** was significantly slower than 2-MAG samples synthesized by enzymatic hydrolysis and incubated in hexane at 30 °C, where 2-palmitoyl-*sn*-glycerol reached migration equilibrium in ~100 h [16]. Also, **1a** synthesized by enzymatic methanolysis in *tert*-butanol/phosphate buffer solutions and ethanolysis in aqueous ethanol solutions and incubated in the reaction solutions reached migration equilibrium in ~50 h at 25 and 30 °C [13, 15]. The rates of **1a–c** acyl migration were very similar to the rate of 2-MAG synthesized from soybean oil, which contained a mixture of 20 % C18:1, 70 % C18:2 and 8 % C18:3 at the *sn*-2 position and was studied neat over a similar temperature range [17].

The acyl migration data in Fig. 3 were modeled using a first-order reaction scheme based on the concentration of 2-MAG, [2-MAG], the concentration of 1-MAG, [1-MAG], and $k_1$ and $k_2$ which were the respective forward and reverse rate constants (Eq. 1) [17].

$$[\text{2-MAG}] \underset{k_2}{\overset{k_1}{\rightleftarrows}} [\text{1-MAG}] \quad (1)$$

Based on a first-order rate law, Compton et al. [17] detail the derivation of an explicit expression of [2-MAG] as a function of time where $[\text{2-MAG}]_0$ is the 2-MAG concentration at 0 h, $[\text{2-MAG}]_e$ is the 2-MAG concentration at equilibrium, $C = (K + 1)/K$, the equilibrium constant $K = k_2/k_1$, and $t$ is time (Eq. 2). This expression was reached based on the rate of migration being zero at equilibrium and using an integrated solution of a [2-MAG] dependent expression of time [17, 26].

$$[\text{2-MAG}] = (([\text{2-MAG}]_0 - [\text{2-MAG}]_e / \exp(Ck_1t)) + [\text{2-MAG}]_e \quad (2)$$

From the kinetics data (Fig. 3) the 2-MAG mol% for **1a–c** was found to be 11.5, 10.6, and 10.6 mol%

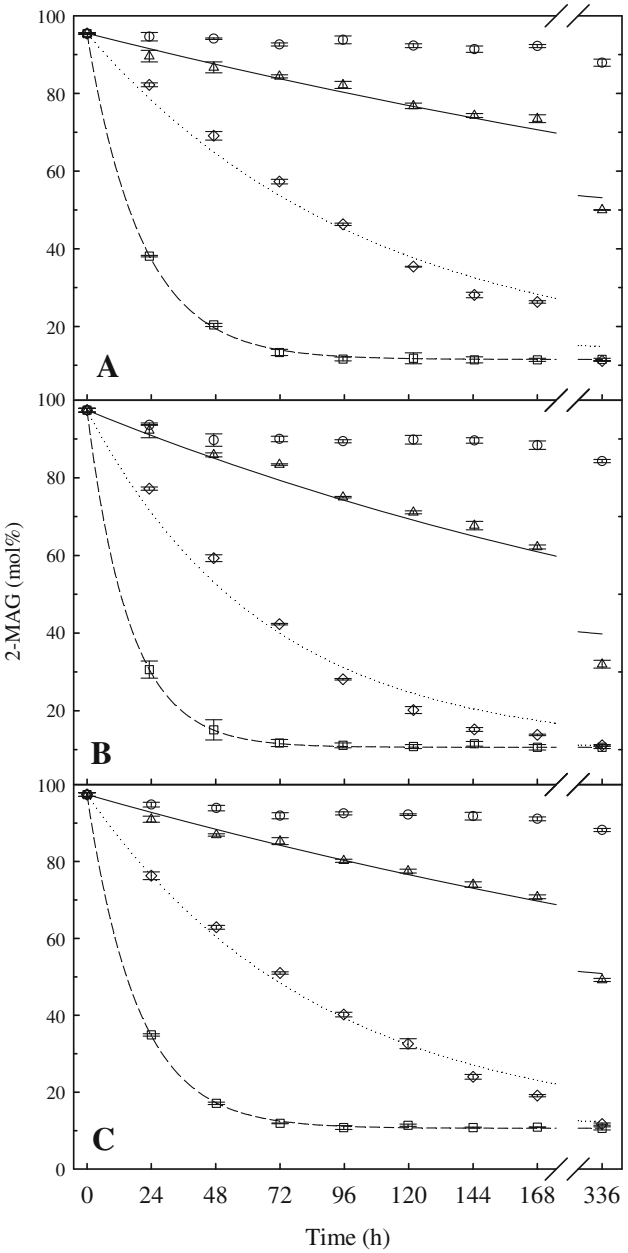



**Fig. 3** Acyl migration of 2-MAG to form 1-MAG: **1a** (**a**), **1b** (**b**) and **1c** (**c**); 20 °C (*circles*), 40 °C (*triangles*), 60 °C (*diamonds*), and 80 °C (*squares*). Data are the mean of $n = 3$ trials with *error bars* denoting one standard deviation from the mean. The *lines* represent values derived from the kinetics model (Eq. 2) and fitted parameters (Table 2)

