Syntheses of Polymerizable Monoacylglycerols and 1,2-Diacyl-*sn*-glycerols

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The first chemical syntheses of polymerizable monoacylglycerol and 1,2-diacyl-*sn*-glycerol are reported. The monodienoylglycerol is obtained in 80% yield from 1,2-*O*-isopropylidene-*sn*-glycerol and dienoyl fatty acid. The dienoic acid is accessible in 60% yield from the base-catalyzed hydrolysis of dienoyl ester, which is synthesized from the Wittig−Horner reaction of aldehyde and trimethyl 4-phosphonocrotonate. The acylation is carried out in the presence of 4-(dimethylamino)pyridine and dicyclohexylcarbodiimide. The use of excess protected glycerol relative to fatty acid affords the acylated product in high yield. The final step is the deprotection of isopropylidene group using dilute HCl solution. The 1,2-diacyl-*sn*-glycerol is synthesized by acylation of 3-(4-methoxybenzyl)-*sn*-glycerol with dienoyl fatty acid in the presence of 4-(dimethylamino)pyridine and dicyclohexylcarbodiimide. The removal of the 4-methoxybenzyl group by dimethylboron bromide catalyzed hydrolysis is especially useful in the synthesis of polymerizable lipids because the deprotection proceeds without any apparent effect on the dienoyl polymerizable group and without detectable isomerization of 1,2-diacylglycerol to 1,3-diacylglycerol. The overall yield for the synthesis of the polymerizable 1,2-diacyl-*sn*-glycerol from the dienoyl fatty acid is ca. 50%.

Introduction

During the past decade the polymerization of preformed lipid assemblies has been successfully utilized to modify the chemical and physical properties of two-dimensional lamellar assemblies, e.g., monolayer, multilayer, and bilayer vesicles.[1] A variety of lipids containing polymerizable groups such as diacetylene, dienoyl, sorbyl, vinyl, styryl, and acryloyl have been designed and synthesized.[2,3] At certain conditions of concentration, temperature, and pressure, biological and synthetic lipids can form hydrated nonlamellar assemblies such as inverted cubic (Q_{II}) and inverted hexagonal (H_{II}) phases. It has been proposed that some of these nonlamellar lipid structures may be important in biological process.[4−6] Various Q_{II} phases have been reported to exist between the lamellar and the H_{II} phases.[4,5] Several inverted cubic phases are possible, and those which are bicontinuous with respect to the polar (aqueous) and nonpolar (lipid) regions can be considered as organic zeolites. The potential use of these lipid assemblies depends at least in part on the prospects for stabilization of their nonlamellar architecture in order to expand their useful temperature and concentration range. Recently, we prepared a polymerizable phosphatidylethanolamine (PE) and a phosphatidylcholine (PC) in order to stabilize a bicontinuous cubic phase.[7] A 3:1 molar mixture of a monodienoylphosphatidylethanolamine (mono-DenPE) and bis-dienoylphosphatidylcholine (bis-DenPC) form a Q_{II} phase belonging to the *Pn*3*m* space group (at ca. 60 °C). Redox-initiated radical polymerization of these lipids stabilized this Q_{II} phase in a manner that permitted it to exist at temperatures as low as ca. 0 °C.[8] This strategy for the stabilization of nonlamellar phases relies on the design of suitable polymerizable lipids, which upon hydration form nonlamellar assemblies. The nonlamellar phase is then fixed by cross-linking polymerization.

In order to demonstrate the generality of this approach, we have designed other classes of polymerizable lipids that are likely to form nonlamellar phases. The formation of nonlamellar phases from monoacylglcerols (MAG) is well documented.[9−11] The phase behavior of monoacylglycerols alone or mixed with other lipids, e.g., diacylglycerols, dioleoylPC, has been extensively investigated and utilized as a model membrane system for the physicochemical investigation of lipid structures and dynamics.[9−12] The temperature−composition phase diagrams of various monoacylglycerols in water have been constructed on the basis of data from X-ray diffraction.[9−11] In addition to lamellar phases, these hydrated lipids form various Q_{II} phases, e.g., monoolein at room temperature forms a Q_{II} phase with *Ia*3*d* symmetry at 25−33 wt % water, whereas at higher water content the phase with *Pn*3*m* symmetry is favored.[9]

On the basis of the literature precedents, we designed a monoacylglycerol with a polymerizable group incorporated into the acyl chain. Such a reactive monoacylglycerol may have the polymerizable moiety located either at a position near the lipid backbone or at the hydrophobic chain end. We have prepared lipids with dienoyl groups at either position. When the polymerizable diene

