Gukulenins A and B, Cytotoxic Tetraterpenoids from the Marine Sponge *Phorbas gukulensis*

Su Young Park,[†] Hyukjae Choi,[‡] Hoosang Hwang,[‡] Heonjoong Kang,[‡] and Jung-Rae Rho*,[†]

Department of Oceanography, Kunsan National University, Jeonbuk, 673-701, South Korea, and Center for Marine Natural Products and Drug Discovery, School of Earth and Environmental Sciences, Seoul National University, NS-80, Seoul, 151-747, South Korea

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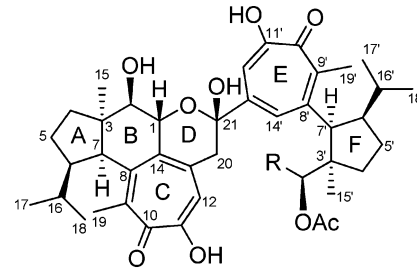
Gukulenins A (**1**) and B (**2**), both having an unprecedented skeleton with a bis-tropolone moiety, were isolated from the Korean marine sponge *Phorbas gukulensis*. They exhibited significant cytotoxicity against human pharynx, stomach, colon, and renal cancer cell lines in the range 0.05−0.80 μM.

Marine sponges have been recognized as a major source of structurally novel classes of secondary metabolites with diverse biological activities.[1−3] Among marine sponges, the genus *Phorbas* has produced potent bioactive compounds with unique structures since the first isolation of phorbazoles in 1994.[4−8] In the course of our continuing search for bioactive compounds from Korean marine species, we isolated two new bis-tropolone tetraterpenoids, gukulenins A (**1**) and B (**2**), along with the known gagunins,[9,10] from *Phorbas gukulensis*, which was recently re-collected and newly assigned. The two new compounds exhibited significant activity in human colon, renal, pharynx, and stomach cancer cell lines. Herein, we report the isolation and structural elucidation of these new compounds and their cytotoxic evaluation.

A specimen of *P. gukulensis* was collected at a depth of 20−25 m off shore of Gageo Island, South Korea, in 2007. The sponge was extracted twice with MeOH. The combined extract was partitioned between CH_2Cl_2 and H_2O, and the organic layer was further repartitioned between 15% aqueous MeOH and *n*-hexane. The polar layer (2 g) was fractionated by reversed-phase flash column chromatography, followed by reversed-phase HPLC, to afford gukulenins A (**1**, 25 mg) and B (**2**, 8 mg).

Gukulenin A (**1**) was determined to have a molecular formula of $C_{42}H_{54}O_{10}$ on the basis of high-resolution FABMS data ($[M + H]^+$ peak at m/z = 719.3792), consistent with 16 degrees of unsaturation. The ^{1}H NMR spectrum of **1** measured in methanol-d_4 showed some broad and overlapping signals, while that in DMSO-d_6 displayed well-separated resonances corresponding to a single compound. The presence of carbonyl and hydroxy groups was confirmed by FTIR, which showed strong absorption bands at 1616, 1723, and 3375 cm^{-1}. The edited HSQC and ^{1}H NMR spectra of **1** in DMSO-d_6 revealed that the compound consisted of nine methyls, five methylenes, 13 methines, and, also from the ^{13}C NMR spectrum and molecular formula, 15 quaternary carbons including 12 quaternary sp^2-hybridized carbons.

