Glycolipids from the Formosan Soft Coral *Lobophytum crassum*

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Three glycolipids (1—3), possessing a sugar moiety at C-2 of glycerol ether, have been isolated from the Formosan soft coral *Lobophytum crassum*. Their structures were elucidated by spectroscopic methods, particularly in 1D- and 2D-NMR experiments. The absolute configurations on the sugar portion and lipid aglycon of 1—3 were determined by methanolysis, chemical transformation and the application of Mosher's method on 1 and 3. Compounds 1—3 exhibited weak cytotoxic activities.

Key words *Lobophytum crassum*; glycolipid; coral; *R*-batyl alcohol; *R*-chimyl alcohol; arabinopyranoside

During the course of our investigation on the bioactive chemical constituents from marine invertebrates,[1—6] we have investigated a soft coral *Lobophytum crassum* collected from Taiwanese waters. Earlier studies of the genus *Lobophytum* have led to the isolation of terpenoids, of which some have shown cytotoxic,[7—10] anti-HIV,[11] and antibacterial[12] activities. In this paper we report the isolation and structural elucidation of three new glycolipids **1**—**3** (Fig. 1) from this organism. The absolute configurations of **1**—**3** were determined by the comparison of specific optical rotations of sugar and aglycon moieties with known compounds, chemical conversions, and the application of Mosher's method. Cytotoxicity of metabolites **1**—**3** against the growth of a limited panel of cancer cells of HepG2, Hep3B (human liver carcinoma), MDA-MB-231 (human breast carcinoma), and Ca9-22 (human gingival carcinoma) is also discussed.

Results and Discussion

(2*R*)-1-Hydroxy-3-hexadecyloxy-propyl-β-D-arabinopyranoside (**1**) was isolated as amorphous white solid. Its HR-ESI-MS exhibited a pseudomolecular ion peak at *m/z* 471.3296 $[M+Na]^+$ (Calcd for $C_{24}H_{48}O_7Na$, 471.3298), corresponding to the molecular formula $C_{24}H_{48}O_7$, indicating one degree of unsaturation. The 1 : 2 ratio of carbon and proton atoms and high oxygen content in its molecular formula and strong absorption bands at ν_{max} 3391, 1142, 1075, and 1007 cm^{-1} in IR spectrum suggested that **1** might be a glycolipid. An anomeric glycoside proton resonance was observed at δ 5.67 (1H, d, J=3.3 Hz), corresponding to a carbon resonance at δ 100.4 (d) in the HMQC spectrum. Except for the above carbon (C-1′), the ^{13}C-NMR spectrum of **1** exhibited eight oxygenated carbons, including four oxymethylenes at δ_C 71.7 (t), 71.1 (t), 64.5 (t), and 63.0 (t), and four oxymethines at δ_C 78.5 (d), 71.0 (d), 70.8 (d), and 70.1 (d) (Table 1). Above data together with the methyl group resonanting at δ_H 0.85 (3H, t, J=6.6 Hz) and methylenes at δ_H 1.26 (br s) revealed the structure of **1** to be a glycoside with a long aliphatic chain.

The gross structure of metabolite **1** was further established by the 2D-NMR studies, particularly in ^{1}H–^{1}H COSY, HMQC and HMBC experiments. The correlations of ^{1}H–^{1}H COSY revealed proton–proton sequences, from H-1′ to H-5′, H_2-1 to H_2-3, H_2-1″ to H_2-2″, and H_2-15″ to H_3-16″, as shown in Fig. 2. The HMBC correlations from H-1′ to C-5′ and C-2, as well as other correlations illustrated in Fig. 2, established a pyranose and a glycerol ether moiety. The pyranose was identified as β-arabinopyranose by analysis of coupling constants of the related protons (Table 1). HR-ESI mass spectrum and the above 2D-NMR spectroscopic analysis led to the establishment of the aglycon to be chimyl alcohol.

