Synthesis of a Novel Lysophosphatidylcholine

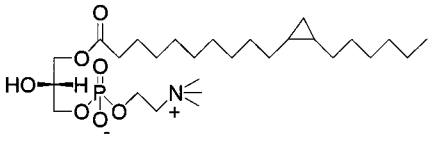
Changjin Zhu,*,† Tatsuya Morimoto,‡ Shuhei Nakajima,‡ Teruhiko Nitoda,‡ and Naomichi Baba‡

Department of Natural Science, Graduate School of Okayama University, Okayama 700-8530, Japan, and Faculty of Agriculture, Okayama University, Okayama 700-8530, Japan

Received May 5, 2000

The novel lysophosphatidylcholine (**1**), which naturally occurs in the marine sponge *Spirastrella abata* and was reported to inhibit cholesterol biosynthesis in the Chang liver cell, has been synthesized in four steps from methyl *cis*-11-octadecenoate (**2**).

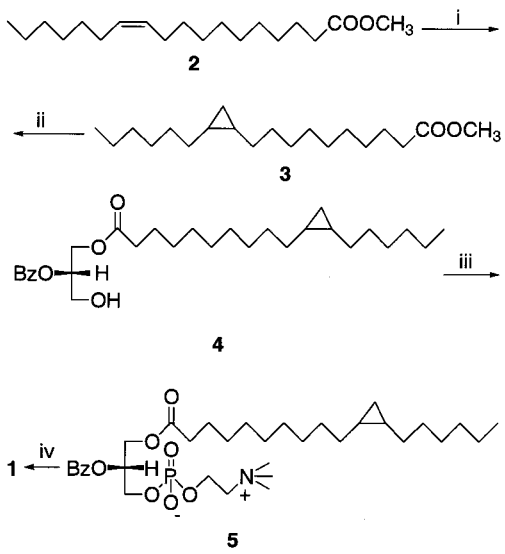
In 1999, Shin et al. disclosed the isolation and structure elucidation of several marine sponge (*Spirastrella abata*) phospholipids with inhibitory activity against cholesterol biosynthesis in the Chang liver cell.[1] These compounds specifically blocked the conversion of lanosterol into cholesterol, which is quite *downstream* in the cholesterol biosynthetic pathway, as compared to the steps inhibited by the well-known mevinolin and its analogues, as well as by zaragozic acids (squalestatins). Mevinolin and its analogues inhibit HMG CoA reductase, while zaragozic acids, which have recently been widely studied, inhibit squalene synthase.[2] Among the phospholipids, lysophosphatidylcholine (**1**) showed an IC_{50} value of 60 μg/mL. It bears a cyclopropane moiety important in a number of natural and synthetic products[3] as well as in molecules used to probe biological processes.[4] This phospholipid may become a leading compound for the development of more potent inhibitors of cholesterol biosynthesis. In our studies, other lysophosphatidylcholines (lyso-PCs) have been prepared and used as intermediates for the syntheses of bioactive oxidized phospholipids.[5] In this report, we describe the synthesis of the new lyso-PC **1**.



1

As shown in Scheme 1, treatment of methyl *cis*-11-octadecenoate (**2**) with CH_2I_2 and Et_2Zn under an N_2 atmosphere produced the *cis*-cyclopropane-containing ester **3** in quantitative yield. This modified Simmons-Smith reaction has been demonstrated to result in the stereospecific cyclopropanation of the corresponding olefin.[6] The geminal proton signals at δ 0.56 and −0.34 and two methine protons at δ 0.63 in the ^{1}H NMR spectrum of **3** confirmed formation of the cyclopropane. However, the product might be a mixture of diastereomers (11*S*,12*R* and 11*R*,12*S*) because of two chiral centers at C-11 and C-12. The lipase PS-catalyzed transesterification of **3** with 2-*O*-benzylglycerol in $CHCl_3$ afforded the monoester (**4**) in 49% yield, with a very little diacylated side-product (0.7%) and 48% recovery of the methyl ester (**3**). When diisopropyl ether was used as solvent, the reaction went fast but produced much of the diester (6%), while the yield of the monoester was only 20%. The enzyme has been employed in the transesterification reaction in which the stereoselective acylation of 2-*O*-benzyl or 2-methoxyethoxymethyl glycerol yields the (*S*)-monoester predominantly, even using various acyl donor esters.[5a,b,7] Consequently, monoester **4** was suggested to have the *S* superior to *R* configuration at C-2. This compound showed an optical activity with $[\alpha]^{20}_D$ −6.3°, and an optical purity with 60% ee determined with Mosher ester[8] of **4** by HPLC analysis. Direct determination of **4** by ^{1}H NMR experiments in the presence of tris{3-[(heptafluoropropyl)hydroxymethylene]-(+)-camphorato}europium (III) derivative [Eu(hfc)$_3$] also provided a result for optical purity consistent with that obtained by Mosher method. Reaction of the monoester **4** successively with $POCl_3$, choline tosylate, and water, followed by aqueous workup, gave PC **5** in 79% yield. Palladium-catalyzed deprotection of **5** under an H_2 atmosphere gave the desired compound (**1**) in 90% yield. The overall yield for the four-step sequence leading to **1** was 35%.