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(1) O'Brien, D. F. *Trends Polym. Sci.* **1994**, *2*, 183−188.
(2) O'Brien, D. F.; Ramaswami, V. *Encyclopedia of Polymer Science and Engineering*, 2nd ed.; Wiley: New York, 1989; pp 108−135.
(3) (a) Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem., Int. Ed. Engl.* **1988,** *27*, 113−158. (b) Singh, A.; Schnur, J. M. Polymerizable Phospholipids. In *Phospholipids Handbook*; Cevc, G., Ed.; Marcel Dekker: New York, 1993; pp 233−291.
(4) Lindblom, G.; Rilfors, L. *Biochim. Biophys. Acta* **1989**, *988*, 221−256.
(5) Seddon, J. M. *Biochim. Biophys. Acta* **1990**, *1031*, 1−69.
(6) Tate, M. W.; Eikenberry, E. F.; Turner, D. C.; Shyamsunder, E.; Gruner, S. M. *Chem. Phys. Lipids* **1991**, *57*, 147−164.
(7) Lee, Y.-S.; Srisiri, W.; Devanathan, S.; O'Brien, D. F. Submitted for publication.
(8) Lee, Y.-S.; Yang, J.; Sisson, T. M.; Frankel, D. A.; Gleeson, J. T.; Aksay, E.; Keller, S. L.; Gruner, S. M.; O'Brien, D. F. *J. Am. Chem. Soc.* **1995**, *117*, 5573−5578.
(9) Hyde, S.; Andersson, S.; Ericsson, B.; Larsson, K. *Z. Kristallogr.* **1984**, *168*, 213−219.
(10) Briggs, J.; Caffrey, M. *Biophys. J.* **1994**, *66*, 573−587.
(11) Briggs, J.; Caffrey, M. *Biophys. J.* **1994**, *67*, 1594−1602.
(12) Nilsson, A.; Holmgren, A.; Lindblom, G. *Biochemistry* **1991**, *30*, 2126−2133.

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is conjugated to the acyl carbonyl, the formation of the polymer does not directly affect the motions of the hydrophobic lipid tail. Polymerization of the diene-substituted lipids can be accomplished with the aid of either thermal or redox initiators or by direct photopolymerization.[13,14]

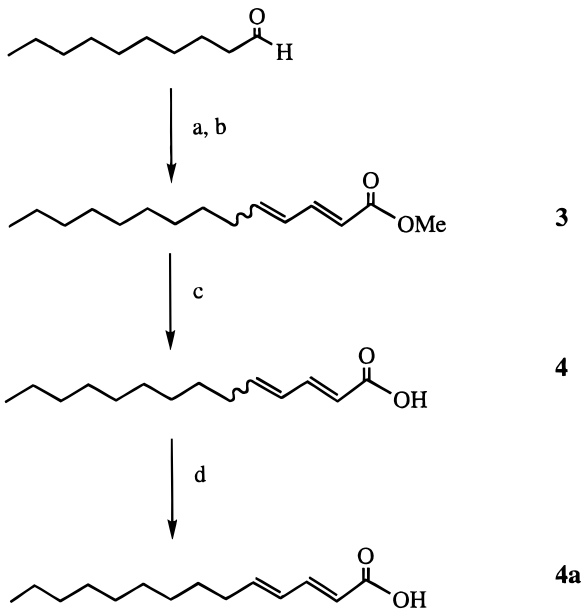
Recently Martin et al.[15] reported general methods for the synthesis of various glycerophospholipids starting from 1,2-diacyl-*sn*-glycerol, which was treated with alkyl dichlorophosphite in the presence of alcohol and then oxidized and deprotected to yield the desired phospholipid derivatives. Diacylglycerols (DAGs) are second messengers produced in the receptor-mediated hydrolysis of inositol phospholipids.[16] They play important roles in signal transduction of a variety of extracellular messengers taking place at the cell surface through activation of protein kinase C.[17] Diacylglycerols are also known to induce a variety of structural changes in membranes, including alternation of membrane curvature,[18] lateral phase separation,[19] the formation of ripple phase,[20] the promotion of membrane fusion,[21] and the production of nonlamellar phases.[22] Here we describe a convenient synthetic route to polymerizable 1,2-diacyl-*sn*-glycerols. In addition to inducing structural changes, this polymerizable DAG could also serve as a cross-linking reagent for polymerizable MAGs to stabilize lipid assemblies. Stabilized cubic phases are expected to be useful models for the investigation of bicontinuous cubic phases, drug or reagent carriers, and the organic analogs of zeolite. In addition, by adaptation of Martin et al.[15] procedures, polymerizable 1,2-diacyl-*sn*-glycerols are a good starting point for the preparation of a variety of other polymerizable lipids.

In this paper, we describe the total synthesis of the polymerizable mono(2,4-(*E*,*E*)-tetradecadienoyl)glycerol (**1**) and 1,2-bis[(2,4-(*E*,*E*)-tetradecadienoyl)-*sn*-glycerol (**2**). A study of the effect of polymerization on the phase behavior of these lipids will be described elsewhere.