respectively, at equilibrium. Based on these values, $K$ for **1a–c** was set to 7.70, 8.43, and 8.43, respectively, independent of temperature. It must be mentioned that the van't Hoff relationship [27] dictates that a different $K$ value would be expected for each 2-MAG species at each temperature. Each of the 2-MAG species, however reached the same $[2\text{-MAG}]_e$ at both 60 and 80 °C as determined by $^1$H-NMR spectroscopy. It was therefore assumed that any change in $K$ for a specific 2-MAG species over the temperature range used for this study was minimal and within the error of measurement. Thus, the same $K$ value was used for calculations at all temperatures for each specific 2-MAG species.

The initial **1a–c** mol% was 95.4, 97.4, and 97.4 mol%, respectively, and these $[2\text{-MAG}]_0$ values were used for each temperature. The rate constant $k_1$ was determined for **1a–c** at each temperature by minimizing the sum of the squared residuals, the difference between the observed and calculated [2-MAG] using Eq. 2 [17]. The fitting algorithm failed to converge for the 20 °C data for all three 2-MAG species. The $k_1$ values and half-life estimates, $t_{1/2} = (k_1 + k_2)^{-1}\ln(2)$, for the acyl migration of **1a–c** to **2a–c** at 40, 60, and 80 °C are reported in Table 2. The $k_1$ values were used to construct the [2-MAG] decay curves fitted to the data in Fig. 3. The $k_1$ values for each 2-MAG species reported in Table 2 exhibited an Arrhenius relationship, illustrated in Fig. 4. The relative activation energies $E_a$ determined as the negative slope of the Arrhenius plot for each 2-MAG species and the corresponding Arrhenius plot correlation factors are reported in Table 3.

Considering only **1a** (the most studied 2-MAG), the rate of acyl migration of the neat 2-MAG at 40 °C 0.0018 $h^{-1}$ was 84-fold slower than **1a** rates in *tert*-butanol at 37 °C [15]. In fact the acyl migration rate of neat **1a** can be concluded to always be slower than in solution, at least 2.5-fold slower than **1a** migration rates in primary through tertiary alcohols and as much as two orders of magnitude slower than in alkane-halides (e.g. tetrachloromethane) [28]. The relative energy of activation of neat **1a** 73.3 kJ $mol^{-1}$ was nearly twofold higher than that reported for **1a** in isooctane, 40 kJ $mol^{-1}$ [13]. Compared to neat 2-MAG from soybean oil, **1a** essentially had the same $E_a$, 79.0 versus 73.0 kJ $mol^{-1}$, respectively [17]. These comparisons confirm that the kinetics data reported herein for the conversion of **1a–c** to **2a–c** were within the expected values reported in the literature.

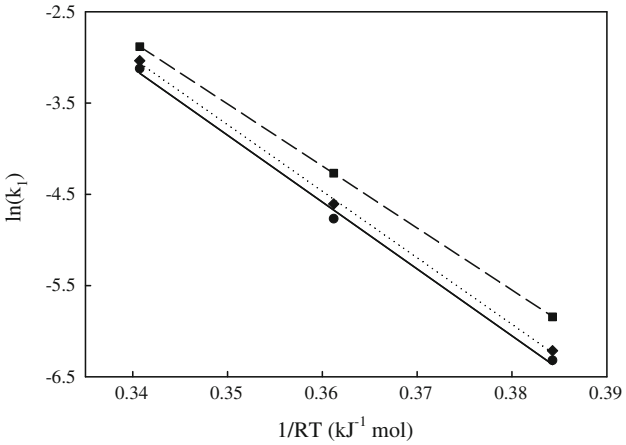



**Fig. 4** Arrhenius relationships determined from the $k_1$ values reported in Table 2 for the acyl migration of **1a** to **2a** (*circles*), **1b** to **2b** (*diamonds*), and **1c** to **2c** (*squares*). *Lines* represent the linear regression of the data at 99 % confidence intervals: **1a** to **2a** (*solid*), **1b** to **2b** (*dashed*), and **1c** to **2c** (*dotted*)

