Syntheses of a Glycerophospholipid, C16-Platelet Activating Factor and a Palmitoyl Analogue of M-5, an Anti-inflammatory Glyceroglycolipid[1)]

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From a chiral C4-epoxide (−)-3, which is one of the synthons in our synthetic strategy for complex lipids, a glycerophospholipid C16-platelet activating factor (C16-PAF, 1) and a palmitoyl analogue (2) of an anti-inflammatory glyceroglycolipid M-5, which was previously isolated from the Okinawan marine sponge *Phyllospongia foliascens*, have been synthesized.

Keywords complex lipid; glycerolipid; glycerophospholipid; chiral C4-epoxide; glycerol derivative optically active; platelet activating factor C16; anti-inflammatory glyceroglycolipid; metal radical epoxide-ring opening

In the previous paper,[1)] we reported a new and versatile synthetic method for sphingoglycolipids in which chiral C4-epoxides were utilized as common synthons. In our continuing synthetic studies on complex lipids, we have next tried to synthesize glycerophospholipid and glyceroglycolipid using those chiral C4-epoxides as starting compounds. This paper deals with total syntheses of a glycerophospholipid, C16-platelet activating factor (C16-PAF, **1**),[2)] and a palmitoyl analogue (**2**) of an anti-inflammatory glyceroglycolipid M-5,[3)] which was previously isolated by us from the Okinawan marine sponge *Phyllospongia foliascens* (PALLAS) through bioassay-guided analysis.

Our synthetic strategy for glycerophospholipids and glyceroglycolipids is shown in Chart 1. An allylic epoxide (**ii**), prepared from a chiral C4-epoxide (**i**) by oxidation followed by a one-carbon introducing Wittig reaction, is converted to an allylic alcohol (**iii**) by two electron reduction effected by treatment with sodium metal, resulting in double bond migration and subsequent epoxide-ring opening, while the configuration at C-2 (with an asterisk) is retained. Then, the C_2 unit in the allylic alcohol (**iii**) is removed by ozonolysis to afford an optically active glycerol derivative (**iv**), which may be transformed into glycerophospholipids through successive introductions of appropriate alkyl and phosphoryl residues. On the other hand, the allylic alcohol (**iii**) is first glycosylated to provide a C5-glycoside (**v**), which is then converted to glyceroglycolipids by removal of the C_2 unit and stepwise acylation of the resulting glyceroglycoside.

Synthesis of C16-Platelet Activating Factor The *E*-type C4-epoxide [(−)-**3**] protected with a methoxymethyl (MOM) group[1)] was used as a starting compound for synthesizing C16-platelet activating factor (**1**), since the epoxide (−)-**3** was obtained in a satisfactory chemical yield (82%) with a high optical yield (ee 93%),[1)] and further the MOM group seemed to be inert to treatment with dissolving metal.

Swern oxidation of the C4-epoxide [(−)-**3**] followed by methylenation with methyl triphenylphosphorane furnished an allylic epoxide (**4**) in 71% yield. Treatment of the allylic epoxide (**4**) with sodium radical[4)] in liquid ammonia at −78 °C and subsequent quenching of the reaction with benzyl bromide provided an allyl benzyl ether (**5**) in 92% yield. The *E*-geometry of the double bond in **5** was determined by the coupling constant (J=15 Hz) between two olefinic proton signals in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum.

The allyl benzyl ether (**5**) was then subjected to oxidation by ozonized oxygen treatment at −78 °C followed by reduction of the ozonide with sodium borohydride to afford an optically active glycerol derivative (**6**) in 93% yield. It should be noted here that the three hydroxyl functions in the glycerol derivative (**6**) are mutually distinguishable due to their differences as regards protecting groups. In other words, **6** was considered a useful