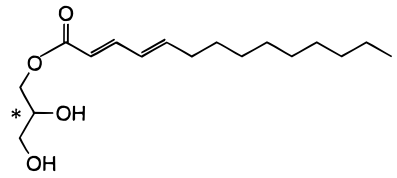
Results and Discussion

The polymerizable monoacylglycerol synthesized was 3-(2,4-(*E*,*E*)-tetradecadienoyl)-*sn*-glycerol (**1**). It was obtained from the acylation of 1,2-*O*-isopropylidene-*sn*-glycerol with 2,4-(*E*,*E*)-tetradecadienoic acid (**4a**). The dienoic acid **4a** was accessible in two steps in 60% yield from commercially available decanal (Scheme 1). The Wittig−Horner reaction of decanal and trimethyl 4-phosphonocrotonate[23] in THF gave methyl 2,4-tetradecadienoate (**3**) in 75% yield. The ^{1}H NMR spectrum indicated

Scheme 1[a]



[a] (a) NaH, THF; (b) trimethyl 4-phosphonocrotonate; (c) 85% KOH, MeOH; (d) urea inclusion.



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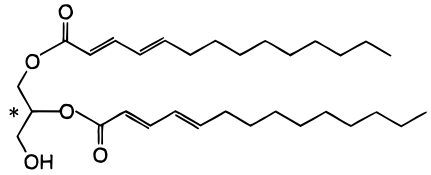


2

that the methyl ester **3** was composed of ca. 80% (*E*,*E*)-isomer and ca. 20% (*E*,*Z*)-isomer. The multiplet peaks at 7.55−7.68 ppm are characteristic of the vinyl proton of the (*E*,*Z*)-isomer, while the multiplet peaks at 7.29−7.40 ppm are due to the vinyl proton of the (*E*,*E*)-isomer. This corresponds well with the previously observed proton NMR spectra of (*E*,*E*)- and (*E*,*Z*)-16-methyl-2,4-octadecadienoic acids.[7] The separation of (*E*,*E*)- and (*E*,*Z*)-isomers was accomplished after the esters were converted to the corresponding acids, since some isomerization of the carbon−carbon double bonds may take place during the hydrolysis process. The hydrolysis of the methyl dienoate **3** was catalyzed by 1.5 molar equiv of KOH in methanol[24] at reflux for 4−5 h. The reaction was followed by TLC using hexane/ethyl acetate (97/3) as the mobile phase. Alternative methods to hydrolyze these methyl esters by acidic (formic acid) or neutral (iodotrimethylsilane)[25] procedures were less satisfactory.

Urea is known for its ability to form a crystalline inclusion complex with certain compounds.[26,27] The hydrogen-bonded urea molecules in methanol orient in

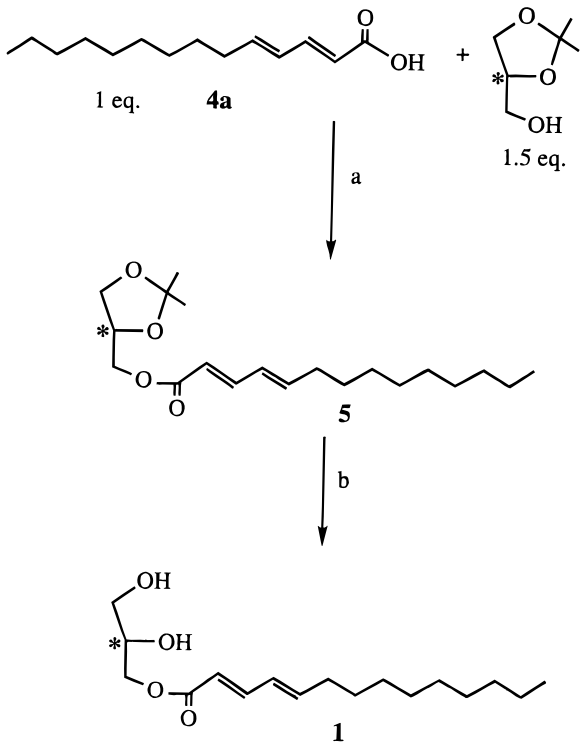
(13) Tsuchida, E.; Hasegawa, E.; Kimura, N.; Hatashita, M.; Makino, E. *Macromolecules* **1992**, *25*, 207−212.
(14) Lamparski, H. G.; O'Brien, D. F. *Macromolecules* **1995**, *28*, 1786−1794.
(15) Martin, S. F.; Josey, J. A.; Wong, Y.-L.; Dean, D. W. *J. Org. Chem.* **1994**, *59*, 4805−4820.
(16) Majerus, P. W.; Connolly, T. M.; Deckmyn, H.; Ross, T. E.; Ishii, H.; Bansal,U.S.; Wilson, D. B. *Science* **1986**, *234*, 1519−1526.
(17) Berridge, M. J. *Annu. Rev. Biochem.* **1987**, *56*, 159−193.
(18) Ohki, T.; Sekiya, T.; Yamauchi, T.; Nozawa, T. *Biochim. Biophys. Acta* **1982**, *693*, 341−350.
(19) Ortiz, A.; Villalain, J.; Gomez-Fernandez, J. C. *Biochemistry* **1988**, *27*, 9030−9036.
(20) Lopez-Garcia, F.; Villalain, J.; Gomez-Fernandez, J. C.; Quinn, P. J. *Biophys. J.* **1995**, *68*, 558−566.
(21) Siegel, D. P.; Banschbach, J.; Alford, D.; Ellens, H.; Lis, L. J.; Quinn, P. J.; Yeagle, P. L.; Bentz, J. *Biochemistry* **1989**, *28*, 3703−9.
(22) Siegel, D. P.; Banschbach, J.; Yeagle, P. L. *Biochemistry* **1989**, *28*, 5010−5019.
(23) Dorn, K.; Klingbiel, R. T.; Specht, D. P.; Tyminiski, P. N.; Ringsdorf, H.; O'Brien, D. F. *J. Am. Chem. Soc.* **1984,** *106*, 1627−1633.
(24) Allen, C. F.; Kalm, K. J. *Org. Synth.* **1963**, *4*, 608−611.
(25) Jung, M. E.; Lyster, M. A. *J. Am. Chem. Soc.* **1977**, *99*, 968−969.

