JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Total Synthesis of Cardiolipins Containing Chiral Cyclopropane Fatty Acids

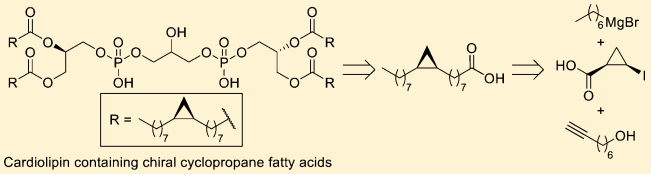
Shinsuke Inuki,[†] Ippei Ohta,[†] Shunichi Ishibashi,[†] Masayuki Takamatsu,[‡] Koichi Fukase,[‡] and Yukari Fujimoto*[,†]

[†]Department of Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan

[‡]Department of Chemistry, Graduate School of Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka, Osaka 560-0043, Japan

Supporting Information

ABSTRACT: Cardiolipin (CL) is a phospholipid located in both the eukaryotic mitochondrial inner membrane and the bacterial cell membrane. Some bacterial CLs are known to contain cyclopropane moieties in their acyl chains. Although the CLs are thought to be involved in the innate immune response, there have been few attempts at chemical synthesis of the CLs, and detailed studies of their biological activities are scarce. Thus, we have developed a synthetic route to CLs containing chiral cyclopropane moieties.



Cardiolipin containing chiral cyclopropane fatty acids

INTRODUCTION

Cardiolipin (CL) is a phospholipid located in both the eukaryotic mitochondrial inner membrane and in the bacterial cell membrane.[1] The structure consists of two phosphatidyl moieties linked by a glycerol bridge and characteristic four acyl groups (Figure 1). The fatty acid composition of CLs has been

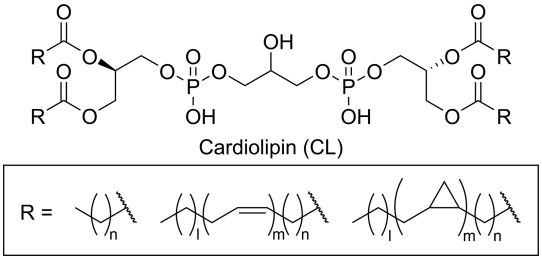


Figure 1. Structure of cardiolipins.

shown to be critical for their biological activities.[2] In animal tissues, the CLs, which contain some kinds of fatty acid variations, such as length and saturation, are involved in either the regulation of ATP biosynthesis or the initiation of the apoptotic program.[3] Recently, Rauch et al. reported that CLs bind to CD1d, a nonpolymorphic MHC class I-like molecule, and activate CD1d-restricted $\gamma\delta$ T cells.[4] Bacterial CLs often contain various cyclopropane fatty acids as acyl groups.[5] Cyclopropane fatty acids were also commonly found in membrane components of bacteria and protozoa, including phospholipids or glycolipids. In the biosynthetic pathway, cyclopropane fatty acids are generally formed by the methylenation of *cis*-unsaturated fatty acids with *S*-adenosylmethionine[6] Recently, Williams et al. reported that cyclopropane fatty acid-containing glycolipid, GL1 from *Lactobacillus plantarum*, could be recognized by the glycolipid pattern recognition receptor Mincle that plays important roles in the innate immune system.[7] We are interested in these kinds of bacterial CLs because of their potential as immunomodulators. However, their detailed structure−activity relationships have not been examined to date. In addition, although several synthetic studies of CLs with saturated and unsaturated fatty acids have been reported,[8] few attempts have been made at synthesizing cyclopropane-containing CLs. Thus, we planned to synthesize these CLs containing chiral cyclopropane moieties in order to elucidate their biological functions and activities in detail.

The key steps for the total synthesis of CLs are the construction of the phosphate linkage between phosphatidyl moieties and glycerol, and the synthesis of enantiomerically pure cyclopropane fatty acids. In terms of the construction of the phosphate linkage, Ahmad and colleagues reported a phosphoramidite approach enabling the synthesis of large quantities of CL analogues.[8a,9] Miyoshi and colleagues developed a concise procedure for the synthesis of CLs having different fatty acid combinations.[8b,c] On the other hand, some groups have reported synthetic methods for chiral cyclopropyl fatty acids. Kobayashi et al. synthesized chiral cyclopropyl fatty acids starting from a homochiral cyclopropa-γ-lactone.[10] Minnikin et al. also reported a related synthetic route to chiral lactobacillic acid from a homochiral cyclopropane linchpin.[11] Nicolaou et al. reported the synthesis of a fatty acid containing a chiral cyclopropane using a chiral borate ester.[12] Corey et al. developed a synthetic route to 9*R*,10*S*-dihydrosterculic acid

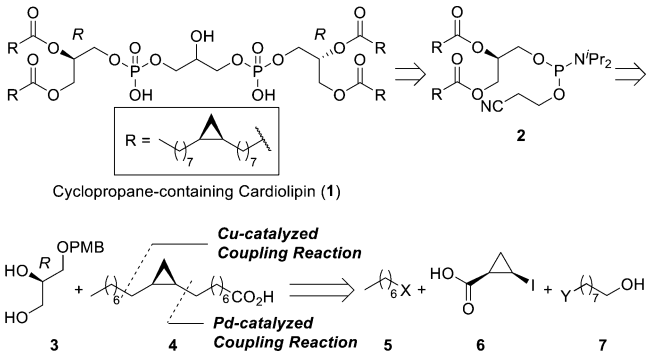
Received: April 20, 2017
Published: July 6, 2017

DOI: 10.1021/acs.joc.7b00945
J. Org. Chem. 2017, 82, 7832−7838

through an Rh(II) catalyzed enantioselective cyclopropanation.[13] Katsuki et al. reported the enantioselective synthesis of the *cis*-9*R*,10*S*-methylenehexadecanoic acid methyl ester in the use of a chiral Ir-salen catalyst.[14] Nishizaki accomplished the synthesis of a fatty acid with two sequential chiral cyclopropanes through enzymatic resolution.[15] Manthorpe et al. reported the synthesis of 9*R*,10*S*-dihydrosterculic acid using the Corey−Chaykovsky cyclopropanation of an alkylidene bis(sulfoxide).[16] More recently, Williams et al. developed a synthetic route to the chiral cyclopropane fatty acid by using chiral auxiliaries.[17] In this study, optically pure chiral cyclopropyl fatty acid was required for biological investigation of the CLs. Thus, we planned to develop a synthetic route to the chiral cyclopropane fatty acid.

The retrosynthetic analysis of cyclopropane-containing CLs **1** is shown in Scheme 1. We postulated that the phosphate linkage can be constructed using phosphoramidite intermediates **2**, which can be derived from (*R*)-3-[(4-methoxybenzyl)oxy]-propane-1,2-diol **3** and cyclopropane-containing fatty acid **4**. The stepwise cross-coupling reactions of alkyl (**5**), chiral iodocyclopropane (**6**), and functionalized alkane moieties (**7**) could be feasible for synthesizing the fatty acid **4**. The chiral iodocyclopropane **6** could be accessed from cyclopropane carboxylic acid, following published procedures.[18] This synthetic route can be applied to the synthesis of fatty acids with plural cyclopropane rings and can enable the introduction of cyclopropane moieties at various positions in the fatty acid portion.