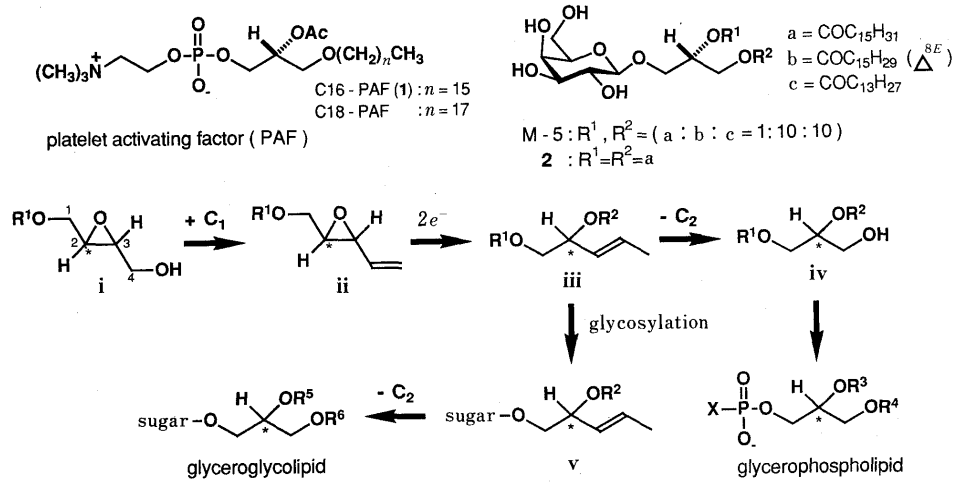

Chart 1

intermediate for the synthesis of glycerophospholipids having various acyl residues.

Treatment of **6** with potassium hydride and palmityl methanesulfonate in the presence of dibenzo-18-crown-6 and subsequent acidic hydrolysis gave in 87% yield a monoalkyl ether (**7**) which corresponded to the intermediate in the synthesis of PAF by Ohno and his group.[5] The monoalkyl ether (**7**) was then converted to a betaine compound (**8**)[5] in 59% overall yield through successive reactions which were carried out under somewhat modified conditions from those of Ohno's procedure: 1) phosphorylation with 2-bromoethyl phosphoryl dichloride and treatment with 2) triethylamine-H_2O, 3) trimethylamine, and 4) silver carbonate. Finally, **8** was transformed in a good yield into C16-PAF (**1**) by catalytic hydrogenation to remove the benzyl group and acetylation. The physical data including the optical rotation for C16-PAF thus prepared were identical with those reported in the literature.[5]

It seems likely that C18-platelet activating factor (C18-PAF) may also be synthesized *via* the present synthetic procedure by employing stearyl methanesulfonate in place of palmityl methanesulfonate. Furthermore, it is presumed that the antipodes of C16-PAF and C18-PAF may be synthesized by using (+)-**3**, an enantiomer of (−)-**3**, as the starting epoxide. The C4-epoxide (+)-**3** was synthesized previously in a good chemical yield (83%) with a high optical yield (ee 93%).[1]

Synthesis of a Palmitoyl Analogue of M-5 The allyl benzyl ether (**5**), used as the intermediate for the synthesis of C16-PAF (**1**), was hydrolyzed under acidic conditions to restore the primary hydroxyl group and subsequently was subjected to glycosylation with *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)trichloroacetimidate and boron trifluoride-etherate[6] to afford an acetylated β-D-galactoside (**9**) in a moderate yield. The β-configuration of the anomeric carbon in **9** was indicated by the ^{1}H-NMR coupling constant (J=8.0 Hz) of the anomeric proton. Then, the acetylated galactoside (**9**) was converted to a silylated galactoside (**10**) by alkaline hydrolysis and subsequent treatment with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDSCl) in the presence of pyridine.

The silylated galactoside (**10**) was derivatized, in 50% yield, into a palmitoyl analogue (**2**) of M-5 through the following reaction sequences: 1) ozonolysis followed by sodium borohydride reduction to generate a primary hydroxyl function, 2) catalytic hydrogenation to remove the benzyl group, thus restoring the secondary hydroxyl function, 3) treatment with palmitoyl chloride and triethylamine to introduce two palmitoyl residues, and finally 4) treatment with tetra-*n*-butylammonium fluoride to remove the silyl protecting group. The product (**2**) thus obtained was shown to move with the same *Rf* value on thin-layer chromatography (TLC) as the natural M-5 isolated from the marine sponge.[3]

