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Stereochemical Determination of 2-O-Methoxyethoxymethylglycerol Bearing Polyunsaturated Fatty Acyl Group at *sn*-1 Position

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A correlation of optical rotation, optical purity and configuration of the asymmetric center was done for 2-O-methoxyethoxymethylglycerol bearing polyunsaturated fatty acyl group at *sn*-1 position. This compound was enzymatically prepared and is an important starting material for the syntheses of optically active glycerophospholipids having polyunsaturated fatty acyl groups.

Key words : phospholipid, polyunsaturated fatty acid, methoxyethoxymethylglycerol, synthesis

In recent years, polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and icosapentaenoic acid (EPA) have attracted wide interest because of their diverse but prominent biological functions. These fatty acids usually have carbon numbers of 18-22. On the other hand, a number of rather less common unsaturated fatty acids have been discovered whose carbon chain length are longer by 2-34 or more carbon atoms than abundant ones. These fatty acids are called very long chain fatty acids (VLCFA) and are found mostly as glycerophospholipids and sphingolipids in the mature supermatozoa of a number of mammalian species, the retina, the human brain etc.[1)] Also, VLCFA of n-6 series were reported to occur in rat seminiferous tubules.[2)] Studies on their biological roles have just started and thus it is essential to develop synthetic methods for preparing these biomolecules since it is not easy to isolate them in a pure state and in a large amount from the complex mixtures of biological materials. With this in mind, a synthetic route for a phospholipid bearing two kinds of polyunaturated fatty acyl group has been developed by us, and stereochemical assignment of the asymmetric carbon was also conducted [3)]. The route for the stereochemical assignment starts from lipase-catalyzed stereoselective mono-acylation of 2-*O*-methoxyethoxymethylglycerol **1** (2-*O*-MEMG) to afford an optically active monoacyl glycerol **2**. To determine optical purity and configuration of the asymmetrc carbon, this chiral glycerol was converted to 1-acyl-2, 3-*di*-*O*-MEMG (not shown) followed by reductive cleavage of the ester group at the *sn*-1 position to give 2, 3-*di*-*O*-MEMG. We determinated the relation between its optical rotation and its stereochemical configuration as well as its optical purity[4)]. Their accuracy and reliability, however, were not satisfactory, since **1** and **2** with a number of oxygen atoms were found to be very hygroscopic and it was therefore difficult to determine their accurate weight. Inherently low specific rotation of **2**

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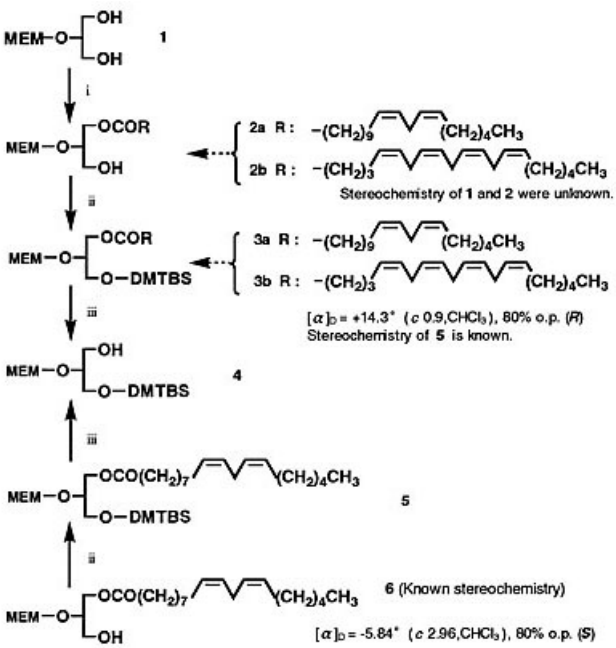
also has prevented active glycerophospholipids bearing polyunsaturated fatty acyl group at *sn*-1 and 2 positions.