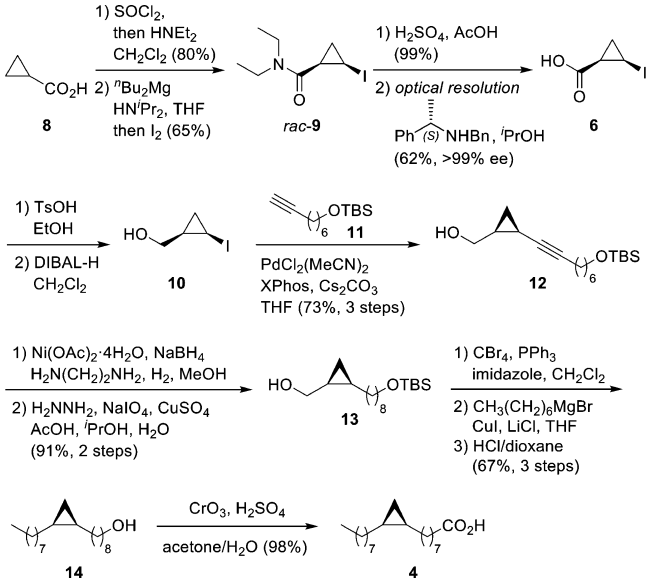
Scheme 1. Retrosynthetic Analysis of Cyclopropane-Containing CL (1)



RESULTS AND DISCUSSION

The synthesis of the chiral cyclopropane fatty acid **4** is shown in Scheme 2. The chiral iodocyclopropane carboxylic acid **6** was prepared from cyclopropane carboxylic acid **8**, following a modified published procedure.[18] The cyclopropane carboxylic acid **8** was converted to the corresponding diisopropylamide, followed by *cis*-selective iodination at the β-position of the amide group using magnesium amide.[19] Hydrolysis of **9** with H_2SO_4 and AcOH gave the racemic iodocyclopropane carboxylic acid *rac*-**6**. Optical resolution using the chiral amine provided the chiral iodocarboxylic acid **6** (>99% ee).[18] Esterification and reduction with DIBAL-H produced the known alcohol **10**.[12a] Next, we investigated the coupling reaction between **10** and the functionalized alkane moiety **7** (Scheme 1) using palladium catalysts. After investigating the palladium-catalyzed coupling reaction, we found that the copper-free Sonogashira reaction condition reported by

Scheme 2. Synthesis of the Chiral Fatty Acid 4



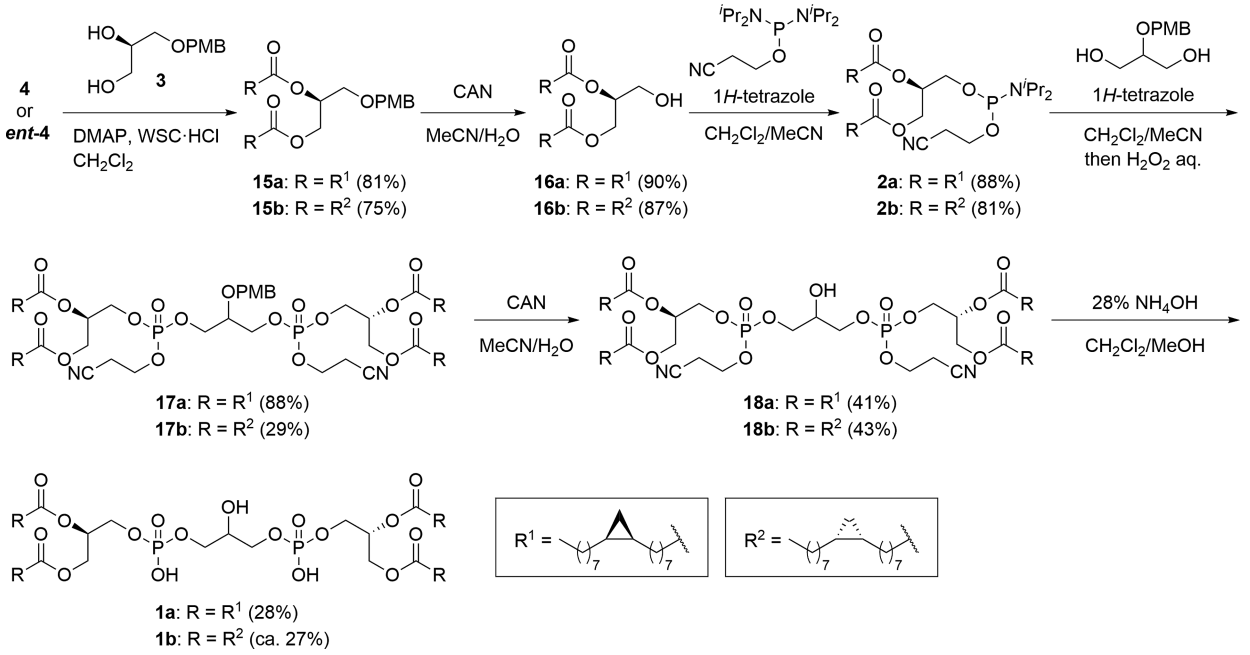
Meyer, Cossy, and co-workers[20] permitted successful generation of the desired product. The reaction of **10** with the functionalized alkyne **11**, $PdCl_2(MeCN)_2$, 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos), and Cs_2CO_3 in THF gave the desired alkyne **12** with a high yield (73%, three steps from **6**). Next, we examined the reduction of alkyne **12** under several conditions using Pd/C, palladium−fibroin (Pd/Fib), Raney Ni, and Wilkinson's catalyst, but these conditions led to insufficient conversion. Moreover, ring-opening side reactions of cyclopropane occurred. We therefore performed a two-step reduction of the alkyne instead. Treating **12** with $Ni(OAc)_2{\cdot}4H_2O$, $NaBH_4$, and $H_2N(CH_2)_2NH_2$ under a H_2 atmosphere[21] followed by diimide reduction[22] provided the desired product **13** (91%, two steps). After **13** was elaborated to form the bromide derivative, copper-mediated alkylation reaction with Grignard reagent and removal of the TBS group gave the alcohol **14**, which was subsequently reacted with CrO_3 to give the corresponding carboxylic acid **4**.[17] Using our optimized route, we have synthesized over 1 g of the chiral cyclopropane fatty acid **4** for subsequent use.

With the chiral cyclopropane fatty acid **4** in hand, we converted the fatty acid into CLs (Scheme 3). The esterification of (*R*)-3-[(4-methoxybenzyl)oxy]-propane-1,2-diol **3** with the fatty acid **4** and subsequent removal of the PMB group gave the diester **16a**. Next, treating **16a** with the phosphitylating reagent (2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropyl phosphorodiamidite)[23] in the presence of 1*H*-tetrazole generated the phosphoramidite intermediate **2a** with a 88% yield as a diastereomixture with a stereogenic phosphorus atom. Subsequently, the coupling reaction between intermediate **2a** and 2-[(4-methoxybenzyl)oxy]-propane-1,3-diol gave the phosphite trimester, which was subjected to oxidation with H_2O_2 to generate the protected CL **17a** as a diastereomixture with two stereogenic phosphorus atoms. Finally, total synthesis of CLs containing the chiral cyclopropane fatty acid **1a** was completed by removing the PMB and cyanoethyl groups.

To investigate the effect of cyclopropane chiralities on their biological activities, we went on to synthesize the diastereomeric CL (**1b**) containing the enantiomeric chiral cyclopropane fatty acid *ent*-**4** in the same manner, starting from the

DOI: 10.1021/acs.joc.7b00945
J. Org. Chem. 2017, 82, 7832−7838

Scheme 3. Total Synthesis of CLs Containing Chiral Cyclopropane Fatty Acid 1a and 1b



iodocyclopropane *ent*-**6** (Scheme 2). The *ent*-**4** was converted into the CL **1b** over six steps (Scheme 3). The final compounds **1a** and **1b** contained impurities, which were quite difficult to remove at the final stage of the purification.

In conclusion, we have successfully completed the total syntheses of CLs containing a chiral cyclopropane fatty acid. Our synthesis highlights a stepwise cross coupling reaction involving three building blocks, namely, alkyl Grignard reagents, chiral iodocyclopropanes, and functionalized alkyne moieties. Another key feature is a two-step reduction of the alkyne attached to the cyclopropane moiety to provide the desired cyclopropane derivative at high yield. The cyclopropane-containing CLs can be used as chemical tools to explore the mechanism of immune responses. The determination of their biological activities is now underway.

EXPERIMENTAL SECTION

General Methods. All moisture-sensitive reactions were performed using syringe-septum cap techniques under an argon atmosphere, and all glassware was dried in an oven at 80 °C for 2 h prior to use. Analytical thin-layer chromatography (TLC) was performed on Silica gel 60 F_{254} plates (0.25 mm thickness). For flash chromatography, Silica gel 60 N [spherical neutral (40−50 μm)] was employed. Optical rotations were measured with a polarimeter. All NMR spectral data were recorded on a NMR spectrometer for ^{1}H (400 MHz) and ^{13}C (100 MHz). Chemical shifts are reported in δ (ppm) relative to TMS in $CDCl_3$ as internal standard (^{1}H NMR) or the residual $CHCl_3$ signal (^{13}C NMR). ^{1}H NMR spectra are tabulated as follows: chemical shift, multiplicity (b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), number of protons, and coupling constant(s). Exact mass (HRMS) spectra were recorded on an electrospray ionization quadrupole time-of-flight (ESI-QTOF) mass spectrometer.