In the above-described synthesis of the palmitoyl analogue (**2**) of M-5, two acyl (palmitoyl) residues were simultaneously introduced onto two hydroxyl functions

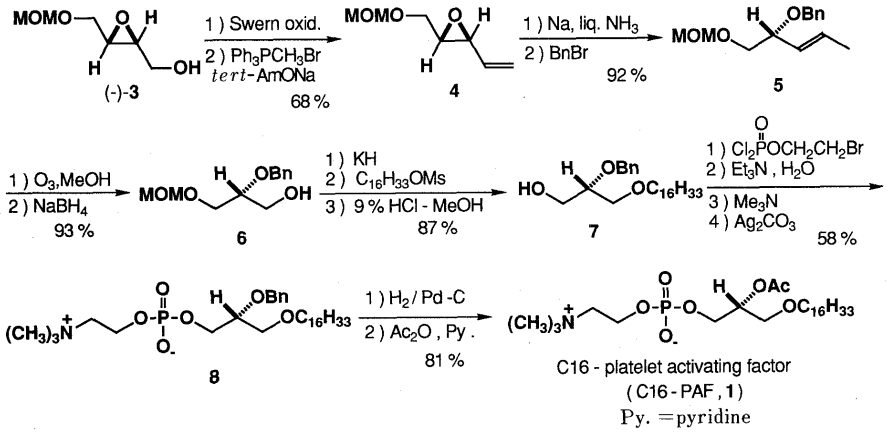

Chart 2

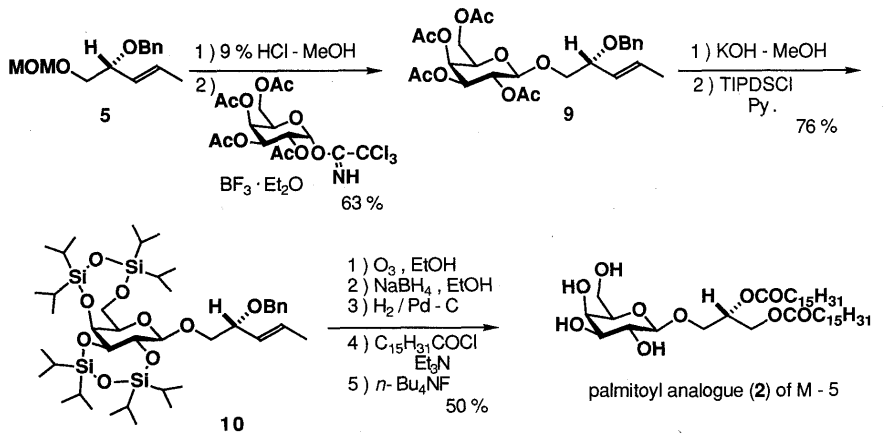

Chart 3

which were generated through successive removal of the C_2 unit from **10** by ozonolysis and of the benzyl group by catalytic hydrogenation. Provided that various acyl residues are introduced stepwise onto the newly generated hydroxyl functions [*e.g.* i) first after ozonolysis and then ii) after removal of the benzyl group], a variety of analogues of M-5 may be synthesized.

In conclusion, we have developed a new synthetic method using a chiral C4-epoxide for complex lipids (*i.e.* sphingoglycolipid,[1] glycerophospholipid and glyceroglycolipid) possessing arbitrary fatty acid residues or alkyl moieties. The present synthetic procedure seems to be versatile for synthesizing a variety of glycerophospholipids and glyceroglycolipids which may be of use for biochemical investigation of complex lipids.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as in our previous paper.[1]