a helical crystal lattice in such a way as to leave a narrow cylindrical channel with the diameter of 5.3 Å. Compounds with a small cross-section diameter can reside in these clathrate channels. Straight-chain normal alkanes having seven or more carbon atoms such as *n*-hexadecane form complexes with urea, but bulky, branched-chain hydrocarbons such as 2,2,4-trimethylpentane do not. The single-crystal X-ray diffraction shows that the guest molecule hydrocarbon was not bonded to the host urea, but merely trapped in the channel.[28] This urea inclusion complexation has also been utilized for the separation of oleic acid from linoleic acid,[29] because the two cis double bonds at the 9- and 12-positions in linoleic acid render it too bulky to lie inside the clathrate channel. We utilized urea inclusion complexation to separate the (*E*,*E*)-dienoic acid from its (*E*,*Z*)-isomer. The optimum ratio of the host urea to the guest dienoic acid was predetermined to be 14. The mixed-isomer dienoic acid sample was added to a methanolic solution of urea. The more linear (*E*,*E*)-dienoic acid formed an inclusion complex with the urea and precipitated. The bent (*E*,*Z*)-isomer apparently did not fit in the urea clathrate channel and stayed in the solution. Upon filtration and then extraction of the inclusion complex with ether, the recovered dienoic acid was exclusively the (*E*,*E*)-isomer. The characteristic vinyl proton of (*E*,*Z*)-isomer at 7.55−7.68 ppm was not observed in the ^{1}H NMR spectrum.

The acylation of 1,2-*O*-isopropylidene-*sn*-glycerol with dienoic acid **4a** using DCC and DMAP in chloroform gave the protected monodienoylglycerol **5** (Scheme 2). The amount of protected glycerol and acid **4a** employed is quite crucial to the yield of the product. When equimolar amounts of the protected glycerol and acid **4a** were used, the product **5** was obtained in ca. 40% yield along with acid anhydride and acid−DCC complex side products. However, the yield could be increased to 96% based on dienoic acid when excess protected glycerol (1.5 molar equiv) was employed. The deprotection of the isopropylidene group proceeded in high yield upon treating **5** with a 1 N HCl solution in methanol at rt. The monodienoylglycerol **1** was obtained as a white solid in 80% yield after purification by column chromatography. The purity of this polymerizable lipid was confirmed by elemental analysis and ^{1}H NMR. It was further characterized by UV/vis spectroscopy. Lipid **1** in water shows a λ_{max} at 256 nm with an extinction coefficient of 1.24×10^4 L/(mol cm).

The successful synthesis of 1,2-bis[2,4-(*E*,*E*)-tetradecadienoyl]-*sn*-glycerol depends on effectiveness of C3 hydroxy group protection in the glycerol (Scheme 3). The requirements for this protecting group are (1) stability during the acylation step, (2) ready removal without affecting the polymerizable dienoyl group, and (3) deprotection under nonbasic conditions in order to avoid acyl chain migrations leading to the formation of the 1,3-diacylglycerol. Several groups have reported the use of 3-*O*-benzyl-*sn*-glycerol derivatives as intermediates in the synthesis of various phospholipids and diacylglycerols.[30] Benzyl ethers can be removed by catalytic hydrogenation

Scheme 2[a]