The absolute configurations on the sugar portion and lipid aglycon were determined on the basis of methanolysis, chemical transformation and the application of the modified Mosher's method. As shown in Chart 1, methanolysis of **1** with 1 N $HCl_{(aq)}$–MeOH (1 : 1) yielded the methyl arabinopyranoside and the chimyl alcohol. The glycerol-based portion was found to be 2*R*-chimyl alcohol, $[\alpha]_D^{22}$ −2°.[13] The sugar-containing portion was further treated with 1 N $HCl_{(aq)}$ and the sugar thus obtained was shown to be (−)-D-arabinose,

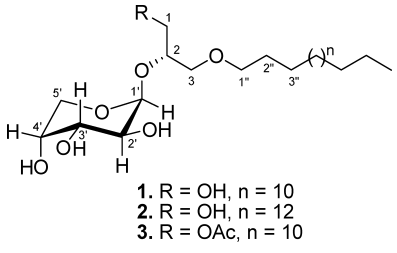

Fig. 1. Structures of Metabolites **1**—**3**

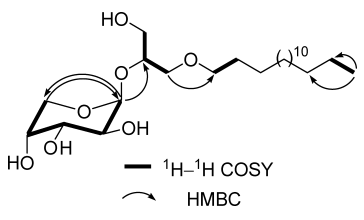

Fig. 2. Selective ^{1}H–^{1}H COSY and HMBC Correlations of **1**

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **1**—**3**

C #	**1**		**2**		**3**	
	^{1}H[a)]	^{13}C[b)]	^{1}H[a)]	^{13}C[b)]	^{1}H[a)]	^{13}C[b)]
1	4.14 br s	63.0 (t)[d)]	4.14 br s	63.0 (t)	4.55 dd (11.4, 3.9) 4.46 dd (11.4, 6.6)	65.1 (t)
2	4.37 m	78.5 (d)	4.37 m	78.5 (d)	4.36 m	74.9 (d)
3	3.89 dd (10.2, 4.2)[c)] 3.85 dd (10.2, 5.4)	71.1 (t)	3.89 dd (10.2, 4.2) 3.85 dd (10.2, 5.4)	71.1 (t)	3.75 dd (10.2, 5.4) 3.67 dd (10.2, 5.4)	70.2 (t)
1′	5.67 d (3.3)	100.4 (d)	5.67 d (3.3)	100.4 (d)	5.55 d (3.6)	100.7 (d)
2′	4.62 dd (9.0. 3.3)	70.8 (d)	4.62 dd (9.0. 3.3)	70.8 (d)	4.61 dd (9.0. 3.6)	70.5 (d)
3′	4.50 dd (9.0, 3.3)	71.0 (d)	4.50 dd (9.0, 3.3)	71.0 (d)	4.47 m	70.9 (d)
4′	4.36 br s	70.1 (d)	4.36 br s	70.1 (d)	4.40 br s	70.2 (d)
5′	4.47 dd (12.0, 1.5) 4.07 dd (12.0, 2.7)	64.5 (t)	4.47 dd (12.0, 1.5) 4.07 dd (12.0, 2.7)	64.5 (t)	4.36 dd (12.0, 1.8) 4.09 dd (12.0, 2.4)	64.5 (t)
1″	3.46 t (6.3)	71.7 (t)	3.46 t (6.3)	71.7 (t)	3.43 t (6.3)	71.7 (t)
2″	1.54 m	30.1 (t)	1.54 m	30.1 (t)	1.55 m	30.0 (t)
3″	1.26 m	26.4 (t)	1.26 m	26.4 (t)	1.26 m	26.4 (t)
4″—13″	1.26 br s	29.6—30.0 (t)	1.26 br s	29.6—30.0 (t)	1.26 br s	29.6—30.0 (t)
14″	1.26 br s	32.1 (t)	1.26 br s	29.6—30.0 (t)	1.26 br s	32.1 (t)
15″	1.26 br s	22.9 (t)	1.26 br s	29.6—30.0 (t)	1.26 br s	22.9 (t)
16″	0.85 t (6.6)	14.2 (q)	1.26 br s	32.1 (t)	0.86 t (6.6)	14.3 (q)
17″			1.26 br s	22.9 (t)		
18″			0.85 t (6.6)	14.2 (q)		
OAc					2.02 s	20.7 (q) 170.7 (s)

a) Spectra recorded at 300 MHz in pyridine-d_5. *b*) Spectra recorded at 75 MHz in pyridine-d_5. *c*) *J* values (in Hz) in parentheses. *d*) Multiplicity deduced by DEPT and indicated by usual symbols.