Preparation of Allylic Epoxide (4) from Chiral C4-Epoxide [(−)-3] A solution of dimethyl sulfoxide (0.48 ml, 6.53 mmol) in dry CH_2Cl_2 (4.0 ml) was added to a solution of oxalyl chloride (0.44 ml, 4.90 mmol) in dry CH_2Cl_2 (4.0 ml) at −78 °C, and the whole mixture was stirred further at the same temperature for 15 min. A solution of C4-epoxide (−)-**3** (483 mg, 3.26 mmol) in dry CH_2Cl_2 (2.0 ml) was added, and the resulting mixture was stirred at −78 °C for a further 30 min. Then, triethylamine (2.4 ml, 16.3 mmol) was added at once and the whole was warmed slowly to room temperature. After addition of Et_2O (20 ml) to the reaction mixture, the whole was passed through a Florisil column. The eluate was concentrated under reduced pressure to give an aldehyde (528 mg). A solution of the aldehyde in dry tetrahydrofuran (THF) (5.0 ml) was added to methyl triphenylphosphorane reagent [prepared from 60% NaH (243 mg, 6.08 mmol), *tert*-AmOH (1.6 ml), triphenylphosphine methylbromide (2.4 g, 6.53 mmol) and dry THF (10 ml)] and the whole mixture was stirred at room temperature for 30 min. After addition of Et_2O (20 ml) to the reaction mixture, the whole was again passed through a Florisil column. The eluate was evaporated under reduced pressure to give a product (3.5 g). Purification of the product by column chromatography (SiO_2 60 g, *n*-hexane: EtOAc = 10 : 1) afforded **4** (334 mg, 2.23 mmol, 68% yield).

4: Colorless oil. $[\alpha]_D^{25}$ −26° (*c* = 2.4, MeOH). IR (film) cm^{-1}: 3095, 881. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 3.0—3.4 (2H, m, –HC(O)CH–), 3.38 (3H, s, OCH_3), 3.5—3.9 (2H, m, MOMO–CH_2–), 4.66 (2H, s, –OCH_2–OCH_3), 5.2—5.6 (3H, m, olefinic protons). FAB-MS *m/z*: 145 $(M+H)^+$. *Anal.* Calcd for $C_7H_{12}O_3$: C, 58.31; H, 8.39. Found: C, 58.29; H, 8.39.

Treatment of 4 with Sodium in Liquid NH_3 Followed by Benzylation Giving 5 A solution of **4** (95 mg, 0.66 mmol) in dry THF (2.0 ml) was added to a mixture of sodium (200 mg, 8.7 mmol) and liquid NH_3 (*ca.* 10 ml) at −78 °C. The mixture was stirred at −78 °C for 10 min, then treated with benzyl bromide (1.2 ml, 10.1 mmol) with stirring at the same temperature for a further 20 min. After removal of NH_3, the residue was extracted with EtOAc. The EtOAc extract was washed with brine, and dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a product (350 mg). Purification of the product by column chromatography (SiO_2 10 g, *n*-hexane: EtOAc = 15 : 1) afforded an allyl benzyl ether (**5**, 143 mg, 0.61 mmol, 92% yield).

5: Colorless oil. $[\alpha]_D^{25}$ −35° (*c* = 1.0, MeOH). IR (film) cm^{-1}: 1670, 970, 730. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.76 (3H, dd, *J* = 6.0, 1.0 Hz, –CH=CH–CH_3), 3.37 (3H, s, OCH_3), 3.62 (2H, d, *J* = 5.0 Hz, –O–CH_2–CH(OBn)–CH=), 3.8—4.1 (1H, m, –C–CH_2–CH(OBn)–CH=), 4.67 (2H, s, –OCH_2–OCH_3), 4.34, 4.72 (2H, ABq, *J* = 12.0 Hz, –CH_2–Ph), 5.43 (1H, ddq, *J* = 7.5, 15.0, 1.0 Hz, –CH=CH–CH_3), 5.79 (1H, dq, *J* = 15.0, 6.0 Hz, –CH=CH–CH_3), 7.35 (5H, br s, aromatic protons). FAB-MS *m/z*: 237 $(M+H)^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{14}H_{20}O_3$+H: 237.1491. Found: 237.1456 $(M+H)^+$.