Synthesis of *cis*-2-Iodocyclopropyl-*N*,*N*-diethylcarboxamide (*rac*-9). The title compound was prepared according to the procedure reported by Shang et al.[18] To a flask charged with thionyl chloride (18.6 mL, 256 mmol) was added cyclopropanecarboxylic acid **8** (20.0 mL, 253 mmol) dropwise below 5 °C. After being stirred for 1 h below 5 °C, the reaction mixture was warmed to room temperature and stirred for 1 h. Another flask was charged with a solution of diethylamine (53.8 mL, 527 mmol) in anhydrous CH_2Cl_2 (80 mL) and cooled to 0 °C. The acid chloride solution was added dropwise to the cooled diethylamine solution via a cannula. After being stirred at room temperature for 1 h, the reaction was quenched with water, and the extract was washed with 1 M HCl, saturated $NaHCO_3$ aq., and brine. The extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by vacuum distillation to give cyclopropyl-*N*,*N*-diethylcarboxamide (28.6 g, 80%) as a slight yellow oil: bp 113 °C (26 mmHg). All the spectral data were in agreement with those reported by Shang et al.:[18] ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.49 (q, J = 7.2 Hz, 2H), 3.39 (q, J = 7.2 Hz, 2H), 1.73−1.67 (m, 1H), 1.25 (t, J = 7.2 Hz, 3H), 1.11 (t, J = 7.2 Hz, 3H), 1.00−0.96 (m, 2H), 0.76−0.71 (m, 2H). To a flask charged with dibutylmagnesium (1.0 M solution in heptane, 74.4 mL, 74.4 mmol) was added diisopropylamine (10.5 mL, 74.4 mmol) dropwise, keeping the internal temperature below 40 °C. The reaction mixture was stirred without external heating for 1 h then heated to reflux for 15 min and allowed to cool to room temperature. A solution of cyclopropyl-*N*,*N*-diethylcarboxamide (10.0 g, 70.8 mmol) in anhydrous THF (120 mL) was added to the above mixture via a cannula, and the mixture was refluxed for 70 min. Another flask was charged with a solution of I_2 (53.9 g, 212 mmol) in anhydrous THF (180 mL), and the solution was cooled to −15 °C. The reaction mixture containing the cyclopropane derivative was cooled to 0 °C before being added dropwise to the I_2 solution via a cannula. After complete addition, the reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched with 1 M H_2SO_4 (12.0 mL), and the whole was extracted with CH_2Cl_2 three times. The combined extract was washed with saturated $Na_2S_2O_3$ aq. three times and brine once and dried over anhydrous Na_2SO_4. The extract was concentrated under reduced pressure, and the crude oil was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2:1) to give *cis*-2-iodocyclopropyl-*N*,*N*-diethylcarboxamide *rac*-**9** (12.2 g, 65%) as a brown oil. All the spectral data were in agreement with those reported by Shang et al.:[18] ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.70−3.55 (m, 2H), 3.37 (m, 1H), 3.23 (m, 1H), 2.82 (ddd, J = 8.2, 8.2, 6.0 Hz, 1H), 1.94 (ddd, J = 8.2, 8.2, 6.4 Hz, 1H), 1.57 (m, 1H), 1.41 (ddd, J = 8.2, 8.2, 6.4 Hz, 1H), 1.26 (t, J = 7.2 Hz, 3H), 1.17 (t, J = 7.2 Hz, 3H).

Synthesis of *cis*-2-Iodocyclopropanecarboxylic Acid (*rac*-6). A mixture of *cis*-2-iodocyclopropyl-*N*,*N*-diethylcarboxamide *rac*-**9** (9.24 g, 34.6 mmol), acetic acid (60 mL), and 3 M H_2SO_4 (120 mL) was refluxed for 10 days. The whole was extracted with EtOAc

DOI: 10.1021/acs.joc.7b00945
J. Org. Chem. 2017, 82, 7832−7838

five times and washed with brine. The extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give racemic *cis*-2-iodocyclopropanecarboxylic acid **6** (7.28 g, 99%) as a brown solid. All the spectral data were in agreement with those reported by Shang et al.:[18] ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 10.30 (br s, 1H), 2.91−2.87 (m, 1H), 1.92 (dd, J = 14.6, 8.3 Hz, 1H), 1.60 (dd, J = 14.6, 8.3 Hz, 1H), 1.45−1.41 (m, 1H).

Optical Resolution of *cis*-2-Iodocyclopropanecarboxylic Acid (6). The process of optical resolution was carried out according to the procedure reported by Shang et al.[18] To a solution of racemic acid **6** (4.60 g, 21.7 mmol) in 2-propanol (54 mL) was added (*S*)-(−)-*N*-benzyl-1-phenylethylamine (4.6 mL, 21 mmol). The mixture was stirred at 70 °C for 10 min and then cooled gradually to room temperature. The resulting mixture was left overnight, and the crystalline solid was collected by vacuum filtration. The solid was washed with 2-propanol and dried under reduced pressure to give a crude crystalline solid (3.80 g, 9.00 mmol). A mixture of the crystalline solid (3.80 g) and 2-propanol (28 mL) was stirred at 70 °C for 10 min and then cooled gradually to room temperature. The precipitated crystal was collected by vacuum filtration, washed with 2-propanol, and dried under reduced pressure to give a crystalline solid (3.01 g, 7.11 mmol). A solution of the crystalline solid (3.01 g) in CH_2Cl_2 (9 mL) and NaOH aq. (0.5 M, 18 mL) was stirred for 5 min. After the organic layer was separated, the aqueous solution was extracted with CH_2Cl_2 three times and then acidified with 1 M HCl. The whole was extracted with *tert*-butylmethyl ether five times. The combined ether solutions were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give (1*R*,2*R*)-2-iodocyclopropanecarboxylic acid **6** (1.43 g, 62% yield for the (1*R*,2*R*)-isomer, > 99% ee [determined by chiral HPLC; Chiralpak-IA column, eluting with *n*-hexane/EtOH/TFA (97:3:0.1) at 0.5 mL/min, λ = 254 nm, t_1 = 27.0 min for (1*S*,2*S*)-isomer, t_2 = 28.5 min for (1*R*,2*R*)-isomer]). By a procedure identical with that described above, the enantiomeric carboxylic acid *ent*-**6** was resolved using (*R*)-(+)-*N*-benzyl-1-phenylethylamine.

Synthesis of Alkynyl-Substituted Cyclopropylmethylalcohol (12). A solution of (1*R*,2*R*)-2-iodocyclopropanecarboxylic acid **6** (2.99 g, 14.1 mmol) and *p*-toluenesulfonic acid monohydrate (134 mg, 0.705 mmol) in anhydrous EtOH (36 mL) was refluxed overnight. The reaction mixture was then concentrated to ca. 1/5 volume under reduced pressure and diluted with water. The whole was extracted with EtOAc three times, washed with saturated $NaHCO_3$ aq. and brine once, and then dried over anhydrous Na_2SO_4. The extract was concentrated under reduced pressure to give ethyl (1*R*,2*R*)-2-iodocyclopropanecarboxylate (3.15 g) as a crude oil, which was used in the next reaction without further purification. To a solution of the ethyl ester (2.70 g) in anhydrous CH_2Cl_2 (10 mL) was added DIBAL-H (1.03 M in hexane, 27 mL) at −78 °C. After the mixture was stirred at this temperature for 15 h, the reaction was quenched with EtOAc and the resulting mixture was diluted with saturated aqueous solution of Rochelle salt. The mixture was extracted with Et_2O five times, washed with brine once, and dried over anhydrous Na_2SO_4. The extract was concentrated under reduced pressure to give (1*R*,2*R*)-2-iodocyclopropylmethylalcohol **10** (2.06 g) as a crude oil, which was used to the next reaction without further purification. To a flask charged with the crude alcohol **10** (1.95 g), $PdCl_2(MeCN)_2$ (76.6 mg, 0.295 mmol), XPhos (423 mg, 0.886 mmol), and Cs_2CO_3 (8.02 g, 24.6 mmol) was added a solution of TBS-protected alkynol **11** (3.26 g, 13.6 mmol) in anhydrous THF (49 mL). After being stirred at 60 °C overnight, the reaction mixture was filtered through Celite and concentrated under reduced pressure to obtain a crude oil, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 8:1) to give alkynyl-substituted cyclopropylmethylalcohol **12** (2.58 g, 73% in three steps) as a colorless oil. $[\alpha]^{25}_D$ = −57.1 (*c* 0.83, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.93−3.85 (m, 1H), 3.60 (t, J = 6.5 Hz, 2H), 3.56 (m, 1H), 2.14 (td, J = 7.0, 1.9 Hz, 2H), 1.91 (br s, 1H), 1.55−1.41 (m, 5H), 1.40−1.29 (m, 5H), 0.96−0.92 (m, 1H), 0.89 (s, 9H), 0.57−0.52 (m, 1H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 79.4, 78.3, 64.1, 63.1, 32.7, 29.0, 28.6, 25.9 (3C), 25.3, 20.1, 18.7, 18.3, 12.6, 4.5, −5.3 (2C). HRMS (ESI-QTOF): calcd $C_{18}H_{34}NaO_2Si$ $[M + Na]^+$, 333.2220; found $[M + Na]^+$, 333.2226.