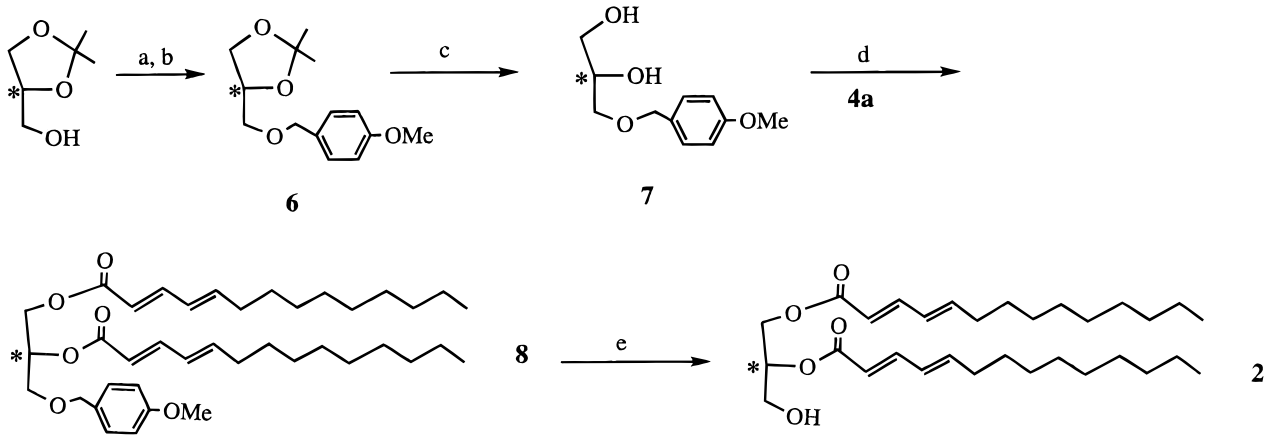
[a] (a) 1 equiv of DCC, 1 equiv of DMAP, $CHCl_3$, rt; (b) 1 N HCl, MeOH, rt, 2 h.

or by hydrolysis using dimethylboron bromide at 10 °C. However deprotection via hydrogenation will affect the dienoyl group. The deprotection of benzyl ether with dimethylboron bromide usually does not proceed completely and sometimes affords the deprotected product in only low yield. Another possibility is 2,2,2-trichloroethoxycarbonate (Troc) which can readily be removed by activated zinc,[31,32] which does not interfer with the polymerizable dienoyl group. However the acylation of the Troc-protected glycerol also liberated 2,2,2-trichloroethanol leading to several side reactions. Recently, Hebert et al. reported the synthesis of an unusual macrocyclic and bolaform phosphatidylcholine using 3-(4-methoxybenzyl)-*sn*-glycerol as the starting material and a new reagent for the removal of the 4-methoxybenzyl ether (PMB) protecting group.[33] The subsequent acid-catalyzed hydrolysis of the electron-rich PMB using dimethylboron bromide proceeded rapidly at low temperature (−78 °C), giving the deprotected product in high yield. In our synthesis of the polymerizable 1,2-bis-(dienoyl)-*sn*-glycerol **2**, we found that PMB was an effective hydroxyl protecting group which could be removed without isomerization of the desired 1,2-diacylglycerol.

1,2-Bis[2,4-(*E*,*E*)-tetradecadienoyl]-*sn*-glycerol (**2**) was synthesized by acylation of 3-(4-methoxybenzyl)-*sn*-glycerol with (*E*,*E*)-dienoyl fatty acid **4a** (Scheme 3). 3-(4-Methoxybenzyl)-*sn*-glycerol (**7**)[33] was obtained in two steps starting from 1,2-*O*-isopropylidene-*sn*-glycerol.

(26) Fetterly, L. C. *Non-Stoichiometric Compounds*; Mandelcorn, L., Ed.; Academic Press: New York, 1965; Chapter 8.
(27) (a) Farina, N. *Inclusion Compounds*; Atword, J. L., et al., Eds.; Academic Press: New York, 1984; Vol. 2, p 69. (b) Reference 27a, Vol. 3, p 297.
(28) Smith, A. E. *Acta. Crystallogr.* **1952**, *5*, 224.
(29) (a) Fieser, L. F. *J. Chem. Educ.* **1963**, 457; (b) **1965**, *42*, 408.
(30) Kadali, D. R.; Tereyak, A.; Fahey, D. A.; Small, D. M. *Chem. Phys. Lipids* **1990**, *52*, 163−175.
(31) Pfeiffer, F. R.; Cohen, S. R.; Weisbach, J. A. *J. Org. Chem.* **1969**, *34*, 2795−2796.
(32) Pfeiffer, F. R.; Miao, C. K.; Weisbach, J. A. *J. Org. Chem.* **1970**, *35*, 221−224.
(33) Hebert, N.; Beck, A.; Lennox, R. B.; Just, G. *J. Org. Chem.* **1992**, *57*, 1777−1783.

Scheme 3[a]



[a] (a) NaH, THF; (b) 4-methoxybenzyl chloride; (c) 1 N HCl, MeOH; (d) **3**, DMAP, DCC, $CHCl_3$; (e) Me_2BBr, −78 °C, CH_2Cl_2.