Ozonolysis of 5 Followed by $NaBH_4$ Reduction Giving 6 A solution of **5** (100 mg, 0.42 mmol) in dry MeOH (5.0 ml) was bubbled with a stream of ozonized oxygen at −78 °C for 30 min. After removal of excess ozone by bubbling N_2 gas, $NaBH_4$ (20 mg, 0.53 mmol) was added to the residue and the whole mixture was stirred at room temperature for 30 min. Work-up of the reaction mixture in a usual manner gave a product (105 mg). Purification of the product by column chromatography (SiO_2 5 g, *n*-hexane: EtOAc = 2 : 1) afforded **6** (89 mg, 0.39 mmol, 93% yield).

6: Colorless oil. $[\alpha]_D^{24}$ +1.8° (*c* = 1.2, MeOH). IR (film) cm^{-1}: 3450, 735, 693. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 3.3—3.6 (5H, m, –O–CH_2–CH(OBn)–CH_2–OH), 3.38 (3H, s, OCH_3), 4.58, 4.79 (2H, ABq, *J* = 12.0 Hz, –CH_2–Ph), 4.64 (2H, s, –OCH_2–OCH_3), 7.35 (5H, br s, aromatic protons). FAB-MS *m/z*: 249 $(M+Na)^+$, 227 $(M+H)^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{12}H_{18}O_4$+H: 227.1283. Found: 227.1280 $(M+H)^+$.

Preparation of 7 from 6 Palmityl methanesulfonate (602 mg, 1.87 mmol) and dibenzo-18-crown-6 (one microspatula-full) were added at room temperature to a stirred solution of **6** (352 mg, 1.56 mmol) and KH (95 mg, 2.38 mmol) in dry THF (15 ml), and the reaction mixture was stirred further at room temperature for 7 h. After quenching of the reaction with aqueous saturated NH_4Cl, the whole was extracted with EtOAc. The EtOAc extract was washed with brine, and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave an alkyl ether (1.24 g). The alkyl ether was treated with 9% HCl–MeOH (1.5 ml) at room temperature for 2 h. After neutralization with Ag_2CO_3 powder, the precipitate was removed by filtration. The solvent was evaporated off from the filtrate under reduced pressure to give a product (1.18 g). Purification of the product by column chromatography (SiO_2 20 g, *n*-hexane : EtOAc = 10 : 1) afforded a mono alkyl ether (**7**, 624 mg, 1.36 mmol, 87% yield).

7: Colorless oil. $[\alpha]_D^{24}$ −1.4° (*c* = 0.9, MeOH). IR (film) cm^{-1}: 3450, 731, 696. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.89 (3H, t, *J* = 6.0 Hz, CH_3), 1.27 (28H, br s, $CH_2 \times 14$), 3.45 (2H, t, *J* = 6.0 Hz, –OCH_2–), 3.5—3.7 (5H, m, HO–CH_2–CH(OBn)–CH_2–O–), 4.52, 4.69 (2H, ABq, *J* = 11.0 Hz, –CH_2–Ph), 7.35 (5H, br s, aromatic protons). FAB-MS *m/z*: 429 $(M+Na)^+$, 407 $(M+H)^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{26}H_{46}O_3$+H: 407.3468. Found: 407.3505 $(M+H)^+$.

Preparation of 8 from 7 A solution of Et_3N (0.06 ml, 0.41 mmol) and 2-bromoethyl phosphoryl dichloride (85 mg, 0.39 mmol) in dry Et_2O (1.6 ml) was added to a solution of **7** (145 mg, 0.36 mmol) in dry Et_2O (3.2 ml), and the whole mixture was stirred at room temperature for 8 h. After addition of Et_3N (0.16 ml) and H_2O (0.03 ml) to the reaction mixture, the whole was heated under reflux for 2 h. After cooling, the reaction mixture was acidified with 5% aqueous HCl and extracted with Et_2O. The Et_2O extract was washed with brine and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product (226 mg), which was purified by column chromatography (SiO_2 8 g, $CHCl_3$: MeOH = 6 : 1) to afford a phosphoryl compound (182 mg, 0.31 mmol). The phosphoryl compound was dissolved in $CHCl_3$–iso-PrOH–DMF (3 : 5 : 5, 3.2 ml) and treated in an ice-water bath with Me_3N gas [prepared from $Me_3N \cdot HCl$ (2.91 g, 3.14 mmol), NaOH (1.26 g, 3.14 mmol) and H_2O (16 ml)]. The resulting mixture was heated at 50 °C for 2 h, then allowed to cool, and Ag_2CO_3 powder (60 mg, 0.23 mmol) was added. The reaction mixture was heated again under reflux for 1 h. After cooling, the precipitate was removed by filtration. The filtrate was concentrated under reduced pressure to give a product (205 mg). Purification of the product by column chromatography (SiO_2 2 g, $CHCl_3$: MeOH = 1 : 1 → 3 : 7) afforded a betaine compound (**8**, 120 mg, 0.21 mmol, 58% yield).