Synthesis of Alkynyl-Substituted Cyclopropylmethylalcohol (*ent*-12). By a procedure identical with that described for synthesis of **6** from **12**, the carboxylic acid *ent*-**6** (1.15 g) was converted into *ent*-**12** (1.18 g, 70% in three steps) as a colorless oil. $[\alpha]^{25}_D$ = +53.5 (*c* 0.61, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.93−3.88 (m, 1H), 3.60 (t, J = 6.6 Hz, 2H), 3.59−3.53 (m, 1H), 2.14 (td, J = 7.0, 1.9 Hz, 2H), 1.84−1.79 (m, 1H), 1.55−1.43 (m, 5H), 1.38−1.30 (m, 5H), 0.96−0.92 (m, 1H), 0.89 (s, 9H), 0.57−0.53 (m, 1H), 0.05 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 79.5, 78.4, 64.1, 63.2, 32.7, 29.0, 28.7, 26.0 (3C), 25.3, 20.2, 18.7, 18.4, 12.6, 4.5, −5.3 (2C). HRMS (ESI-QTOF): calcd $C_{18}H_{34}NaO_2Si$ $[M + Na]^+$, 333.2220; found $[M + Na]^+$, 333.2226.

Synthesis of Alkyl-Substituted Cyclopropylmethylalcohol (13). To a solution of $Ni(OAc)_2{\cdot}4H_2O$ (516 mg, 2.08 mmol) in MeOH (13 mL) was added $NaBH_4$ (78.8 mg, 2.08 mmol) at 0 °C, and the solution was stirred at room temperature for 5 min. Anhydrous ethylenediamine (275 μL, 4.15 mmol) was then added to the reaction mixture followed by stirring at room temperature for 5 min. After addition of a solution of **12** (2.58 g, 8.31 mmol) in MeOH (15 mL), argon gas was replaced to hydrogen gas and the reaction mixture was stirred at room temperature overnight. The mixture was filtered through Celite and concentrated under reduced pressure. The residual oil was diluted with water, and the whole was extracted with Et_2O three times. The combined extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the alkene intermediate (2.54 g) as a crude oil, which was used in the next reaction without further purification. A mixture of the crude product (2.52 g), 2-propanol (138 mL), acetic acid (1.1 mL), saturated $CuSO_4$ aq. (1.1 mL), and hydrazine monohydride (11 mL) was heated to 70 °C, and a solution of $NaIO_4$ (17.2 g, 80.6 mmol) in hot water (50 mL) was added to the reaction mixture (dropwise, over 90 min). After additional stirring for 90 min, the mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was diluted with water and extracted with Et_2O three times. The combined extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a crude oil, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 8:1) to give alkyl-substituted cyclopropylmethylalcohol **13** (2.35 g, 91% in two steps) as a colorless oil. $[\alpha]^{25}_D$ = −12.5 (*c* 1.01, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.68−3.54 (m, 4H), 1.55−1.36 (m, 6H), 1.29 (br s, 8H), 1.24−1.18 (m, 1H), 1.15−1.04 (m, 1H), 0.89 (s, 9H), 0.87−0.82 (m, 1H), 0.70 (ddd, J = 8.3, 8.3, 4.5 Hz, 1H), 0.05 (s, 6H), −0.01 to −0.06 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 63.3 (2C), 32.8, 30.1, 29.6, 29.5, 29.4, 28.5, 26.0 (3C), 25.8, 18.4, 18.1, 16.1, 9.5, −5.3 (2C). HRMS (ESI-QTOF): calcd $C_{18}H_{38}O_2Si$ $[M + Na]^+$, 337.2533; found $[M + Na]^+$, 337.2541.

Synthesis of Alkyl-Substituted Cyclopropylmethylalcohol (*ent*-13). By a procedure identical with that described for synthesis of **13** from **12**, the alcohol *ent*-**12** (1.16 g, 3.74 mmol) was converted into *ent*-**13** (0.84 g, 71% in two steps) as a colorless oil. $[\alpha]^{25}_D$ = +13.2 (*c* 0.90, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.68−3.55 (m, 2H), 3.60 (t, J = 6.6 Hz, 2H), 1.61−1.37 (m, 6H), 1.29 (br s, 8H), 1.25−1.19 (m, 1H), 1.15−1.05 (m, 1H), 0.89 (s, 9H), 0.87−0.83 (m, 1H), 0.70 (ddd, J = 8.4, 8.4, 4.6 Hz, 1H), 0.05 (s, 6H), −0.01 to −0.06 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 63.4, 63.3, 32.9, 30.1, 29.6, 29.5, 29.4, 28.5, 26.0 (3C), 25.8, 18.4, 18.1, 16.1, 9.5, −5.3 (2C). HRMS (ESI-QTOF): calcd $C_{18}H_{38}NaO_2Si$ $[M + Na]^+$, 337.2533; found $[M + Na]^+$, 337.2540.

Synthesis of Cyclopropane-Containing Alkanol (14). To a solution of cyclopropylmethylalcohol derivative **13** (2.28 g, 7.25 mmol), imidazole (0.99 g, 14.5 mmol), and Ph_3P (3.80 g, 14.5 mmol) in anhydrous CH_2Cl_2 (36 mL) was added CBr_4 (3.61 g, 10.9 mmol) at 0 °C. After being stirred at 0 °C for 3 h, the reaction mixture was concentrated under reduced pressure and reslurried with hexane/Et_2O = 3:1 solution. A sticky solid was removed by filtration, and the filtrate was concentrated under reduced pressure to give the bromocyclopropylmethane derivative (3.33 g) as a crude oil, which was used in the next reaction without further purification. To a mixture of CuI (413

DOI: 10.1021/acs.joc.7b00945
J. Org. Chem. 2017, 82, 7832−7838

mg, 2.18 mmol) and LiCl (0.5 M solution in THF, 58 mL, 29 mmol) was added heptylmagnesium bromide (0.70 M solution in THF, 31.0 mL, 22 mmol) at 0 °C, and the reaction mixture was stirred at this temperature for 10 min. A solution of the crude bromocyclopropylmethane derivative (3.31 g) in anhydrous THF (15 mL) was added to the reaction mixture dropwise. After the mixture was stirred at 0 °C overnight, the reaction was quenched with saturated NH_4Cl aq. and the mixture was diluted with 28% NH_4OH. The whole was extracted with Et_2O three times. The extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the TBS-protected alkanol (3.86 g) as a crude oil, which was used in the next reaction without further purification. A solution of the TBS-protected alkanol (3.77 g) in 4 M HCl in dioxane (6.8 mL, 27.2 mmol) and dioxane (48 mL) was stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with Et_2O three times. The extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude oil, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 7:1) to give the title compound **14** (1.33 g, 67% in three steps) as a colorless oil. $[\alpha]^{25}_D$ = +1.01 (*c* 0.77, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.64 (t, *J* = 6.6 Hz, 2H), 1.61−1.52 (m, 2H), 1.44 (s, 1H), 1.40−1.10 (m, 26H), 0.88 (t, *J* = 6.7 Hz, 3H), 0.67−0.62 (m, 2H), 0.58−0.53 (m, 1H), −0.31 to −0.36 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 63.0, 32.8, 31.9, 30.20, 30.17, 29.7 (2C), 29.64, 29.58, 29.43, 29.35, 28.69, 28.67, 25.73, 22.67, 15.74, 15.71, 14.1, 10.9. HRMS (ESI-QTOF): calcd $C_{19}H_{38}NaO$ $[M + Na]^+$, 305.2815; found $[M + Na]^+$, 305.2815.