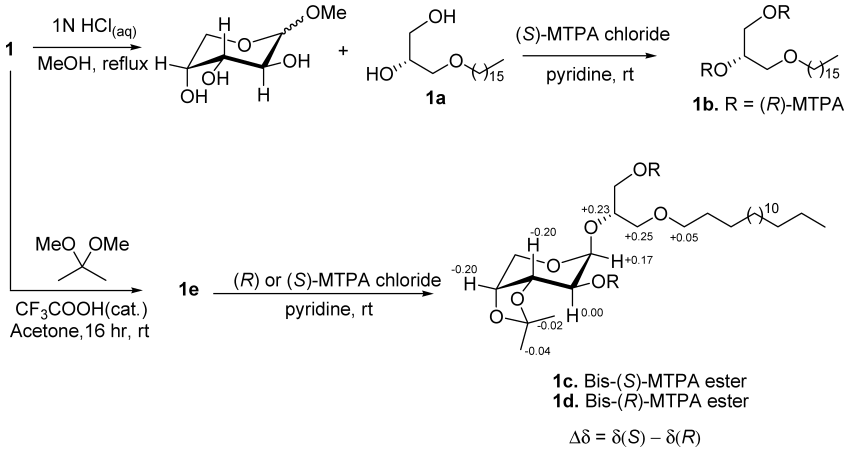

Chart 1. Chemical Conversions from **1** to **1a**—**e** and Chemical Shift Differences of MTPA Esters **1c** and **1d**

$[\alpha]_D^{22}$ −110°,[14)] by comparison of specific optical rotation and TLC analysis with the authentic sample. The absolute configuration of chimyl alcohol (**1a**) was further confirmed by the calculation of the chemical shift difference of two diastereotopic protons of H_2-1 in bis-(*R*)-MTPA ester (**1b**), which was prepared from **1a** with (*S*)-MTPA chloride. Previous study has shown that the chemical shift difference of two diastereotopic protons of H_2-1, resonating as two doublets of doublets, in 2*S*-glycerol ether was 0.26 ppm, while that in 2*R*-glycerol ether was 0.31 ppm.[15)] In the case of **1b**, the above two protons were found to resonate at δ 4.73 and 4.42 (Δ=0.31 ppm), indicating *R* configuration at C-2, the same as that deduced by specific optical rotation. The absolute configuration of the sugar moiety was also determined by the application of Mosher's method[16,17)] on the acetonide derivative **1e**. The chemical shift differences of (*S*)-MTPA ester (**1c**) and (*R*)-MTPA ester (**1d**) were summarized in Chart 1 and indicated the *S*-configuration at C-2′; hence, the sugar moiety of **1** was deduced as D-arabinose.

(2*R*)-1-Hydroxy-3-octadecyloxy-propyl-β-D-arabinopyranoside (**2**) had the molecular formula of $C_{26}H_{52}O_7$, 28 mass units higher than that of **1**, as determined by HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectral data of **2** were found to be very similar with those of **1**. Compound **2** is more strongly retained by the reverse stationary phase in ODS column than **1**, implying that **2** might possess longer aliphatic chain. Inspection of 2D-NMR spectral data of **2** allowed the establishment of the same planar structure as that of **1**, with the slight difference of aliphatic chain length at 3-*O* position. Hence, the glycerol moiety of **2** was derived from batyl alcohol. The absolute stereochemistry of **2** was suggested to be the same as that of **1** due to the biogenetic consideration as well as the