8: A white amorphous solid. $[\alpha]_D^{24}$ +2.0° (*c* = 0.6, MeOH). IR ($CHCl_3$) cm^{-1}: 3390, 3070, 3040, 1660. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.83 (3H, t, *J* = 7.0 Hz, $-CH_3$), 1.21 (28H, br s, $CH_2 \times 14$), 3.10 (9H, s, $-\overset{+}{N}(CH_3)_3$), 3.70 (11H, m, –O–CH_2–CH(OBn)–CH_2–$OC_{16}H_{33}$, $-OCH_2-$, $=\overset{+}{N}-CH_2CH_2-O-$), 4.60 (2H, s, –$CH_2$–Ph), 7.26 (5H, m, aromatic protons). FAB-MS *m/z*: 572 $(M+H)^+$. *Anal.* Calcd for $C_{31}H_{58}NO_6P$: C, 65.12; H, 10.22; N, 2.45. Found: C, 65.20; H, 10.22; N, 2.41.

Preparation of C16-Platelet Activating Factor (1) from 8 A solution of **8** (80 mg, 0.14 mmol) in MeOH (8 ml) containing 10% Pd–C (25 mg) was hydrogenated at room temperature for 8 h. The catalyst was removed by filtration, and the solvent was evaporated from the filtrate under reduced pressure to give a debenzylated product (81 mg). The debenzylated product was treated with Ac_2O (0.5 ml) and pyridine (1.0 ml) at room temperature for 10 h. Work-up of the reaction mixture in a usual manner gave a product (104 mg). Purification of the product by column chromatography (SiO_2 1 g, $CHCl_3$: MeOH : NH_4OH = 10 : 5 : 1) furnished C16-platelet activating factor (**1**, 59 mg, 0.11 mmol, 81% yield).

1: A white amorphous solid. $[\alpha]_D^{24}$ −3.2° (*c* = 1.2, $CHCl_3$: MeOH =

1 : 1). IR ($CHCl_3$) cm^{-1}: 2980, 2860, 1745. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.89 (3H, t, J=7.0 Hz, CH_3), 1.28 (26H, m, $CH_2 \times 13$), 1.53 (2H, m, CH_2), 2.07 (3H, s, $COCH_3$), 3.21 (9H, s, $-\overset{+}{N}(CH_3)_3$), 3.45 (2H, t, J=7.0 Hz, $-O-CH_2-CH_2-$), 3.59 (2H, d, J=5.0 Hz), 3.46 (2H, m) ($-O-CH_2-CH(OAc)-CH_2-O-C_{16}H_{33}$), 4.00, 4.26 (2H each, both m, $N-CH_2CH_2-O-$), 5.13 (1H, quintet, J=5.0 Hz, $-CH(OAc)-$). FAB-MS m/z: 524 $(M+H)^+$. High-resolution FAB-MS m/z: Calcd for $C_{26}H_{54}NO_7P$: 524.3716. Found: 524.3741 $(M+H)^+$.