Scheme 1[a]



[a] (i) CH_2I_2, Et_2Zn, benzene, 70 °C; (ii) 2-*O*-benzylglycerol, lipase, $CHCl_3$, 30 °C; (iii) *a*. $POCl_3$, $CHCl_3$, 0−5 °C; *b*. choline tosylate, room temperature; *c*. H_2O; (iv) Pd−C, H_2, MeOH, H_2O, room temperature.

The formation of **1** was supported by ^{1}H, ^{13}C, and ^{1}H-^{1}H COSY NMR experiments, as well as FABMS and HR-FABMS measurements. The spectral data for the synthesized lysophosphatidylcholine (**1**) are identical to that of the natural one reported by Shin et al.[1] Only coupling constants for the H-19′ geminal protons at δ 0.56 and −0.34 in the ^{1}H NMR spectrum showed some difference.

Experimental Section

General Experimental Procedures. ^{1}H NMR spectra were obtained on a Varian VXR 500 spectrophotometer; ^{13}C

* To whom correspondence should be addressed. Tel.: +81-86-251-8292. Fax: +81-86-251-8388. E-mail: zhuchangjin@hotmail.com.
† Department of Natural Science.
‡ Faculty of Agriculture.

10.1021/np000222a CCC: $20.00
Published on Web 11/18/2000

NMR spectra, on a Varian VXR 200 spectrophotometer at 50 MHz; and chemical shifts were recorded in ppm (δ). Rotation was determined on a JASCO DIP-360 polarimeter. Normal-phase HPLC was carried out on Inertsil SIL column (4.6 × 250 mm, hexane−2-propanol, 100:1) with detection at 255 nm. FABMS and HRFABMS measurements were obtained on an SX102A mass spectrometer.

Chemicals. Chemical reagents were obtained from commercial sources and used directly without further purification. 2-*O*-Benzylglycerol,[7a] choline tosylate,[9] and Mosher *R*(−)-MTPA ester[8] were prepared as described. *R*(−)-α-Methoxy-α-trifluoromethylphenylacetyl chloride [*R*(−)-MTPA] was purchased from Tokyo Kasei Organic Chemicals. Methyl *cis*-11-octadecenoate (**2**) and Eu(hfc)$_3$ were from Sigma Chemical Co. Et_2Zn (1 M in hexane) and Pd−C (palladium, 10 wt % [dry basis] on activated carbon) were from Aldrich. Lipase from *Pseudomonas* species (PS) was from Amano Pharmaceutical Co. All the other chemicals were from Nacalai Tesque Co.

Methyl *cis*-11,12-Methylene-octadecanoate (3). To a stirred solution of methyl *cis*-11-octadecenoate (**2**) (100 mg, 0.34 mmol) in C_6H_6 (10 mL) under dried N_2 atmosphere were added Et_2Zn (420 mg, 3.4 mmol) and CH_2I_2 (1018 mg, 3.8 mmol) dropwise at room temperature. After being stirred at 65 °C for 6 h, the reaction mixture was cooled with ice and poured into 1% HCl (40 mL) with stirring. The organic layer was washed with 30% aqueous $NaHCO_3$ and water and dried over molecular sieves. After filtration and evaporation of the solvent under reduced pressure, the remaining residue was purified by flash column chromatography (Si gel, hexane−EtOAc, 100:3) to give the known compound **3**[10] (105 mg, 0.34 mmol, 100%) as a colorless oil: R_f = 0.27 (hexane−EtOAc, 100:3).