This communication describes a different route as shown in the scheme. First, *t*-butyldimethylsilyl (TBDMS) group was introduced to the hydroxy group at *sn*-3 position in an optically active monoacylglycerol **2** with unknown stereochemistry. As a next step, we attempted to cleave the ester group at *sn*-1 position reductively with lithiumaluminum hydride or methylmagnesium bromide. Unfortunately, however, the former afforded a complex reaction mixture and the latter, no reaction. We found that sodium borohydride could cleave the ester linkage smoothly. This unusual reactivity of sodium borohydride was supposed to be due to the presence of the MEM group with oxygen atoms such as crown ethers whose lone pair may interact with Na^+, allowing activation of the hydride anions. Although the stereochemistry of the glycerol derivative **4** free from acyl group at *sn*-1 position was unknown, the same compound could be obtained from 1-stearoyl-2-*O*-MEMG via an intermediate **5** with known stereochemical configuration (*S*) and optical purity (80% o.p.). The configuration was determined previously by the stereochemical correlation from 2-*O*-benzyl-1-stearoyl-*sn*-glycerol with known stereochemistry.[5] The optical purity of **6** was, however, not so reliable as to be able to be used for the determination of the optical purity of **4**. Therefore, the optical purity of **6** was further confirmed by converting it to an MTPA ester and measuring the diastereomeric ratio by TLC analysis. Thus, **6** was converted to **4** which afforded $[\alpha]_D = +14.3°$ ($CHCl_3$), 80% o.p. with *R*-configuration. Therefore, **6** with 100% o.p. should have $[\alpha]_D = +$or$-17.9°$ ($CHCl_3$) with *R*-or *S*-configuration, respectively. Using this value, the stereochemistry of **4** from unknown **2** could be determined by this correlation since there may be no possibility of racemization at *sn*-2 carbon in each reaction step from **6** to **4** and **2** to **4**. Thus, **2a** and **2b** were found to have~100% o.p. with *R*-configuration and 95% o.p. with *R*, respectively. Obviously, the established maximum specific rotation and the configuration for **4** can be used to determine the optical purity and stereochemistry of **2** with any different acyl group at *sn*-1 position.



Scheme 1 Conversion of Optically Active Monoacylglycerol **1**, **2** with Unknown Stereochemistry and **6** with Known Stereochemistry to a Common Product **4** without Unsaturated Fatty Acyl Group. i. $RCOOCF_3$, Lipase PS; ii. Dimethy-t-butylsilyl Chloride, Imidazole in DMF; iii. Sodiumborohydride.

Experimental

^1H NMR spectra (δH) were recorded on a Varian VXR 200 or 500 spectrophotometer, ion spray mass spectra were taken with an API III triple quadrupole mass spectrometer equipped with an ion spray interface (PE-Sciex), and optical rotations were on a polarimeter, model J-720 (Japan Optics).

1-Icosadienoyl-2-O-MEM-sn-glycerol (**2a**) — A mixture of trifluoroethyl icosadienoate (150 mg, 0.4 mmol) and 2-*O*-MEM-glycerol (72 mg, 0.4

mmol) was dissolved in distilled diisopropyl ether after complete removal of moisture from the ester *in vacuo*. Lipase PS (100 mg, Amano Pharmaceutical Co, Japan) was added and the solution was stirred at 0°C in a nitrogen atmosphere in the dark for 3 h. After this period, the enzyme was removed by filtration and the residue after solvent evaporation was chromatographed on silica gel (hexane/ethyl acetate, 7:3) affording **2a** in almost quantitative yield (220 mg). TLC (hexane/ethyl acetate, 6:4): Rf=0.20. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.35 (m, 8H, $CH=CH\times2$), 4.81 (m, 2H, OCH_2O), 4.20–3.80 (m, 5H, (1-3)-H), 3.65 (m, 2H, OCH_2CH_2O), 3.60 (m, 2H, OCH_2CH_2O), 3.40 (s, 3H, OCH_3), 2.78 (t, J=0.7, 2H, $CH=CHCH_2CH=CH$), 2.35 (dd, J=7.9, 7.9, 2H, α-CH_2), 2.05 (q, J=15.5, 4H, $CH_2CH=CHCH_2CH=CHCH_2$), 1.60 (m, 2H, β-CH_2), 1.50 (m, 18H, 4'-9', 17'-18' and 19'-$CH_2\times9$), 0.88 (t, J=7.6, 3H, ω-CH_3)

1-Arachidonoyl-2-O-MEM-sn-glycerol (**2b**) — ^{1}H NMR (200 MHz, $CDCl_3$) : δ 5.35 (m, 8H, $CH=CH\times4$), 4.83 (s, 2H, OCH_2O), 4.27-3.45 (m, 7H, OCH_2CH_2O and (1-3)-H), 3.38 (s, 3H, OCH_3), 2.80 (dd, J=4.5, 11.8, 6H, $CH=CHCH_2CH=CH$), 2.33 (m, 2H, α-CH_2), 2.06 (m, 4H, 4' and $CH_2CH=CH\times2$), 1.62 (m, 2H, β-CH_2), 1.30 (m, 6H, 17'-19'-$CH_2\times3$), 0.88 (t, J=6.4, 3H, ω-CH_3)