Synthesis of Cyclopropane-Containing Alkanol (*ent*-14). By a procedure identical with that described for synthesis of **14** from **13**, the alcohol *ent*-**13** (0.78 g, 2.67 mmol) was converted into *ent*-**14** (0.22 g, 31% in three steps) as a colorless oil. $[\alpha]^{25}_D$ = −1.92 (*c* 0.36, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.64 (t, *J* = 6.6 Hz, 2H), 1.60−1.54 (m, 3H), 1.40−1.10 (m, 26H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.67−0.62 (m, 2H), 0.58−0.53 (m, 1H), −0.31 to −0.36 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 63.1, 32.8, 31.9, 30.21, 30.19, 29.68 (2C), 29.65, 29.59, 29.44, 29.36, 28.71, 28.69, 25.7, 22.7, 15.8, 15.7, 14.1, 10.9. HRMS (ESI-QTOF): calcd $C_{19}H_{38}NaO$ $[M + Na]^+$, 305.2815; found $[M + Na]^+$, 305.2809.

Synthesis of Chiral Cyclopropane Fatty Acid (4). A solution of cyclopropane-containing alkanol **14** (1.24 g, 4.39 mmol) in acetone (88 mL) was cooled to 0 °C. Another mixture of CrO_3 (1.88 g, 18.8 mmol), sulfuric acid (1.6 mL), and water (7.2 mL) was added to the above solution dropwise. After stirring for 10 min, the reaction mixture was diluted with water and extracted with hexane three times. The extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude oil, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 4:1) to give the title compound **4** (1.07 g, 82%) as a white solid. All the spectral data were in agreement with those reported by Williams et al.:[17] $[\alpha]^{25}_D$ = +0.97 (*c* 0.42, $CHCl_3$) [lit. $[\alpha]^{24}_D$ = +0.95 (*c* 0.55, $CHCl_3$)]. ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.35 (t, *J* = 7.6 Hz, 2H), 1.66−1.61 (m, 2H), 1.41−1.09 (m, 24H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.67−0.62 (m, 2H), 0.58−0.53 (m, 1H), −0.31 to −0.36 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 179.5, 33.9, 31.9, 30.2, 30.1, 29.7, 29.4, 29.4 (2C), 29.3, 29.1, 28.7, 28.7, 24.7, 22.7, 15.8, 15.7, 14.1, 10.9.

Synthesis of Chiral Cyclopropane Fatty Acid (*ent*-4). By a procedure identical with that described for synthesis of **4** from **14**, the alkanol *ent*-**14** (216 mg, 0.765 mmol) was converted into *ent*-**4** (206 mg, 91%) as a white solid. All the spectral data were in agreement with those reported by Williams et al.:[17] $[\alpha]^{25}_D$ = −0.81 (*c* 0.78, $CHCl_3$) [lit. $[\alpha]^{24}_D$ = −0.81 (*c* 0.295, $CHCl_3$)]. ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.35 (t, *J* = 7.5 Hz, 2H), 1.66−1.61 (m, 2H), 1.40−1.10 (m, 24H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.67−0.62 (m, 2H), 0.59−0.53 (m, 1H), −0.31 to −0.36 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 179.6, 33.9, 31.9, 30.2, 30.1, 29.7, 29.4, 29.4 (2C), 29.3, 29.1, 28.7, 28.7, 24.7, 22.7, 15.8, 15.7, 14.1, 10.9.

Synthesis of PMB-Protected Diacylglycerol (15a). To a mixture of chiral cyclopropane fatty acid **4** (150 mg, 507 μmol), PMB-protected glycerol **3** (46.8 mg, 220 μmol), DMAP (8.1 mg, 66 μmol), and CH_2Cl_2 (1.5 mL) was added a solution of WSC·HCl (114 mg, 595 μmol) in CH_2Cl_2 (0.7 mL) at 0 °C. After the mixture was stirred at room temperature overnight, the reaction was quenched with 10% citric acid aq. and the mixture was extracted with CH_2Cl_2 three times. The extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude oil, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 30:1) to give the title compound **15a** (137 mg, 81%) as a colorless oil. $[\alpha]^{25}_D$ = +2.59 (*c* 1.27, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.23 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 5.25−5.20 (m, 1H), 4.51−4.42 (m, 2H), 4.33 (dd, *J* = 11.9, 3.8 Hz, 1H), 4.17 (dd, *J* = 11.9, 6.5 Hz, 1H), 3.80 (s, 3H), 3.55 (d, *J* = 5.2 Hz, 2H), 2.35−2.25 (m, 4H), 1.65−1.57 (m, 4H), 1.41−1.07 (m, 48H), 0.88 (t, *J* = 6.8 Hz, 6H), 0.67−0.61 (m, 4H), 0.58−0.53 (m, 2H), −0.31 to −0.36 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.4, 173.1, 159.3, 129.7, 129.3 (2C), 113.8 (2C), 72.9, 70.0, 67.9, 62.7, 55.2, 34.3, 34.1, 31.9 (2C), 30.20 (2C), 30.15 (2C), 29.7 (2C), 29.5 (2C), 29.4 (4C), 29.13 (2C), 29.10 (2C), 28.71 (2C), 28.66 (2C), 25.0, 24.9, 22.7 (2C), 15.8 (2C), 15.7 (2C), 14.1 (2C), 10.9 (2C). HRMS (ESI-QTOF): calcd $C_{49}H_{84}NaO_6$ $[M + Na]^+$, 791.6160; found $[M + Na]^+$, 791.6169.

Synthesis of PMB-Protected Diacylglycerol (15b). By a procedure identical with that described for synthesis of **15a** from **4**, the fatty acid *ent*-**4** (38.7 mg, 182 μmol) was converted into **15b** (105 mg, 75%) as a colorless oil. $[\alpha]^{25}_D$ = +2.94 (*c* 1.23, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.23 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 5.24−5.20 (m, 1H), 4.51−4.42 (m, 2H), 4.33 (dd, *J* = 11.8, 3.7 Hz, 1H), 4.17 (dd, *J* = 11.8, 6.5 Hz, 1H), 3.80 (s, 3H), 3.55 (d, *J* = 5.2 Hz, 2H), 2.34−2.25 (m, 4H), 1.59 (m, 4H), 1.39−1.09 (m, 48H), 0.88 (t, *J* = 6.7 Hz, 6H), 0.66−0.62 (m, 4H), 0.58−0.53 (m, 2H), −0.32 to −0.36 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.4, 173.1, 159.3, 129.7, 129.3 (2C), 113.8 (2C), 72.9, 70.0, 67.9, 62.7, 55.2, 34.3, 34.1, 31.9 (2C), 30.20 (2C), 30.15 (2C), 29.7 (2C), 29.5 (2C), 29.4 (2C), 29.3 (2C), 29.1 (4C), 28.71 (2C), 28.66 (2C), 25.0, 24.9, 22.7 (2C), 15.8 (2C), 15.7 (2C), 14.1 (2C), 10.9 (2C). HRMS (ESI-QTOF): calcd $C_{49}H_{84}NaO_6$ $[M + Na]^+$, 791.6160; found $[M + Na]^+$, 791.6160.

Synthesis of Diacylglycerol (16a). To a solution of PMB-protected diacylglycerol **15a** (132 mg, 172 μmol) in MeCN/H_2O (v/v = 10/1, 14 mL) was added $Ce(NH_4)_2(NO_3)_6$ (941 mg, 1.72 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 1 h. The mixture was diluted with water and extracted with CH_2Cl_2 three times. The extract was washed with saturated $NaHCO_3$ aq. and saturated $NaHSO_3$ aq., dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude oil, which was purified by silica gel column chromatography (toluene/EtOAc = 10:1) to give the title compound **16a** (105 mg, 94%) as a colorless oil. $[\alpha]^{25}_D$ = −0.89 (*c* 0.14, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.12−5.05 (m, 1H), 4.32 (dd, *J* = 11.9, 4.4 Hz, 1H), 4.24 (dd, *J* = 11.9, 5.6 Hz, 1H), 3.75−3.71 (br m, 2H), 2.38−2.30 (m, 4H), 2.08 (br s, 1H), 1.66−1.59 (m, 4H), 1.41−1.09 (m, 48H), 0.88 (t, *J* = 6.5 Hz, 6H), 0.68−0.61 (m, 4H), 0.59−0.53 (m, 2H), −0.31 to −0.36 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.8, 173.4, 72.1, 62.0, 61.5, 34.3, 34.1, 31.9 (2C), 30.2 (2C), 30.1 (2C), 29.7 (2C), 29.5 (2C), 29.4 (2C), 29.3 (2C), 29.13 (2C), 29.10 (2C), 28.71 (2C), 28.66 (2C), 24.94, 24.89, 22.7 (2C), 15.8 (2C), 15.7 (2C), 14.1 (2C), 10.9 (2C). HRMS (ESI-QTOF): calcd $C_{41}H_{76}NaO_5$ $[M + Na]^+$, 671.5585; found $[M + Na]^+$, 671.5594.