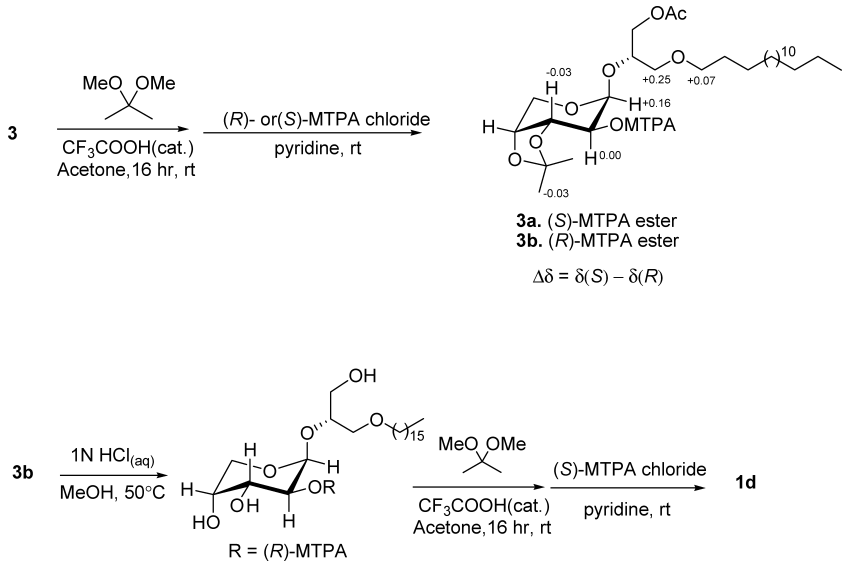

Chart 2. Chemical Conversions from **3** to **1d** and Chemical Shift Differences of MTPA Esters **3a** and **3b**

Table 2. Cytotoxicity Data of Compounds **1**—**3**

Compound	Cell lines IC_{50} (μg/ml)			
	Hep G2	Hep 3B	MDA-MB-231	Ca9-22
1	10.8	12.7	14.5	12.0
2	9.2	9.7	11.1	15.0
3	11.3	9.7	12.4	9.5
Doxorubicin	0.2	0.2	0.2	0.1

same sign of specific optical rotations, and similarity of NMR spectral data.

(2*R*)-1-Acetoxy-3-hexadecyloxy-propyl-β-D-arabinopyranoside (**3**) was obtained as a white powder. Its HR-ESI-MS exhibited a molecular ion peak at *m/z* 513.3404 $[M+Na]^+$ and established a molecular formula of $C_{26}H_{50}O_8$, corresponding to two degrees of unsaturation. The IR spectrum of **3** revealed the presence of hydroxyl (ν_{max} 3418 cm^{-1}) and ester (ν_{max} 1741 cm^{-1}) moieties. The ester was identified as an acetoxyl from the ^{1}H-NMR data at δ 2.02 (3H, s) and the ^{13}C-NMR data at δ 20.7 (q) and 170.7 (s) (Table 1). The attaching of acetoxy group at C-1 was confirmed by the HMBC correlation from H_2-1 to the acetate carbonyl carbon. Therefore, **3** was identified as a C-1 acetoxy derivative of **1**.

To establish the absolute configuration on C-2′ of the sugar moiety of **3**, the modified Mosher's method was applied on the acetonide derivative of **3** (Chart 2). As shown in Chart 2, the chemical conversion from **3** to **1d** through methanolysis of **3b** at 50 °C followed by the treatment with 2,2-dimethoxypropane and then (*S*)-MTPA chloride in pyridine to obtain **1d** demonstrated that both **1** and **3** possess the same absolute configurations at all chiral centers. The chemical shift differences of (*S*)-MTPA ester (**3a**) and (*R*)-MTPA ester (**3b**) were summarized in Chart 2 and also suggested the *S*-configuration at C-2′, the same as that of **1**.

The cytotoxicity of compounds **1**—**3** against HepG2, Hep3B, MDA-MB-231, and Ca9-22 cancer cells was shown in Table 2. It was found that all of **1**—**3** showed cytotoxic activities toward the above cancer cell lines with IC_{50}'s ranged from 9.2 to 15.0 μg/ml.

Experimental

General Procedure Optical rotations were measured on a Jasco P-1020 polarimeter. IR spectra were recorded on Jasco FT/IR-4100 fourier transform infrared spectrophotometer. The NMR spectra were recorded on Varian Mercury-Plus 300 FT-NMR instruments at 300 MHz for ^{1}H, and 75 MHz for ^{13}C in pyridine-d_5, and chemical shifts are referenced to δ_C 135.5 ppm and δ_H 8.71 ppm. Nuclear Overhauser and exchange spectroscopy (NOESY), ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) experiments were performed by using standard Varian pulse sequences. LR-MS and HR-MS were obtained by ESI on a Bruker APEX II mass spectrometer. Silica gel 60 (Merck, 230—400 mesh) was used for column chromatography. Precoated Silica gel plates (Merck Kieselgel 60 F_{254} 0.2 mm) were used for analytical TLC. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-10AT $_{VP}$ apparatus equipped with a Shimadzu SPD-10A $_{VP}$ UV detector and with a Purospher® STAR RP-18e column (250×10 mm, 5 μm). (−)-D-Arabinose was purchased from Aldrich.