Preparation of 9 from 5 The allyl benzyl ether (**5**, 406 mg, 1.72 mmol) was treated with 9% HCl–MeOH (2.0 ml) at room temperature for 2 h. After neutralization with Ag_2CO_3 powder, the precipitate was removed by filtration. The solvent was evaporated under reduced pressure from the filtrate to give a product (380 mg). Purification of the product by column chromatography (SiO_2 10 g, *n*-hexane : EtOAc = 4 : 1) afforded a demethoxymethyl compound (304 mg, 1.58 mmol). A solution of the demethoxymethyl compound in dry CH_2Cl_2 (75 ml) was treated with molecular sieves 4A (11 g) and *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)trichloroacetimidate (1.01 g, 2.05 mmol). The mixture was stirred at −30 °C for 10 min, then BF_3–Et_2O (0.06 ml, 0.49 mmol) was added. The whole mixture was stirred further at the same temperature for 40 min. The reaction mixture was poured into ice-water and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product (1.6 g). Purification of the product by column chromatography (SiO_2 30 g, *n*-hexane : EtOAc = 5 : 2) afforded an acetylated galactoside (**9**, 566 mg, 1.08 mmol, 63% yield).

9: Colorless oil. $[\alpha]_D^{23}$ +12.5° (c=1.2, MeOH). IR (film) cm^{-1}: 1735. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.97, 2.00, 2.05, 2.16 (3H, each, all s, $COCH_3 \times 4$), 1.75 (1H, d, J=6.5 Hz, $-CH=CH-CH_3$), 3.62 (1H, dd, J=8.0, 11.0 Hz), 3.86 (1H, dd, J=3.0, 11.0 Hz) ($-O-CH_2-CH(OBn)-CH=$), 3.89 (1H, t, J=7.0 Hz, 5′-H), 4.02 (1H, ddd, J=3.0, 7.5, 8.0 Hz, $-O-CH_2-CH(OBn)-CH=$), 4.16 (2H, d, J=7.0 Hz, 6′-H_2), 4.43, 4.60 (1H, each, both d, J=12.0 Hz, $-CH_2-Ph$), 4.64 (1H, d, J=8.0 Hz, 1′-H), 5.02 (1H, dd, J=3.0, 10.5 Hz, 3′-H), 5.26 (1H, dd, J=8.0, 10.5 Hz, 2′-H), 5.36 (1H, dd, J=15.5, 7.5 Hz, $-CH=CH-CH_3$), 5.40 (1H, d, J=3.0 Hz, 4′-H), 5.76 (1H, m, $-CH=CH-CH_3$), 7.3–7.4 (5H, m, aromatic protons). FAB-MS m/z: 523 $(M+H)^+$. *Anal.* Calcd for $C_{26}H_{34}O_{11}$: C, 59.76; H, 6.56. Found: C, 59.71; H, 6.52.

Preparation of 10 from 9 A solution of the acetylated galactoside (**9**, 88 mg, 0.17 mmol) in dry MeOH (8.0 ml) was treated with 10% KOH–MeOH (0.8 ml) at 0 °C for 1 h. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. The solvent was evaporated under reduced pressure from the filtrate to give a deacetylated product (60 mg). The deacetylated product was dissolved in pyridine (1.0 ml) and the whole mixture was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.16 ml, 0.51 mmol) at room temperature for 10 min. Work-up of the reaction mixture in a usual manner gave a product (205 mg). Purification of the product by column chromatography (SiO_2 10 g, *n*-hexane : EtOAc = 40 : 1) afforded **10** (107 mg, 0.13 mmol, 76% yield).

10: Colorless oil. $[\alpha]_D^{24}$ +9.4° (c=1.1, MeOH). IR (film) cm^{-1}: 1240. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.06 (56H, br s, $Si-CH(CH_3)_2 \times 8$), 1.72 (3H, d, J=6.5 Hz, $-CH=CH-CH_3$), 3.48 (1H, dd, J=6.0, 10.0 Hz), 3.66 (1H, dd, J=7.0, 10.0 Hz) ($-O-CH_2-CH(OBn)-CH=$), 3.67 (1H, t, J=9.0 Hz, 5′-H), 3.7—3.9 (4H, m, 2′-H, 3′-H, 6′-H_2), 3.99 (1H, ddd, J=6.0, 7.0, 7.5 Hz, $-CH(OBn)-$), 4.20 (1H, d, J=3.0 Hz, 4′-H), 4.31 (1H, d, J=7.0 Hz, 1′-H), 4.47, 4.59 (1H, each, both d, J=12.0 Hz, $-CH_2-Ph$), 5.45 (1H, dd, J=7.5, 15.0 Hz, $-CH=CH-CH_3$), 5.72 (1H, dq, J=15.0, 6.5 Hz, $-CH=CH-CH_3$), 7.30 (5H, br s, aromatic protons). FAB-MS m/z: 861 $(M+Na)^+$, 839 $(M+H)^+$. High-resolution FAB-MS m/z: Calcd for $C_{42}H_{78}O_9Si_4$+H: 839.4801. Found: 839.4816 $(M+H)^+$.