Treating the protected glycerol with NaH base in DMF solvent and then trapping the anion with 4-methoxybenzyl chloride gave 1,2-*O*-isopropylidene-3-(4-methoxybenzyl)-*sn*-glycerol (**6**). The use of DMF facilitates this nucleophilic substitution. The isopropylidene protecting group was then easily removed in dilute HCl solution, to give white solid 3-(4-methoxybenzyl)-*sn*-glycerol (**7**) in quantitative yield. The acylation of protected glycerol **7** with 2,4-(*E*,*E*)-dienoyl fatty acid **4a** in the presence of coupling reagent DCC and DMAP again proceeded in high yield when an excess amount of protected glycerol relative to the fatty acid was used. The final step was the removal of PMB group on the protected glycerol **8.** The PMB group was removed by treating it with excess dimethylboron bromide in dry CH_2Cl_2 at −78 °C for 15 min. The reaction was quenched by diluting with ether and washing with water until the aqueous extracts were neutral. 1,2-Bis[2,4-(*E*,*E*)-tetradecadienoyl)-*sn*-glycerol (**2**) was obtained in 80% yield after purification by column chromatography. The purity of the product was determined by thin layer chromatography and ^{1}H NMR spectroscopy. The solvent system of toluene, chloroform, and methanol (85:15:5) is useful for the separation of 1,2-diacylglycerol and 1,3-diacylglycerol. TLC of the isolated product in this solvent system showed only one spot with a R_f value of 0.24.[34] The ^{1}H NMR spectrum of the product showed distinctive multiplet peaks at chemical shifts of 5.10−5.00 ppm that are characteristic of the proton at the C2 position on glycerol backbone of 1,2-diacylglycerol. The characteristic multiplet peaks at chemical shifts of 4.10−4.00 ppm for the proton at the C2 position of 1,3-diacylglycerol was not observed.[34] The TLC and ^{1}H NMR data show that deprotection of PMB group by Lewis acid catalyzed hydrolysis proceeds without detectable isomerization of the 1,2-diacylglycerol. In contrast attempted deprotection of the PMB group via DDQ oxidation under nearly neutral conditions at room temperature[35] took place slowly and afforded product **2** in only low yield along with its 1,3-isomer.

(34) Ponpipom, M. M.; Bugianesi, R. L. *J. Lipid Res.* **1980**, *21*, 336−339.

(35) Quindon, Y.; Yoakim, C.; Morton, H. E. *J. Org. Chem.* **1984**, *29*, 3912−3920.

Conclusions

In summary, the polymerizable monodienoylglycerol **1** and 1,2-bis(dienoyl)-*sn*-glycerol **2** were designed and synthesized for the future studies of the polymerization of hydrated lipid−water phases. The synthesis utilized for monodiacylglycerol **1** is straightforward, giving the lipid in four steps in ca. 50% yield. The choice of protecting group for hydroxy group on C3 of glycerol backbone was crucial for the successful synthesis of 1,2-bis(dienoyl)-*sn*-glycerol **2**. 4-Methoxybenzyl ether is an excellent choice for this protecting group since the deprotection via Me_2BBr-catalyzed hydrolysis at −78 °C proceeded without affecting with the polymerizable dienoyl group or isomerization of 1,2-diacylglycerol to 1,3-diacylglycerol. A phase investigation of these lipids and their mixtures as well as their polymerization behavior will be reported in due course.

Experimental Section

General Procedure. Decanal, 2,2,2-trichloroethylchloroformate, and (*S*)-(+)-2,2-dimethyl-1,3-dioxalane-4-methanol were obtained from Aldrich Chemical Corp. Trimethyl 4-phosphonocrotonate was purchased from Lancaster Synthesis Inc. THF was distilled from sodium benzophenone ketyl, and chloroform was distilled from CaH_2. Compounds containing UV-sensitive group were handled under yellow light. The reactions were monitored by TLC visualized by UV lamp. NMR spectra were recorded on a 250 MHz magnetic resonance spectrometer in chloroform-*d* with TMS as an internal reference. Melting points were measured and corrected. Elemental analyses were performed by Desert Analytics, Tucson, AZ. FAB-MS was performed by Nebraska Center of Mass Spectrometer, NE. High-resolution mass spectroscopy was performed by Mass Spectroscopy Facility, The University of Arizona.

Methyl 2,4-Tetradecadienoate (3). Hexane (30 mL) was added into the flask containing 1.6 g (45 mmol) of NaH (60% in mineral oil) under argon. The mixture was stirred for 5 min, and the hexane was removed under vacuum. Dry THF (100 mL) was transferred into the flask under argon, and the solution of trimethyl 4-phosphonocrotonate (8.0 g, 38 mmol) in 200 mL of THF was then added dropwise at 0 °C. After 1 h, the solution of decanal (5.0 g, 32 mmol) in 200 mL of THF was added slowly at 0 °C. The reaction was allowed to warm up to rt and monitored by TLC using hexane/EtOAc (97/3) as the mobile phase. After the reaction was completed, excess NaH was killed by slow addition of cold water to the reaction. After evaporation of THF, the residue was diluted by diethyl

ether and extracted with water and brine. The organic layer was dried with anhydrous $MgSO_4$ and concentrated. The product was purified by column chromatography using hexane/EtOAc (97/3) to give methyl ester **2** in 75% yield. IR (NaCl): 2926, 1718, 1647 cm^{-1}. ^1H NMR ($CDCl_3$): 7.68−7.55 ((*E*,*Z*)-isomer, m, 1H) and 7.40−7.29 ((*E*,*E*)-isomer, dd, J = 15.18, 10.01 Hz, 1H), 6.10−6.05 (m, 2H), 5.75−5.68 (d, J = 15.18 Hz, 1H), 3.67 (s, 3H), 2.10−2.04 (m, 2H), 1.33−1.09 (br, 14H), 0.83−0.78 (t, J = 5.05 Hz, 3H) ppm. Anal. Calcd: C, 75.63; H, 10.92. Found: C, 75.30; H, 11.19.