Animal Material The soft coral *L. crassum* was collected by hand using scuba at the coast of Kenting, in January, 2004, at a depth of 10 m, and was stored in a freezer until extraction. A voucher specimen was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University (specimen no. 200401-9).

Extraction and Isolation The soft coral *L. crassum* (1.1 kg fresh wt.) was collected and freeze-dried. The freeze-dried material was minced and extracted exhaustively with EtOH (3×2 l). The organic extract was concentrated to an aqueous suspension and was further partitioned between EtOAc and water. The EtOAc extract (23 g) was fractionated by open column chromatography on silica gel using *n*-hexane–EtOAc and EtOAc–MeOH mixtures of increasing polarity. A fraction eluted with EtOAc–MeOH (4 : 1) was subjected to separation by a Sephadex LH-20 column (2×90 cm) using acetone and followed by reverse phase HPLC (acetone–H_2O, 1 : 4) to afford compounds **1**—**3** (24.6, 14.0, 13.2 mg, respectively).

(2*R*)-1-Hydroxy-3-hexadecyloxy-propyl-β-D-arabinopyranoside (**1**): Amorphous white solid; $[\alpha]_D^{22}$ −26° (*c*=2.46, MeOH); IR (KBr) ν_{max} 3391, 2919, 2851, 1142, 1075, 1007 cm^{-1}; for ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 471 $[M+Na]^+$, HR-ESI-MS *m/z* 471.3296 $[M+Na]^+$ (Calcd for $C_{24}H_{48}O_7Na$, 471.3298).

(2*R*)-1-Hydroxy-3-octadecyloxy-propyl-β-D-arabinopyranoside (**2**): Amorphous white solid; $[\alpha]_D^{22}$ −25° (*c*=1.40, MeOH); IR (KBr) ν_{max} 3391, 2921, 2853, 1142, 1075, 1007 cm^{-1}; for ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 499 $[M+Na]^+$, HR-ESI-MS *m/z* 499.3609 $[M+Na]^+$ (Calcd for $C_{26}H_{52}O_7Na$, 499.3611).

(2*R*)-1-Acetoxy-3-hexadecyloxy-propyl-β-D-arabinopyranoside (**3**):

Amorphous white solid; $[\alpha]_D^{22}$ −20° (c=1.32, MeOH); IR (KBr) ν_{max} 3418, 1741, 1238, 1142, 1079, 1007 cm^{-1}; for ^1H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS m/z 513 $[M+Na]^+$, HR-ESI-MS m/z 513.3404 $[M+Na]^+$ (Calcd for $C_{26}H_{50}O_8Na$, 513.3403).

Acid Hydrolysis of 1 To a mixture of **1** (5.0 mg) and MeOH (0.3 ml) was added 0.3 ml of 1 N $HCl_{(aq)}$. The reaction was carried out under refluxing for 16 h. The reaction mixture was then cooled to room temperature and extracted with *n*-hexane. Hexane layer was concentrated and chromatographed over silica gel using *n*-hexane–CH_2Cl_2 (1 : 2) as eluent to obtain chimyl alcohol (2.5 mg, 68%), $[\alpha]_D^{22}$ −2° (c=0.25, $CHCl_3$). The MeOH–water layer, containing methyl arabinoside (mixture of α- and β-anomers), was evaporated to dryness and followed by treated with 1 N HCl at 190 °C for 1.5 h. Then, the aqueous layer was concentrated under reduce pressure and the residue was chromatographied over silica gel using $CHCl_3$–acetone–MeOH–H_2O (8 : 2 : 3 : 1) as eluent to yield (−)-D-arabinose (0.4 mg, 23%), $[\alpha]_D^{22}$ −110° (c=0.040, H_2O), which was confirmed by co-TLC analysis with authentic sample ($CHCl_3$–acetone–MeOH–H_2O, 6 : 2 : 4 : 1, *Rf* 0.47).