Preparation of a Palmitoyl Analogue (2) of M-5 from 10 A solution of **10** (100 mg, 0.12 mmol) in absolute EtOH (5.0 ml) was treated with ozonized oxygen at −78 °C for 30 min. After removal of excess ozonized oxygen with an N_2 stream, $NaBH_4$ (6 mg, 0.16 mmol) was added to the mixture, and the whole was stirred at room temperature for 30 min. Work-up of the reaction mixture in a usual manner gave a primary alcohol (105 mg). A solution of the primary alcohol in 95% EtOH (4.0 ml) containing 10% Pd–C (20 mg) was hydrogenated at room temperature for 5 h. The catalyst was removed by filtration. The solvent was evaporated under reduced pressure from the filtrate to give a product (110 mg). Purification of the product by column chromatography (SiO_2 1 g, *n*-hexane : EtOAc = 10 : 1) afforded a diol (70 mg, 0.09 mmol). A solution of the diol in dry THF (1.0 ml) was treated with palmitoyl chloride (0.08 ml, 0.28 mmol) and Et_3N (0.13 ml, 0.95 mmol) at room temperature for 1 h. Work-up of the reaction mixture in a usual manner gave a product (150 mg), which was purified by column chromatography (SiO_2 2 g, *n*-hexane : EtOAc = 40 : 1) to afford the dipalmitate (85 mg, 0.07 mmol). Then the dipalmitate, dissolved into dry THF (1.0 ml), was treated with tetra-*n*-butylammonium fluoride (114 mg, 0.44 mmol) at room temperature for 5 h. After addition of pyridine (3 drops) to the reaction mixture, the whole was concentrated under reduced pressure to afford a product (198 mg). Purification of the product by column chromatography (SiO_2 2 g, $CHCl_3$: MeOH = 10 : 1) furnished a palmitoyl analogue of M-5 (**2**, 45 mg, 0.06 mmol, 50% yield).

2: Colorless needles, mp 61.5—62.0 °C (MeOH). $[\alpha]_D^{24}$ +2.3° (c=0.5, EtOH). IR (KBr) cm^{-1}: 3360, 2915, 1725. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.89 (6H, t, J=7.0 Hz, $CH_3 \times 2$), 1.26 (48H, br s, $CH_2 \times 24$), 1.59 (4H, br s, $CH_2 \times 2$), 2.32, 2.34 (2H each, both t, J=7.3 Hz, $-OCOCH_2- \times 2$), 2.72 (2H, br s, OH × 2), 2.92 (1H, br s, OH), 3.01 (1H, br s, OH), 3.5—3.7 (3H, m, galactosyl moiety), 3.76 (1H, dd, J=6.0, 11.0 Hz, Gal–O–$CH_a(H_b)-$), 3.8—3.9 (1H, m, galactosyl moiety), 3.93 (1H, dd, J=6.0, 11.0 Hz, Gal–O–$CH_a(H_b)-$), 3.9—4.0 (2H, m, galactosyl moiety), 4.22 (1H, dd, J=6.5, 12.0 Hz, $-CH_a(H_b)-O-CO-$), 4.29 (1H, d, J=8.0 Hz, 1′-H), 4.40 (1H, dd, J=3.0, 12.0 Hz, $-CH_a(H_b)-O-CO-$), 5.33 (1H, m, $-CH(OCOC_{15}H_{31})-$). FAB-MS m/z: 753 $(M+Na)^+$, 731 $(M+H)^+$. High-resolution FAB-MS m/z: Calcd for $C_{41}H_{78}O_{10}$+H: 731.5674. Found: 731.5663 $(M+H)^+$.

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