2,4-Tetradecadienoic Acid (4). A methanolic solution of methyl ester **3** (5.0 g, 200 mmol in 100 mL) was treated with 1.5 molar equiv of an 85% aqueous solution of KOH. The mixture was refluxed gently until the ester disappeared (about 5 h) as determined by TLC using hexane/ethyl acetate (97/3) as the mobile phase. The methanolic solution was concentrated and then diluted with ether. After the solution was acidified to pH 3 with dilute HCl solution, it was extracted many times with water. The organic layer was dried with anhydrous $MgSO_4$ and then concentrated, affording the crude dienoic acid **4**.

2,4-(*E*,*E*)-Tetradecadienoic Acid (4a). A well-stirred solution of urea (6.0 g, 100 mmol) in methanol (100 mL) was treated with a solution of acid **4** (2.7 g, 12 mmol) in methanol (100 mL). The solution was then kept at 0 °C overnight. The crystalline needles were filtered, washed many times with methanol, and then dried under vacuum. These crystals were dissolved in ether and washed several times with dilute HCl solution and water. The organic layer was combined and dried with anhydrous $MgSO_4$. After concentration, the crude acid was purified by recrystallization from hexane at −30 °C, giving the dienoic acid **4a** as colorless needles in 80% yield. IR (NaCl): 3429, 2922, 1684 cm^{-1}. ^1H NMR ($CDCl_3$): 7.40−7.29 (dd, J = 15.28, 10.26 Hz, 1H), 6.21−6.18 (m, 2H), 5.81−5.75 (d, J = 15.28 Hz, 1H), 2.22−2.14 (m, 2H), 1.43−1.21 (br, 14H), 0.91−0.85 (t, J = 6.50 Hz, 3H) ppm. Anal. Calcd: C, 75.00; H, 14.29. Found: C, 75.30; H, 14.42.

1,2-*O*-Isopropylidene-3-(2,4-(*E*,*E*)-tetradecadienoyl)-*sn*-glycerol (5). (*S*)-(+)-2,2-Dimethyl-1,3-dioxalane-4-methanol (0.7 g, 4.8 mmol), acid **4a** (0.9 g, 4.0 mmol), and DMAP (0.5 g, 4.0 mmol) were dissolved in 20 mL of chloroform, and then 0.8 g (4.0 mmol) of DCC in 10 mL of chloroform was added. After the solution was stirred at rt overnight, the urea was filtered and the filtrate was concentrated. The crude product was purified by column chromatography using hexane/EtOAc (9/1), to give the protected glyceride **5** in 96% yield. ^1H NMR ($CDCl_3$): 7.31−7.21 (m, 1H), 6.14−6.11 (m, 2H), 5.82−5.67 (d, J = 15.29 Hz, 1H), 4.35−4.04 (m, 5H), 2.17−2.09 (m, 2H), 1.41 (s, 6H), 1.39−1.23 (br, 14H), 0.87−0.82 (t, J = 6.85 Hz, 3H) ppm. Anal. Calcd: C, 71.00; H, 10.06. Found: C, 71.36; H, 9.91.

3-(2,4-(*E*,*E*)-Tetradecadienoyl)-*sn*-glycerol (1). A solution of protected glyceride **4** (1.3 g) in 20 mL of methanol was treated with 5 mL of a 1 N HCl solution. The solution was stirred at rt for 2 h and then diluted with 50 mL of ether. The ether solution was washed with saturated $NaHCO_3$ and brine. After being dried with anhydrous $MgSO_4$, the organic layer was concentrated. The crude product was purified by column chromatography using hexane/EtOAc (1/1), affording the monoacylglycerol **1** as a white solid in 81% yield. Mp: 50−51 °C. IR (NaCl): 3279, 2920, 1705 cm^{-1}. ^1H NMR ($CDCl_3$): 7.25−7.19 (m, 1H), 6.13−6.09 (m, 2H), 5.77−5.71 (d, J = 15.27 Hz, 1H), 4.21−4.13 (m, 2H), 3.91−3.88 (m, 1H), 3.67−3.51 (m, 2H), 2.23 (br, 2H), 2.12−2.07 (m, 2H), 1.36−1.20 (br, 14H), 0.84−0.79 (t, J = 6.85 Hz, 3H) ppm. FAB-MS: calcd 298 (M^+), 207 (fatty acid-OH) found 298 (M^+), 207 (fatty acid-OH). High-resolution-MS found.: 299 (M+1), 207 (fatty acid-OH).