Preparation of Bis-(*R*)-MTPA Ester (1b) To a solution of chimyl alcohol (1.0 mg) in dry pyridine (0.4 ml) was added (*S*)-MTPA chloride (20 μl), and the solution was allowed to stand overnight at room temperature for 16 h. The reaction was quenched by the addition of 1.0 ml of water, followed by extraction with CH_2Cl_2 (3×1 ml). The CH_2Cl_2-soluble layers were combined, dried over anhydrous $MgSO_4$ and evaporated. The residue was subjected to short silica-gel column using *n*-hexane–CH_2Cl_2 (1 : 1) to yield bis-(*R*)-MTPA ester (**1b**) (0.2 mg, 8%) in trace amount and the C-1 substituted mono-(*R*)-MTPA ester was the major product (1.5 mg, 89%). ^1H-NMR ($CDCl_3$) of **1b**: δ_H 7.33—7.49 (10H, m, 2×Ph), 5.43 (1H, m, H-2), 4.73 (1H, dd, J=12.3, 2.8 Hz, H-1a), 4.42 (1H, dd, J=12.3, 6.3 Hz, H-1b), 3.49 (2H, m, H-3), 3.48 (3H, s, OCH_3), 3.40 (3H, s, OCH_3), 3.30 (2H, t, J=6.6 Hz, H_2-1″), 1.26 (28H, br s, H_2-2″—H_2-15″), 0.88 t (3H, t, J=6.6 Hz, H-16″); ^1H-NMR ($CDCl_3$) of mono-(*R*)-MTPA ester: δ_H 7.40—7.55 (5 H, m, Ph), 4.38 (2H, d, J=5.0 Hz, H_2-1), 4.03 (1H, m, H-2), 3.57 (3H, s, OCH_3), 3.36—3.49 (4H, m, H_2-3, H_2-1″), 1.26 (28H, br s, H_2-2″—H_2-15″), 0.88 t (3H, t, J=6.6 Hz, H-16″).