## Effect of Desaturation on 2-MAG Acyl Migration

To reiterate, the objective of this study was to determine if desaturation had an effect on 2-MAG to 1-MAG acyl migration rates. A previous study reported that there was no appreciable influence of desaturation on 2-MAG (2-palmitoyl-*sn*-glycerol vs. **1a**) acyl migration rates at 25 and 37 °C in hexane/tris buffer emulsions (model chylomicra solutions) [20]. However, 2-palmitoyl-*sn*-glycerol was reported to have an acyl migration $k_1 = 0.0436$ $h^{-1}$ ($K = 5.3$) in hexane solutions [16] while **1a** was reported to have an acyl migration rate of $k_1 = 0.152$ $h^{-1}$ ($K = 20.7$) in *tert*-butanol solutions [15]. Fureby et al. [13] showed that the acyl migration of **1a** in hexane was tenfold faster than in *tert*-butanol, contrary to the solvent effects observed for 2-palmitoyl-*sn*-glycerol and **1a** in the previous two studies, suggesting that desaturation may have an effect on acyl migration rates.

**Table 2** First-order reaction constants for the acyl migration of 2-MAG (**1a–c**) to 1-MAG (**2a–c**) determined from $^1$H-NMR kinetic data

| Temperature (°C) | Rate constant, $k_1$ ($h^{-1}$) | | | Half-life, $t_{1/2}$ (h) | | |
|---|---|---|---|---|---|---|
| | **1a → 2a** | **1b → 2b** | **1c → 2c** | **1a → 2a** | **1b → 2b** | **1c → 2c** |
| 40 | 0.0018 | 0.0029 | 0.0020 | 330 | 210 | 300 |
| 60 | 0.0085 | 0.014 | 0.010 | 72 | 46 | 60 |
| 80 | 0.044 | 0.056 | 0.048 | 14 | 11 | 13 |

**Table 3** Relative activation energies and correlation factors determined from the linear regressions of the Arrhenius relationships in Fig. 4 for the acyl migration of 2-MAG (**1a–c**) to 1-MAG (**2a–c**)

| Acyl migration | $E_a$ (kJ mol$^{-1}$) | Correlation factor, $r^2$ |
|---|---|---|
| **1a → 2a** | 73.3 | 0.9974 |
| **1b → 2b** | 68.0 | 0.9998 |
| **1c → 2c** | 72.9 | 0.9993 |

Theoretically, through density functional calculations, it was predicted that desaturation would not have an appreciable effect on 2-MAG acyl migration. The energies associated with the intermediates ($\Delta G_{298.15}$) reported in Table 1 suggested that the nonlinearity of the fatty acid chain starting at the C9 carbon did not interfere with the formation of the 5-member ketal ring intermediate (Scheme 2). The theoretical calculations, however were conducted within the parameters of an isolated 2-MAG molecule in vacuo. Thus, the calculations considered only intramolecular interactions and did not account for intermolecular interactions such as differences in close packing due to the increasing nonlinearity of the fatty acid chain and the resultant decrease in bulk viscosity.

The relative energy of activations $E_a$ of the 2-MAG species calculated from the $^1$H-NMR kinetics data inherently include bulk property effects such as intermolecular interactions and viscosities. Compared to the predicted $\Delta G_{298.15}$ values, the measured $E_a$ for the 2-MAG species (Table 3) were 1.9–2.0-fold higher than the theoretical values. This suggested that the intermolecular interactions unaccounted for in the density functional calculations affected actual acyl migration rates, slowing the formation of the transition-state ketal ring. The $E_a$ values of the three 2-MAG species, however were essentially the same (within 5.3 kJ mol$^{-1}$) as predicted by the density functional modeling, suggesting that increased desaturation and intermolecular interactions (e.g. viscosity) had no significant effect on relative acyl migration rates.

## Conclusions

2-MAG are used as intermediates in the synthesis of ABA-type structured lipids, and spontaneous acyl migration of the fatty acid moiety from the *sn*-2 to the *sn*-1(3) position of the glycerol backbone must be considered when using 2-MAG as synthetic intermediates. It was known that a number of variables including the presence of solvent, solvent polarity, temperature, water activity, and acyl chain length influence the rate of acyl migration. Because of conflicting evidence in the literature we investigated the influence of fatty acid desaturation to definitively determine if there was an effect on acyl migration rates.

Theoretical energies of the intermediates and relative energy of activations calculated from kinetic data were determined for the steric family of desaturated 2-MAG: 2-monooleoylglycerol (C18:*cis*-Δ9), 2-monolinoleoylglycerol (C18:*cis*-Δ9,12), and 2-monolinolenoylglycerol (C18:*cis*-Δ9,12,15), were examined. 2-Monostearoylglycerol was excluded from the study, because it was solid over most of the temperature range studied (mp ~ 58 °C). Both the theoretical calculations and the measured kinetics data conducted on neat (i.e. absent solvent) 2-MAG samples showed that increasing the number of double bonds past the C9 carbon on the fatty acid chain of a 2-MAG had no appreciable effect on acyl migration rates.

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