1-*O*-(*cis*-11′,12′-Methylene-octadecanoyl)-2-*O*-benzylglycerol (4). A solution of **3** (90 mg, 0.29 mmol) in $CHCl_3$ (20 mL) was added to the mixture of 2-*O*-benzylglycerol (900 mg, 4.9 mmol) and lipase PS (500 mg) in $CHCl_3$ (2 mL). After being stirred at 30 °C for 6 days, the reaction mixture was filtered through a plug of Celite and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (Si gel, hexane−EtOAc, 3:1) to yield **4** (65 mg, 0.14 mmol, 49%) as a colorless oil: R_f = 0.25 (hexane−EtOAc, 3:1); $[\alpha]^{20}_D$ −6.3° (*c* 3.81, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38−7.28 (5H, m, Ph), 4.73 (1H, d, J = 12.50 Hz, PhCH_2O), 4.60 (1H, d, J = 12.50 Hz, PhCH_2O), 4.23 (2H, d, J = 4.86 Hz, H-1), 3.73−3.68 (3H, m, H-2 and −3a), 3.63 (1H, dd, J = 12.50, 7.64 Hz, H-3b), 2.34 (2H, t, J = 7.47 Hz, H-2′), 1.63−1.58 (2H, m, H-3′), 1.40−1.33 (6H, m, H-9′, -10′a, -13′a, and -14′), 1.33−1.23 (16H, m, H-4′ to H-8′ and H-15′ to H-17′), 1.17−1.08 (2H, m, H-10′b and -13′b), 0.88 (3H, t, J = 7.00 Hz, H-18′), 0.64 (2H, m, H-11′ and -12′), 0.57 (1H, ddd, J = 8.54, 8.54, 4.27 Hz, H-19′b), −0.34 (1H, dd, J = 9.46, 4.73 Hz, H-19′a); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.9 (C-1′), 137.8 (C-1″), 128.5 (C-4″), 128.0 (C-2″ and -6″), 127.9 (C-3″ and -5″), 77.2 (PhCH_2O), 72.1(C-2), 62.7 (C-1), 62.0 (C-3), 34.2 (C-2′), 31.9 (C-16′), 30.2 (C-4′ and -5′), 29.6 (C-6′ and -7′), 29.5 (C-15′), 29.3 (C-8′), 29.2 (C-13′), 29.1 (C-10′), 28.7 (C-9′ and -14′), 24.9 (C-3′), 22.7 (C-17′), 15.8 (C-11′ and -12′), 14.1 (C-18′), 10.9 (C-19′); FABMS *m*/*z* 461 $[M + H]^+$ (40), 443 (23), 369 (91), 353 (72), 297 (5), 277 (35), 181 (7), 91 (45), 55 (100), 43 (56).

1-*O*-(*cis*-11′,12′-Methylene-octadecanoyl)-2-*O*-benzylglycero-3-phosphocholine (5). To $POCl_3$ (25 μL, 0.28 mmol) with stirring was added a solution of **4** (64 mg, 0.14 mmol) with Et_3N (77 μL, 0.56 mmol) in $CHCl_3$ (3 mL) dropwise over 20 min at 0−4 °C. After being stirred at the same temperature for 30 min and subsequently at room temperature for 1 h, to the mixture was added choline tosylate (165 mg, 0.6 mmol) and pyridine (1.05 mL, 3.0 mmol), and it was continually stirred at room temperature for 44 h. The reaction was quenched by the addition of H_2O (0.5 mL). The reaction mixture was washed with H_2O. The organic layer was dried over Na_2SO_4, followed by filtration and evaporation of the solvent under low pressure. The resulting residue was purified by flash column chromatography (Si gel, $CHCl_3$−MeOH−28% aqueous NH_3, 30:15:2) to give benzyl-PC (**5**) (69 mg, 0.11 mmol, 79%) as a colorless amorphous solid: R_f = 0.54 ($CHCl_3$−MeOH−28% aqueous NH_3, 30:15:2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.34−7.22 (5H, m, Ph), 4.62 (2H, dd, J = 11.6, 20.45 Hz, PhCH_2O), 4.25 (1H, dd, J = 11.60, 3.05 Hz, H-1a), 4.20 (2H, m, POCH_2CH_2N), 4.16 (1H, dd, J = 11.59, 7.33 Hz, H-1b), 3.97 (H, m, H-3a), 3.93 (H, m, H-3b), 3.78 (1H, m, H-2), 3.60 (2H, m, POCH_2CH_2N), 3.16 (9H, s, NMe_3), 2.27 (2H, t, J = 7.63 Hz, H-2′), 1.57 (2H, m, H-3′), 1.36 (6H, m, H-9′, -10′a, -13′a, and -14′), 1.33−1.20 (16H, m, H-4′ to H-8′ and H-15′ to H-17′), 1.17−1.08 (2H, m, H-10′b and -13′b), 0.88 (3H, t, J = 7.02 Hz, H-18′), 0.63 (2H, m, H-11′ and -12′), 0.55 (1H, ddd, J = 8.55, 8.55, 4.27 Hz, H-19′b), −0.35 (1H, dd, J = 9.46, 5.50 Hz, H-19′a); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.7 (C-1′), 138.3 (C-1″), 128.4 (C-2″ and -6″), 127.9 (C-3″ and -5″), 127.7 (C-4″), 76.6 (PhCH_2O), 72.1 (C-2), 66.1 (C-3), 64.5 (C-1), 64.2 (POCH_2CH_2N), 59.2 (POCH$_2$$CH_2$N), 54.1 ($NMe_3$), 34.2 (C-2′), 31.9 (C-16′), 30.2 (C-4′), 30.1 (C-5′), 29.7 (C-6′), 29.6 (C-7′), 29.5 (C-15′), 29.3 (C-10′ and -13′), 29.2 (C-8′), 28.7 (C-9′ and -14′), 24.9 (C-3′), 22.7 (C-17′), 15.7 (C-11′ and -12′), 14.1(C-18′), 10.9 (C-19′); FABMS *m*/*z* 626 $[M + H]^+$ (100), 536 (30), 184 (43), 86 (27).