Preparation of MTPA Esters 1c, 1d, 3a and 3b To a solution of **1** (2 mg) in dry acetone (0.3 ml) was added 1,2-dimethoxypropane (0.3 ml) and catalytic amount of CF_3COOH, and the reaction mixture was stirred at room temperature for 16 h. The mixture was concentrated and divided into two equal portions. One portion was converted to (*S*)-MTPA ester (**1c**) (1.5 mg, 73%) with (*R*)-MTPA chloride (20 μl) and the other was converted to (*R*)-MTPA ester (**1d**) (2.0 mg, 97%) with (*S*)-MTPA chloride (20 μl) according to the procedure of the preparation of bis-(*R*)-MTPA ester (**1b**). ^1H-NMR ($CDCl_3$) of **1c**: δ_H 7.36—7.56 (10H, m, 2×Ph), 5.24 (1H, d, J=3.3 Hz, H-1′), 5.09 (1H, dd, J=7.9, 3.3 Hz, H-2′), 4.59 (1H, dd, J=12.0, 3.3 Hz, H-1a), 4.25 (1H, dd, J=12.0, 6.3 Hz, H-1b), 4.07 (1H, m, H-2), 3.99 (1H, dd, J=7.9, 5.4 Hz, H-3′), 3.91 (1H, dd, J=13.7, 2.5 Hz, H-5′a), 3.82 (1H, d, J=13.7 Hz, H-5′b), 3.81 (1H, m, H-4′), 3.55 (3H, s, OCH_3), 3.51 (3H, s, OCH_3), 3.38 (2H, m H-3), 3.30 (2H, t, J=6.6 Hz, H-1″), 1.52 (3H, s, CH_3), 1.47 (1H, m, H-2″a) 1.30 (3H, s, CH_3), 1.26 (1H, br s, H-2″b), 1.26 (26H, br s, H_2-3″—H_2-15″), 0.88 t (3H, t, J=6.6 Hz, H-16″); ^1H-NMR ($CDCl_3$) of **1d**: δ_H 7.39—7.58 (10H, m, 2×Ph), 5.09 (1H, dd, J=7.9, 3.3 Hz, H-2′), 5.07 (1H, br s, H-1′), 4.54 (1H, dd, J=11.7, 2.9 Hz, H-1a), 4.22 (1H, dd, J=11.7, 5.9 Hz, H-1b), 4.19 (1H, dd, J=7.9, 5.4 Hz, H-3′), 4.01 (1H, dd, J=5.4, 2.5 Hz, H-4′), 3.89 (1H, dd, J=13.4, 2.5 Hz, H-5′a), 3.85 (1H, m, H-2), 3.75 (1H, d, J=13.4 Hz, H-5′b), 3.58 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 3.25 (2H, br t, J=6.3 Hz, H-1″), 3.13 (2H, br d, J=6.0 Hz, H-3), 1.54 (3H, s, CH_3), 1.47 (1H, m, H-2″a) 1.34 (3H, s, CH_3), 1.26 (1H, br s, H-2″b), 1.26 (26H, br s, H_2-3″—H_2-15″), 0.88 t (3H, t, J=6.6 Hz, H-16″). The same procedure was treated with **3** (2.5 mg) to obtain the (*S*)-MTPA ester (**3a**) (1.6 mg, 84%) and (*R*)-MTPA ester (**3b**) (1.9 mg, 100%). Selective ^1H-NMR ($CDCl_3$) of **3a**: δ_H 7.40—7.59 (5H, m, Ph), 5.28 (1H, d, J=3.4 Hz, H-1′), 5.18 (1H, dd, J=7.5, 3.4 Hz, H-2′), 4.30 (1H, m, H-3′), 4.00 (1H, m, H-2), 3.55 (3H, s, OCH_3), 3.42 (2H, m, H-3), 3.34 (2H, t, J=6.6 Hz, H-1″), 1.55—1.58 (3H, overlapped with H_2O, CH_3), 1.47 (1H, m, H-2″a), 1.34 (3H, s, CH_3), 1.26 (1H, br s, H-2″b), 1.26 (26H, br s, H_2-3″—H_2-15″), 0.88 t (3H, t, J=6.6 Hz, H-16″); Selective ^1H-NMR ($CDCl_3$) of **3b**: δ_H 7.39—7.60 (5H, m, Ph), 5.15 (1H, dd, J=7.5, 2.2 Hz, H-2′), 5.13 (1H, br s, H-1′), 4.33 (1H, m, H-3′), 3.85 (1H, m, H-2), 3.59 (3H, s, OCH_3), 3.17 (2H, m, H-3), 3.27 (2H, t, J=6.6 Hz, H-1″), 2.03 (3H, s, OAc), 1.55—1.58 (3H, overlapped with H_2O, CH_3), 1.47 (1H, m, H-2″a), 1.37 (3H, s, CH_3), 1.26 (1H, br s, H-2″b), 1.26 (26H, br s, H_2-3″—H_2-15″), 0.88 t (3H, t, J=6.6 Hz, H-16″).

Conversion of 3b to 1d To a mixture of **3b** (1.0 mg) and MeOH (0.3 ml) was added 0.3 ml of 1 N $HCl_{(aq)}$. The reaction was stirred at 50 °C for 4 h. Water (1 ml) was added to the mixture and then extracted with *n*-hexane (3×2 ml). Hexane layer was concentrated and then dried in vacuum. The residue was redissolved in acetone (0.3 ml), and then subsequently added 1,2-dimethoxypropane (0.3 ml) and catalytic amount of CF_3COOH. The reaction mixture was stirred at room temperature for 16 h and then concentrated to dryness. The residue was redissolved in dry pyridine (0.4 ml) and added (*S*)-MTPA chloride (20 μl). The reaction was stirred for another 16 h. The reaction mixture was concentrated and chromatographed over silica gel using *n*-hexane–EtOAc (8 : 1) as eluent to obtain a diester (0.9 mg, 73%), of which the ^1H-NMR data was consistent with that of **1d**.

Cytotoxicity Testing Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays were performed using the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide] colorimetric method.[18,19]

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