Starting from the methyl proton signals of an isopropyl group at δ_H 0.20 and 0.56, sequential couplings in the COSY spectrum readily led to the identity of ring A, in conjunction with the HMBC correlations of a methyl proton at δ_H 1.31 (Me-15) with carbon signals at δ_C 75.5 (C-2), 43.8 (C-3), and 55.5 (C-7). On the basis of this partial structure, the structures of rings B−D were established by HMBC correlations (Table 1). First, the proton doublet at δ_H 3.69 (H-7) showed long-range correlations with three neighboring quaternary sp^2 carbons at δ_C 147.2 (C-8), 132.3 (C-9), and 138.9 (C-14). Similarly, a methyl singlet at δ_H 2.43 (Me-19) also exhibited HMBC correlations with carbon signals at δ_C 147.2 (C-8) and 132.3 (C-9) and a relatively downfield-shifted quaternary carbon at δ_C 167.7 (C-10). Furthermore, the latter carbon was related to a proton singlet at δ_H 6.93 (H-12) by HMBC correlation. Further analysis of the HMBC spectrum showed long-range coupling between H-12 and a downfield-shifted carbon signal at δ_C 168.0 (C-11). The HMBC correlations with the close carbon chemical shifts (δ_C 167.7 and 168.0) were observed by a selective HMBC experiment that focused on a reduced carbon spectral width of 160−180 ppm.[11] Second, a proton doublet at δ_H 4.76 (H-1) coupled with the proton doublet at δ_H 3.39 (H-2) in the COSY spectrum and also correlated with two quaternary sp^2 carbons at δ_C 147.2 (C-8) and 141.0 (C-13) in the HMBC spectrum. This was indicative of the presence of a six-membered ring fused to ring A. Additional HMBC correlations between H-12 and the quaternary sp^2 carbon at δ_C 138.9 (C-14) suggested the presence of a seven-membered ring fused to ring B. This was supported by HMBC correlations of diastereotopic methylene resonances at δ_H 3.11 (H-20) and 3.27 (H-20) with the quaternary sp^2 carbons at C-12, C-13, and C-14. On the basis of the carbon chemical shifts of ring C, it was assigned as the 2-hydroxycycloheptatrien-1-one tropolone moiety.[12] Moreover, the characteristic UV absorption bands at 340, 360, and 373 nm were in good agreement with those of the tropolone moiety.[13] An HMBC correlation between the oxymethine proton H-1 and a hemiketal carbon at δ_C 97.4 (C-21) was also observed. In addition, an HMBC correlation from the hydroxy proton at δ_H 7.14 (OH-21) to the methylene carbon C-20 completed the tetracyclic A−D portion of the molecule.



1 R = CHO
2 R = CH_2OH

The interpretation of COSY, HSQC, and HMBC spectra of the remaining signals revealed the presence of a second isopropylcyclopentane and methyltropolone ring in compound **1**. Different from the fused ring A nature, the tropolone E ring was connected with the cyclopentane ring F through a single bond. This was recognized by HMBC correlations of H-7′ with two quaternary carbons at δ_C 149.3 (C-8′) and 140.4 (C-9′), as well as a methine sp^2 carbon at δ_C 124.4 (C-14′). HMBC correlations with the remaining tertiary methyl protons at δ_H 1.05 (Me-15′) showed C-3′ was connected to an oxymethine carbon at δ_C 82.4 (C-2′). In succession, the proton singlet at δ_H 4.30 (H-2′) attached to C-2′ correlated to the formyl and acetyl carbonyl carbons at δ_C 199.1, and 168.7, respectively,

* To whom correspondence should be addressed. Tel: +82-63-469-4606. Fax: +82-63-469-4990. E-mail: jrrho@kunsan.ac.kr.
† Kunsan National University.
‡ Seoul National University

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Table 1. NMR Data (500 MHz, DMSO-d_6) for Gukulenin A (**1**)