1,2-*O*-Isopropylidene-3-(4-methoxybenzyl)glycerol (6).[33] A solution of 1,2-*O*-isopropylideneglycerol (2 g, 15 mmol) in anhydrous DMF was added dropwise to a suspension of NaH (1 g, 60% dispersion in mineral oil, 25 mmol) in 100 mL of DMF. The reaction mixture was refluxed for 30 min, and 4-methoxybenzyl chloride (2.37 g, 15 mmol) was added in two portions. The reaction was followed by TLC using hexane/EtOAc (3/1) as the mobile phase. As soon as the 4-methoxybenzyl chloride was totally consumed, the reaction mixture was allowed to cool to rt, and water was added slowly to quench the excess NaH. The reaction mixture was then concentrated. The residue was dissolved in ether and extracted many times with water and brine. The organic layers were combined, dried with anhydrous $MgSO_4$, and concentrated. The crude product was purified by column chromatography using hexane/EtOAc (3/1) as the eluent (90% yield). IR (NaCl): 2988, 1612 cm^{-1}. ^1H NMR ($CDCl_3$): 7.27−7.24 (d, J = 8.71 Hz, 2H), 6.89−6.86 (d, J = 8.71 Hz, 2H), 4.50−4.49 (d, J = 3.73 Hz, 2H), 4.31−4.25 (m, 1H), 4.07−4.01 (m, 1H), 3.79 (s, 3H), 3.75−3.68 (m, 1H), 3.55−3.40 (m, 2H), 1.42 (s, 3H), 1.36 (s, 3H) ppm.

3-(4-Methoxybenzyl)-*sn*-glycerol (7).[33] A dilute solution of 1 N HCl was added into the solution of compound **6** in methanol solvent, and the reaction was stirred for 2 h. After extraction with ether and removal of solvent, the residue was purified by column chromatography using hexane/EtOAc (3/1) as the eluent. The product was obtained in quantitative yield as a white solid. IR (NaCl): 3426, 1605 cm^{-1}. ^1H NMR ($CDCl_3$): 7.27−7.24 (d, J = 8.71 Hz, 2H), 6.91−6.87 (d, J = 8.71 Hz, 2H), 4.49 (s, 2H), 3.49−3.89 (m, 1H), 3.81 (s, 3H), 3.76−3.49 (m, 4H) ppm.

1,2-Bis[(*E*,*E*)-2,4-tetradecadienoyl]-3-(4-methoxybenzyl)-*sn*-glycerol (8). 4-Methoxybenzyl-protected glycerol **7** (0.3 g, 1.4 mmol), acid **4a** (0.4 g, 1.8 mmol), and DMAP (0.2 g, 1.8 mmol) were dissolved in 20 mL of chloroform, and then 0.4 g (41.8 mmol) of DCC in 10 mL of chloroform was added. After the solution was stirred at rt overnight, the urea was filtered and the filtrate was concentrated. The crude product was purified by column chromatography using hexane/EtOAc (9/1) to give the protected glyceride **5** in 50% yield. ^1H NMR ($CDCl_3$): 7.19−7.15 (m, 4H), 6.81−6.77 (d, J= 8.69 Hz, 2H), 6.10−6.05 (m, 4H), 5.76−5.65 (dd, J= 12.65, 15.20, 2H), 5.25−5.23 (m, 1H), 4.42−4.40 (d, J= 13.62 Hz, 2H), 4.31−4.25 (m, 2H), 3.72 (s, 3H), 3.55−3.53 (d, J= 5.14 Hz, 2H), 2.13−2.05 (m, 4H), 1.61−1.19 (b, 28H), 0.83−0.78 (t, J = 6.53 Hz, 6H) ppm.

1,2-Bis[(*E*,*E*)-2,4-tetradecadienoyl]-*sn*-glycerol (2). A the solution of the protected glycerol **8** (0.1g, 0.16 mmol) in dry dichloromethane at −78 °C was treated with dimethylboron bromide (0.05 mL, 0.51 mmol) by addition through a syringe under argon. The reaction mixture was stirred for 15 min while the dry-ice bath was removed. The reaction was then diluted with ether and quenched with water slowly. The organic layer was washed many times with water until the aqueous part was neutral. After evaporation of the solvent, the crude 1,2-bis(dienoyl)-*sn*-glycerol **2** was purified by column chromatography using hexane/EtOAc (1/1) (80% yield). ^1H NMR ($CDCl_3$): 7.35−7.23 (m, 2H), 6.18−6.15 (m, 4H), 5.84−5.76 (m, 2H), 5.19−5.15 (m, 1H), 4.62−4.37 (d, J = 5.08 Hz, 2H), 3.81−3.77 (m, 2H), 2.48 (b, 1H), 2.20−2.13 (m, 4H), 1.45−1.27 (b, 28H), 0.91−0.85 (t, J = 6.52 Hz, 6H) ppm. FAB-MS: calcd 504 (M^+), 224 (fatty acid), found 504 (M^+), 224 (fatty acid). High-resolution-MS: found 505 (M + 1), 487 (M^+ − OH), 281 (487 − tetradecadienoyl), 207 (fatty acid-OH).

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