1-*O*-(*cis*-11′,12′-Methylene-octadecanoyl)glycero-3-phosphocholine (1). To a solution of **5** (62 mg, 0.10 mmol) in 6 mL mixed solvent of MeOH and H_2O (10:1) was added Pd−C (50 mg) at room temperature. The mixture was stirred under H_2 at the same temperature for 24 h, filtered through Celite, and concentrated by evaporation of the solvent under low pressure. The resulting residue was purified by flash column chromatography (Si gel, $CHCl_3$−MeOH-28% aqueous NH_3, 30:15:2) to yield the desired lyso-PC (**1**) as a colorless amorphous solid (47 mg, 0.09 mmol, 90%): R_f = 0.12 ($CHCl_3$−MeOH-28% aqueous NH_3, 30:15:2); $[\alpha]^{28}_D$ −0.96° (*c* 4.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.31 (2H, m, POCH_2CH_2N), 4.06 (2H, m, H-1), 3.94 (1H, m, H-2), 3.92 (1H, m, H-3a), 3.83 (1H, m, H-3b), 3.79 (2H, m, POCH$_2$$CH_2$N), 3.33 (9H, s, NMe_3), 2.30 (2H, t, J = 7.63 Hz, H-2′), 1.57 (2H, m, H-3′), 1.36 (6H, m, H-9′, -10′a, -13′a, and -14′), 1.33−1.20 (16H, m, H-4′ to H-8′ and H-15′ to H-17′), 1.17−1.08 (2H, m, H-10′b and -13′b), 0.88 (3H, t, J = 7.02 Hz, H-18′), 0.63 (2H, m, H-11′ and -12′), 0.55 (1H, ddd, J = 8.55, 8.55, 4.27 Hz, H-19′b), −0.34 (1H, dd, J = 9.46, 5.50 Hz, H-19′a);^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.9 (s, C-1′), 68.6 (d, 5.6, C-2), 67.2 (d, 3.5, C-3), 66.1 (t, 3.4, POCH_2CH_2N), 65.2 (br, C-1), 59.4 (d, 4.4, POCH$_2$$CH_2$N), 54.2 (br, NMe_3), 34.1 (s, C-2′), 31.9 (s, C-16′), 30.3 (s, C-4′), 30.2 (s, C-5′), 29.8 (s, C-6′), 29.7 (s, C-7′), 29.6 (s, C-15′), 29.5 (s, C-8′), 29.3 (s, C-10′ and -13′), 28.7 (s, C-9′ and -14′), 24.9 (s, C-3′), 22.7 (s, C-17′), 15.7 (s, C-11′ and -12′), 14.1 (s, C-18′), 10.9 (s, C-19′); FABMS *m*/*z* 536 $[M + H]^+$ (100), 184 (72), 104 (27); HRFABMS *m*/*z* 536.3699 (calcd for $C_{27}H_{55}O_7NP$, 536.3716).

Supporting Information Available: NMR data for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP000222A