1-Icosadienoyl-2-O-MEM-3-O-TBDMS-sn-glycerol (**3a**) — ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.35 (m, 4H, $CH=CH\times2$), 4.75 (s, 2H, OCH_2O), 4.20-3.90 (m, 5H, (1-3)-H), 3.65 (m, 2H, OCH_2CH_2O), 3.47 (m, 2H, OCH_2CH_2O), 3.38 (s, 3H, OCH_3), 2.76 (dd, J=7.2, 7.2, 6H, $CH=CH=CH_2CH=CH$), 2.31 (m, 2H, α-CH_2), 2.04 (q, J=7.3, 4H, $CH_2CH=CHCH_2CH=CHCH_2$), 1.62 (m, 2H, β-CH_2), 1.30 (m, 18H, 4'-9' and 17'-19'-$CH_2\times9$), 0.85 (m, 12H, ω-CH_3 and OSi$(CH_3)_2(CH_3)_3$), 0.03 (s, 6H, OSi$(CH_3)_2$)

2-O-MEM-3-O-TBDMS-sn-glycerol (**4**) — A mixture of **2a** (140 mg, 0.26 mmol) and $NaBH_4$ (15 mg, 0.39 mmol) in methanol (5 ml) was refluxed for 1 day. After the reaction, water (3 ml) was added and the product was extracted with diethyl ether 3 times. After removing the solvent, the residue was chromatographed on silica gel (hexane/ethyl acetate, 7:3) to give 4 in 61% yield. TLC (hexane/ethyl acetate, 8:2): Rf=0.18. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.80 (s, 2H, OCH_2O), 3.85-3.40 (m, 7H, OCH_2CH_2O and (1-3)-H), 3.38 (s, 3H, OCH_3), 0.8 (s, 9H, OSi $(CH_3)_2C(CH_3)_3$), 0.03 (s, 6H, OSi $(CH_3)_2$)

1-Linoleoyl-2-O-MEM-3-O-TBDMS-sn-glycerol (**5**) — ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.35 (m, 5H, $CH=CH\times2$), 4.80 (s, 2H, OCH_2O), 4.20-4.05 (m, 5H, (1-3)-H), 3.75 (m, 2H, OCH_2CH_2O), 3.70 (m, 2H, OCH_2CH_2O), 2.76 (t, J=7.5, 2H, $CH=CHCH_2CH=CH$), 2.31 (m, 2H, α-CH_2), 2.04 (q, J=7.5, 2H, $CH_2CH=CHCH_2CH=CHCH_2$), 1.62 (m, 2H, β-CH_2), 1.30 (m, 14H, 4-7' and 15'-17'-$CH_2\times7$), 0.88 (m, 12H, 18'-H and OSi$(CH_3)_2$ $(CH_3)_3$), 0.03 (s, 6H, OSi $(CH_3)_2(CH_3)_3$)

1-*Arachidonoyl-2-O-MEM-3-O-TBDMS-sn-glycerol* (**3b**) — ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.35 (s, 8H, $CH=CH\times2$), 4.80 (s, 2H, OCH_2O), 4.20-4.00 (m, 5H, (1-3)-H), 3.65 (m, 2H, OCH_2CH_2O), 3.47 (m, 2H, OCH_2CH_2O), 3.38 (s, 3H, OCH_3), 2.88 (dd, J=5.0, 13.0, $CH=CHCH_2CH=CH$), 2.31 (dd, J=8.0, 8.0, 2H, α-CH_2), 2.06 (m, 4H, $CH_2CH=CH\times2$), 1.62 (m, 2H, β-CH_2), 1.30 (m, 6H, 17'-19'-CH_2), 0.80 (s, 12H, 20' and OSi $(CH_3)_2(CH_3)_3$), 0.03 (s, 6H, OSi $(CH_3)_2$)

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Sn-1位に多価不飽和脂肪酸を結合する 2-O-methoxyethoxymethylglycerol の立体配置決定

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自然界に広く存在し，重要な生理機能を担っている多価不飽和脂肪酸結合リン脂質の化学的合成に必要な出発原料としての2-*O*-methoxyethoxymethylglycerolの立体配置と光学純度を，立体配置・光学純度既知の物質に化学的に誘導し，それらの比旋光度をお互いに比較する事により決定した．

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