Synthesis of Diacylglycerol (16b). By a procedure identical with that described for synthesis of **16a** from **15a**, the PMB-protected diacylglycerol **15b** (77.5 mg, 101 μmol) was converted into **16b** (56.8 mg, 87%) as a colorless oil. $[\alpha]^{25}_D$ = +12.0 (*c* 0.10, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.11−5.06 (m, 1H), 4.32 (dd, *J* = 11.9, 4.3 Hz, 1H), 4.23 (dd, *J* = 11.9, 5.6 Hz, 1H), 3.75−3.71 (m, 2H), 2.37−2.30 (m, 4H), 2.00 (br s, 1H), 1.66−1.60 (m, 4H), 1.40−1.10 (m, 48H), 0.88 (t, *J* = 6.5 Hz, 6H), 0.67−0.61 (m, 4H), 0.59−0.53 (m, 2H), −0.32 to −0.36 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.8, 173.4, 72.1, 62.0, 61.5, 34.3, 34.1, 31.9 (2C), 30.2 (2C), 30.1 (2C), 29.7 (2C), 29.4 (2C), 29.34 (2C), 29.30 (2C), 29.12 (2C), 29.09 (2C), 28.70 (2C), 28.65 (2C), 24.93, 24.87, 22.7 (2C), 15.74 (2C), 15.69 (2C), 14.1 (2C), 10.9 (2C). HRMS (ESI-QTOF): calcd $C_{41}H_{76}NaO_5$ $[M + Na]^+$, 671.5585; found $[M + Na]^+$, 671.5594.

DOI: 10.1021/acs.joc.7b00945
J. Org. Chem. 2017, 82, 7832−7838

Synthesis of Diacylglycerol-Phophoramidite (2a). A mixture of diacylglycerol **16a** (28.6 mg, 44.1 μmol), 1*H*-tetrazole (6.2 mg, 88.5 μmol), 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphophordiamidite (28 μL, 27 mg, 88 μmol) and CH_2Cl_2/MeCN (v/v = 2:3, 0.55 mL) was stirred at room temperature for 3 h. The reaction was quenched with saturated $NaHCO_3$ aq., and the whole was extracted with CH_2Cl_2 three times. The combined extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude oil, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 10:1, 3% Et_3N) to give the title compound **2a** (36.3 mg, 88%, ∼1:1 diastereomeric mixture) as a colorless oil. $[\alpha]^{25}_D$ = +3.14 (*c* 0.44, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.23−5.16 (m, 1H), 4.39−4.29 (m, 1H), 4.21−4.12 (m, 1H), 3.89−3.74 (m, 3H), 3.73−3.66 (m, 1H), 3.65−3.54 (m, 2H), 2.64 (t, *J* = 6.4 Hz, 2H), 2.35−2.28 (m, 4H), 1.65−1.58 (br m, 4H), 1.40−1.11 (m, 60H), 0.88 (t, *J* = 6.9 Hz, 6H), 0.67−0.61 (m, 4H), 0.59−0.53 (m, 2H), −0.31 to −0.36 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.4, 173.0, 117.5, 70.6, 62.4, 61.6, 58.4, 43.2, 43.1, 34.3, 34.1, 31.9 (2C), 30.20 (2C), 30.15 (2C), 29.7 (2C), 29.5 (2C), 29.4 (4C), 29.14 (2C), 29.12 (2C), 28.70 (2C), 28.66 (2C), 24.92, 24.89, 24.6 (2C), 24.5 (2C), 22.7 (2C), 20.4, 15.74 (2C), 15.69 (2C), 14.1 (2C), 10.9 (2C). ^{31}P NMR ($CDCl_3$, 161 MHz) δ: 150.2, 150.0. HRMS (ESI-QTOF): calcd $C_{50}H_{93}N_2NaO_6P$ $[M + Na]^+$, 871.6663; found $[M + Na]^+$, 871.6670.

Synthesis of Diacylglycerol-Phophoramidite (2b). By a procedure identical with that described for synthesis of **2a** from **16a**, the diacylglycerol **16b** (47.1 mg) was converted into **2b** (50.1 mg, 81%, ∼1:1 diastereomeric mixture) as a colorless oil. $[\alpha]^{25}_D$ = +3.70 (*c* 1.12, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.22−5.17 (m, 1H), 4.38−4.30 (m, 1H), 4.22−4.13 (m, 1H), 3.88−3.75 (m, 3H), 3.72−3.67 (m, 1H), 3.63−3.54 (m, 2H), 2.64 (t, *J* = 6.4 Hz, 2H), 2.33−2.29 (m, 4H), 1.64−1.59 (m, 4H), 1.40−1.14 (m, 60H), 0.88 (t, *J* = 6.4 Hz, 6H), 0.66−0.62 (m, 4H), 0.59−0.53 (m, 2H), −0.31 to −0.36 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.4, 173.0, 117.5, 70.6, 62.3, 61.6, 58.4, 43.2, 43.1, 34.3, 34.1, 31.90, 31.88, 30.2 (2C), 30.1 (2C), 29.7 (2C), 29.5 (2C), 29.3 (4C), 29.13 (2C), 29.11 (2C), 28.69 (2C), 28.65 (2C), 24.90, 24.87, 24.6 (2C), 24.5 (2C), 22.7 (2C), 20.4, 20.3, 15.72 (2C), 15.67 (2C), 14.1 (2C), 10.9 (2C). ^{31}P NMR ($CDCl_3$, 161 MHz) δ: 150.2, 150.0. HRMS (ESI-QTOF): calcd $C_{50}H_{93}N_2NaO_6P$ $[M + Na]^+$, 871.6663; found $[M + Na]^+$, 871.6671.

Synthesis of PMB/Cyanoethyl-Protected Cardiolipin (17a). A mixture of diacylglycerol-phophoramidite **2a** (120 mg, 145 μmol), PMB-protected glycerol (14.0 mg, 66.1 μmol), 1*H*-tetrazole (13.9 mg, 198 μmol), and CH_2Cl_2/MeCN (v/v = 2:1, 0.72 mL) was stirred at room temperature for 2 h. H_2O_2 aq. (30 wt %, 37 μL) was then added to the mixture, and the reaction mixture was stirred for 15 min. The reaction was quenched with saturated $Na_2S_2O_3$ aq., and the whole was extracted with CH_2Cl_2 three times. The combined extract was washed with saturated NH_4Cl aq., saturated $NaHCO_3$ aq., and brine; dried over anhydrous Na_2SO_4; and concentrated under reduced pressure to give a crude oil, which was purified by silica gel column chromatography ($CHCl_3$/MeOH = 50:1) to give the title compound **17a** (101 mg, 88% based on PMB-protected glycerol, ∼1:1 diastereomeric mixture) as a colorless oil. $[\alpha]^{25}_D$ = +2.25 (*c* 0.80, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.29 (d, *J* = 8.4 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 2H), 5.27−5.21 (m, 2H), 4.60 (s, 2H), 4.35−4.07 (m, 16H), 3.87−3.81 (m, 1H), 3.80 (s, 3H), 2.81−2.66 (m, 4H), 2.36−2.28 (m, 8H), 1.64−1.57 (m, 8H), 1.40−1.09 (m, 96H), 0.88 (t, *J* = 6.8 Hz, 12H), 0.67−0.61 (m, 8H), 0.59−0.53 (m, 4H), −0.31 to −0.36 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.2 (2C), 172.8 (2C), 159.5, 129.7, 129.7, 129.1, 116.4 (2C), 113.9 (2C), 75.7, 72.0, 69.23, 69.15, 65.92 (2C), 65.86 (2C), 62.2 (2C), 61.5 (2C), 55.2, 34.1 (2C), 34.0 (2C), 31.9 (4C), 30.2 (4C), 30.1 (4C), 29.67 (2C), 29.65 (2C), 29.5 (4C), 29.4 (4C), 29.3 (4C), 29.12 (4C), 29.09 (4C), 28.68 (4C), 28.65 (4C), 24.8 (4C), 22.7 (4C), 19.5 (2C), 15.72 (4C), 15.66 (4C), 14.1 (4C), 10.9 (4C). ^{31}P NMR ($CDCl_3$, 161 MHz) δ: −0.83, −0.88, −0.96, −1.04. HRMS (ESI-QTOF): calcd $C_{99}H_{172}N_2NaO_{18}P_2$ $[M + Na]^+$, 1762.1973; found $[M + Na]^+$, 1762.1979.

Synthesis of PMB/Cyanoethyl-Protected Cardiolipin (17b). By a procedure identical with that described for synthesis of **17a** from **2a**, the diacylglycerol-phophoramidite **2b** (12.7 mg) was converted into **17b** (3.6 mg, 29% based on PMB-protected glycerol (1.5 mg, 21 μmol), ∼1:1 diastereomeric mixture) as a colorless oil. $[\alpha]^{25}_D$ = +4.15 (*c* 0.14, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.29 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 5.26−5.21 (m, 2H), 4.60 (s, 2H), 4.33−4.10 (m, 16H), 3.86−3.81 (m, 1H), 3.80 (s, 3H), 2.74−2.66 (m, 4H), 2.35−2.29 (m, 8H), 1.62−1.57 (m, 8H), 1.39−1.10 (m, 96H), 0.88 (t, *J* = 6.8 Hz, 12H), 0.66−0.61 (m, 8H), 0.58−0.53 (m, 4H), −0.32 to −0.36 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.2 (2C), 172.8 (2C), 159.6, 129.7, 129.0, 128.2, 116.4 (2C), 113.9 (2C), 72.0, 69.3, 69.2, 66.0 (2C), 65.9 (2C), 62.2 (2C), 61.6 (2C), 55.3, 53.4, 34.1 (2C), 34.0 (2C), 31.9 (4C), 30.21 (4C), 30.16 (4C), 29.69 (2C), 29.68 (2C), 29.5 (4C), 29.38 (4C), 29.36 (4C), 29.2 (4C), 29.1 (4C), 28.71 (4C), 28.67 (4C), 24.8 (4C), 22.7 (4C), 19.6, 19.5, 15.74 (4C), 15.69 (4C), 14.1 (4C), 10.9 (4C). ^{31}P NMR ($CDCl_3$, 161 MHz) δ: −0.80, −0.86, −0.96, −1.02. HRMS (ESI-QTOF): calcd $C_{99}H_{172}N_2NaO_{18}P_2$ $[M + Na]^+$, 1762.1973; found $[M + Na]^+$, 1762.1977.

Synthesis of Cyanoethyl-Protected Cardiolipin (18a). To a solution of PMB/cyanoethyl-protected cardiolipin **17a** (23.5 mg, 13.5 μmol) in MeCN/H_2O (v/v = 10/1, 1.35 mL) was added $Ce(NH_4)_2(NO_3)_6$ (74.0 mg, 135 μmol) at 0 °C, and the reaction mixture was stirred at room temperature for 3 h. The mixture was diluted with water and extracted with CH_2Cl_2 three times. The extract was washed with saturated $NaHCO_3$ aq. and saturated $NaHSO_3$ aq., dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude oil, which was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100:1) to give the title compound **18a** (9.0 mg, 41%) as a colorless oil. $[\alpha]^{25}_D$ = +1.50 (*c* 0.51, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.29−5.25 (m, 2H), 4.36−4.10 (m, 17H), 2.79 (t, *J* = 5.8 Hz, 4H), 2.37−2.31 (m, 8H), 1.65−1.58 (m, 8H), 1.40−1.10 (m, 96H), 0.88 (t, *J* = 6.7 Hz, 12H), 0.67−0.62 (m, 8H), 0.59−0.53 (m, 4H), −0.31 to −0.36 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.3 (2C), 173.0 (2C), 116.5 (2C), 69.2 (2C), 68.48 (2C), 68.45 (2C), 66.1 (2C), 62.4 (2C), 61.5, 34.1 (2C), 34.0 (2C), 31.9 (4C), 30.2 (4C), 30.1 (4C), 29.7 (4C), 29.5 (4C), 29.3 (8C), 29.12 (4C), 29.08 (4C), 28.68 (4C), 28.65 (4C), 24.8 (4C), 22.7 (4C), 19.7, 19.6, 15.72 (4C), 15.67 (4C), 14.1 (4C), 10.9 (4C). ^{31}P NMR ($CDCl_3$, 161 MHz) δ: −0.26, −0.51. HRMS (ESI-QTOF): calcd $C_{91}H_{164}N_2NaO_{17}P_2$ $[M + Na]^+$, 1642.1397; found $[M + Na]^+$, 1642.1403.

Synthesis of Cyanoethyl-Protected Cardiolipin (18b). By a procedure identical with that described for synthesis of **18a** from **17a**, the cardiolipin derivative **17b** (7.2 mg) was converted into **18b** (2.9 mg, 43%) as a colorless oil. $[\alpha]^{25}_D$ = +2.83 (*c* 0.15, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.29−5.25 (m, 2H), 4.36−4.10 (m, 17H), 2.79 (t, *J* = 5.8 Hz, 4H), 2.37−2.31 (m, 8H), 2.01 (br s, 1H), 1.65−1.58 (m, 8H), 1.40−1.10 (m, 96H), 0.88 (t, *J* = 6.7 Hz, 12H), 0.67−0.62 (m, 8H), 0.59−0.53 (m, 4H), −0.31 to −0.36 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.3 (2C), 172.9 (2C), 116.5 (2C), 69.2 (2C), 68.5 (2C), 68.1 (2C), 66.1 (2C), 62.5 (2C), 61.5, 34.1 (2C), 34.0 (2C), 31.9 (4C), 30.21 (4C), 30.16 (4C), 29.7 (4C), 29.5 (4C), 29.4 (8C), 29.14 (4C), 29.11 (4C), 28.71 (4C), 28.66 (4C), 24.8 (4C), 22.7 (4C), 19.7, 19.6, 15.74 (4C), 15.69 (4C), 14.1 (4C), 10.9 (4C). ^{31}P NMR ($CDCl_3$, 161 MHz) δ: −0.27, −0.51. HRMS (ESI-QTOF): calcd $C_{91}H_{164}N_2NaO_{17}P_2$ $[M + Na]^+$, 1642.1397; found $[M + Na]^+$, 1642.1395.

Synthesis of Cardiolipin Containing Chiral Cyclopropane Moiety (1a). To a solution of cyanoethyl-protected cardiolipin **18a** (5.4 mg, 3.3 μmol) in CH_2Cl_2/MeOH (v/v = 1/1, 830 μL) was added 28% NH_3(aq.) (830 μL). After being stirred at room temperature for 1 h, the reaction mixture was lyophilized with dioxane to give the crude compound **1a** (5.6 mg) as a colorless oil, part of which (0.8 mg) was purified by silica gel column chromatography ($CHCl_3$/MeOH/28% NH_3(aq.) = 7:2:0.3) to obtain the title compound **1a** (0.2 mg, 28%). $[\alpha]^{25}_D$ = +2.72 (*c* 0.22, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.28−5.23 (m, 2H), 4.45−3.35 (m, 21H), 2.39−2.25 (m, 8H), 1.70−1.60 (m, 8H), 1.43−1.09 (m, 96H), 0.88 (t, *J* = 5.7 Hz, 12H), 0.66−0.62 (m, 8H), 0.59−0.53 (m, 4H), −0.31 to −0.36 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.8 (2C), 173.4 (2C), 72.1 (2C), 68.4 (2C), 65.0 (2C), 62.0 (2C), 61.5, 34.3 (2C), 34.1 (2C), 31.9 (4C), 30.2

DOI: 10.1021/acs.joc.7b00945
J. Org. Chem. 2017, 82, 7832−7838

(4C), 30.1 (4C), 29.7 (4C), 29.5 (2C), 29.43 (2C), 29.36 (2C), 29.32 (4C), 29.29 (2C), 29.12 (4C), 29.09 (4C), 28.71 (4C), 28.65 (4C), 24.9 (4C), 22.7 (4C), 15.74 (4C), 15.69 (4C), 14.1 (4C), 10.9 (4C). ^{31}P NMR ($CDCl_3$, 161 MHz) δ: 1.82. HRMS (ESI-QTOF): calcd $C_{85}H_{156}O_{17}P_2$ $[M-2NH_4]^{2-}$, 755.5414; found $[M-2NH_4]^{2-}$, 755.5417.

Synthesis of Cardiolipin Containing Chiral Cyclopropane Moiety (1b). By a procedure identical with that described for synthesis of **1a** from **18a**, the cardiolipin derivative **18b** (2.7 mg) was converted into **1b** (2.5 mg) as a colorless oil, part of which (1.1 mg) was purified by silica gel column chromatography ($CHCl_3$/MeOH/28% NH_3(aq.) = 7:2:0.3) to give the title compound **1b** (0.3 mg, ca. 27%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.28−5.17 (m, 2H), 4.40−3.59 (m, 21H), 2.35 (t, J = 7.6 Hz, 8H), 2.01 (br s, 1H), 1.65−1.59 (m, 8H), 1.40−1.10 (m, 96H), 0.88 (t, J = 6.6 Hz, 12H), 0.67−0.62 (m, 8H), 0.59−0.53 (m, 4H), −0.31 to −0.36 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 174.0 (2C), 173.9 (2C), 72.1 (2C), 68.4 (2C), 65.0 (2C), 62.0 (2C), 61.5, 34.3 (2C), 34.1 (2C), 31.9 (4C), 30.2 (4C), 30.1 (4C), 29.7 (4C), 29.5 (2C), 29.43 (2C), 29.35 (2C), 29.33 (4C), 29.29 (2C), 29.12 (4C), 29.09 (4C), 28.71 (4C), 28.65 (4C), 24.9 (4C), 22.7 (4C), 15.8 (4C), 15.7 (4C), 14.1 (4C), 10.9 (4C). ^{31}P NMR ($CDCl_3$, 161 MHz) δ: 2.75. HRMS (ESI-QTOF): calcd $C_{85}H_{156}O_{17}P_2$ $[M-2NH_4]^{2-}$, 755.5414; found $[M-2NH_4]^{2-}$, 755.5419.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b00945.

^{1}H and ^{13}C NMR spectral data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: fujimotoy@chem.keio.ac.jp.

ORCID

Yukari Fujimoto: 0000-0001-5320-3192

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was financially supported by grants from the JSPS KAKENHI (Nos. JP26282211, JP26102732, JP26882036, and JP16K16638), NEXT Program (LR025) from JSPS and CSTP, the Mizutani Foundation for Glycoscience, and the Nagase Science Technology Foundation and Protein Research Foundation. We thank Prof. K. Suenaga for assistance in measuring the chiroptical data.

■ REFERENCES

(1) Schlame, M. J. *J. Lipid Res.* **2008**, *49*, 1607.

(2) (a) Claypool, S. M.; Koehler, C. M. *Trends Biochem. Sci.* **2012**, *37*, 32. (b) Maguire, J. J.; Tyurina, Y. Y.; Mohammadyani, D.; Kapralov, A. A.; Anthonymuthu, T. S.; Qu, F.; Amoscato, A. A.; Sparvero, L. J.; Tyurin, V. A.; Planas-Iglesias, J.; He, R. R.; Klein-Seetharaman, J.; Bayir, H.; Kagan, V. E. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **2017**, *1862*, 8.

(3) (a) Santiago, E.; López-Moratalla, N.; Segovia, J. *Biochem. Biophys. Res. Commun.* **1973**, *53*, 439. (b) Schug, Z. T.; Gottlieb, E. *Biochim. Biophys. Acta, Biomembr.* **2009**, *1788*, 2022. (c) Paradies, G.; Paradies, V.; De Benedictis, V.; Ruggiero, F. M.; Petrosillo, G. *Biochim. Biophys. Acta, Bioenerg.* **2014**, *1837*, 408.

(4) Dieude, M.; Striegl, H.; Tyznik, A. J.; Wang, J.; Behar, S. M.; Piccirillo, C. A.; Levine, J. S.; Zajonc, D. M.; Rauch, J. *J. Immunol.* **2011**, *186*, 4771.

(5) (a) Yokota, K.; Kanamoto, R.; Kito, M. *J. Bacteriol.* **1980**, *141*, 1047. (b) Hoch, F. *Biochim. Biophys. Acta, Rev. Biomembr.* **1992**, *1113*, 71.

(6) (a) Cronan, J. E., Jr *Curr. Opin. Microbiol.* **2002**, *5*, 202. (b) Bao, X.; Katz, S.; Pollard, M.; Ohlrogge, J. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 7172. (c) Fontecave, M.; Atta, M.; Mulliez, E. *Trends Biochem. Sci.* **2004**, *29*, 243.

(7) Shah, S.; Nagata, M.; Yamasaki, S.; Williams, S. J. *Chem. Commun.* **2016**, *52*, 10902.

(8) (a) Ali, S. M.; Ahmad, M. U.; Koslosky, P.; Kasireddy, K.; Murali Krishna, U.; Ahmad, I. *Tetrahedron* **2006**, *62*, 6990. (b) Abe, M.; Kitsuda, S.; Ohyama, S.; Koubori, S.; Murai, M.; Miyoshi, H. *Tetrahedron Lett.* **2010**, *51*, 2071. (c) Abe, M.; Nakano, M.; Kosaka, A.; Miyoshi, H. *Tetrahedron Lett.* **2015**, *56*, 2258 and references cited therein.

(9) Krishna, U. M.; Ahmad, M. U.; Ahmad, I. *Tetrahedron Lett.* **2004**, *45*, 2077.

(10) Kobayashi, S.; Tokunoh, R.; Shibasaki, M.; Shinagawa, R.; Murakami-Murofushi, K. *Tetrahedron Lett.* **1993**, *34*, 4047.

(11) Coxon, G. D.; Knobl, S.; Roberts, E.; Baird, M. S.; Al Dulayymi, J. R.; Besra, P. J.; Brennan, P. J.; Minnikin, D. E. *Tetrahedron Lett.* **1999**, *40*, 6689.

(12) (a) Charette, A. B.; Juteau, H.; Lebel, H.; Molinaro, C. *J. Am. Chem. Soc.* **1998**, *120*, 11943. (b) Nicolaou, K. C.; Li, J.; Zenke, G. *Helv. Chim. Acta* **2000**, *83*, 1977.

(13) Lou, Y.; Horikawa, M.; Kloster, R. A.; Hawryluk, N. A.; Corey, E. J. *J. Am. Chem. Soc.* **2004**, *126*, 8916.

(14) Suematsu, H.; Kanchiku, S.; Uchida, T.; Katsuki, T. *J. Am. Chem. Soc.* **2008**, *130*, 10327.

(15) Nishizaki, T. PKC-ε Activator. Patent WO 2012/067111 A1, 2012.

(16) Palko, J. W.; Buist, P. H.; Manthorpe, J. M. *Tetrahedron: Asymmetry* **2013**, *24*, 165.

(17) Shah, S.; White, J. M.; Williams, S. J. *Org. Biomol. Chem.* **2014**, *12*, 9427.

(18) Cai, S.; Dimitroff, M.; McKennon, T.; Reider, M.; Robarge, L.; Ryckman, D.; Shang, X.; Therrien, J. *Org. Process Res. Dev.* **2004**, *8*, 353.

(19) (a) Vu, V. A.; Marek, I.; Polborn, K.; Knochel, P. *Angew. Chem., Int. Ed.* **2002**, *41*, 351. (b) Zhang, M.-X.; Eaton, P. E. *Angew. Chem., Int. Ed.* **2002**, *41*, 2169.

(20) de Carne-Carnavalet, B.; Archambeau, A.; Meyer, C.; Cossy, J.; Folleas, B.; Brayer, J.-L.; Demoute, J.-P. *Org. Lett.* **2011**, *13*, 956.

(21) Gansäuer, A.; Fan, C.-A.; Keller, F.; Keil, J. *J. Am. Chem. Soc.* **2007**, *129*, 3484.

(22) Al Dulayymi, J. a. R.; Baird, M. S.; Roberts, E. *Tetrahedron* **2005**, *61*, 11939.

(23) (a) Gu, Q.-M.; Prestwich, G. D. *J. Org. Chem.* **1996**, *61*, 8642. (b) Browne, J. E.; Driver, M. J.; Russell, J. C.; Sammes, P. G. *J. Chem. Soc., Perkin Trans. 1* **2000**, 653.

DOI: 10.1021/acs.joc.7b00945
J. Org. Chem. 2017, 82, 7832−7838