position	δ_C, mult	δ_H (J in Hz)	HMBC[a]	NOE[b]
1	72.4, CH	4.76, d (9.3)	2, 8, 13, 21	4b, 5a, 16, 20a, OH-21
2	75.5, CH	3.39, dd (9.3, 3.2)		15, OH-2
3	43.8, qC			
4a	33.3, CH_2	1.29, m		4b, 5b, OH-2
4b		2.14, dt (12.7,6.4)	2, 3, 6	1
5a	26.5, CH_2	1.39, m		6
5b		1.67, dq (12.7,6.4)	3, 4, 6, 7	1, 17
6	51.0, CH	2.34, m		7, 18
7	55.5, CH	3.69, d (9.5)	3, 5, 6, 8, 9, 14, 15	2, 6, 15, 19
8	147.2, qC			
9	132.3, qC			
10	167.7, qC			
11	168.0, qC			
12	123.6, CH	6.93, s	10, 11, 14, 20	20b
13	141.0, qC			
14	138.9, qC			
15	30.3, CH_3	1.31, s	2, 3, 4, 7	2, 7
16	26.4, CH	1.02, m		
17	23.8, CH_3	0.56, d (6.6)	6, 16, 18	
18	18.6, CH_3	0.20, d (6.6)	6, 16, 17	
19	18.8, CH_3	2.43, s	8, 9, 10	7, 16
20a	47.2, CH_2	3.11, d (15.2)	12, 13, 14, 21, 13′	1, OH-2
20b		3.27, d (15.2)		12, 12′, 14′
21	97.4, qC			
OH-2		5.16, d (3.4)		1′
OH-21		7.14, s	20, 21, 13′	1, 20a, 12′, 14′
1′	199.1, CH	9.43, s	2′	2′, 4′a, 4′b, 14′, OH-2
2′	82.4, CH	4.30, s	1′, 3′, 7′, 15′, C=O	1′, 4′b, 7′, 14′, 15′,
3′	49.9, qC			
4′a	33.7, CH_2	1.60, ddd (13.7, 9.3, 3.4)		2′, 4′b, 15′
4′b		2.39, m		1′, 2′, 4′a, 14′
5′a	29.6, CH_2	1.14, m		
5′b		1.94, m		5′b, 14′
6′	49.7, CH	2.08, m	8′	15′, 17′, 18′
7′	56.1, CH	3.36, d (7.8)	4′, 5′, 8′, 9′, 14′, 15′	2′, 6′, 15′, 19′, Ac(CH_3)
8′	149.3, qC			
9′	140.4, qC			
10′	175.6, qC			
11′	162.1, qC			
12′	115.5, CH	7.46, s	21, 10′, 11′, 13′, 14′	OH-21
13′	147.9, qC			
14′	124.4, CH	6.82, s	21, 7′, 9′, 12′, 13′	2′, 4′b, 5′a, 16′,20b, OH-21
15′	24.6, CH_3	1.05, s	2′, 3′, 4′, 7′	2′, 4′a, 5′b, 6′, 7′, Ac(CH_3)
16′	29.9, CH	0.52, m		
17′	21.3, CH_3	0.39, d (6.4)	6′, 16′, 18′	
18′	22.3, CH_3	0.37, d (6.4)	6′, 16′, 17′	
19′	18.1, CH_3	2.42, s	8′, 9′, 10′	17′, Ac(CH_3)
Ac (C=O)	168.7, qC			
Ac (CH_3)	19.9, CH_3	1.88, s	C=O	2′, 7′, 15′, 19′

[a] HMBC correlations are from proton(s) stated to the indicated carbon. [b] NOE correlations are to proton(s).

in the HMBC experiment. The correlations of the 1D and 2D NMR spectra allowed us to assign all the proton and carbon signals in two substructures (tetacyclic A−D part and bicyclic E, F part) of **1**, accounting for all degrees of unsaturation. Finally, two substructures could be assembled to a single compound by a single bond connecting C-21 in ring D with C-13′ in ring E. This was unambiguously confirmed by HMBC correlations of two broad proton singlets at H-12′ and H-14′ with the hemiketal carbon at C-21. Accordingly, **1** was characterized as a dimeric variant due to the similarity of the two substructures.

From the structure of **1**, it is speculated that the complexity of ^{1}H NMR signals in methanol-d_4 might be due to tautomerism occurring in the two tropolone moieties.[14] However, in DMSO-d_6 only one tautomer was observed. Unobservable hydroxy protons in the two tropolone moieties made it difficult to determine the position of the ketone and hydroxy groups in a given tautomer. One plausible approach to the problem was to observe, after acetylation of **1**, NOE correlations between the corresponding acetyl methyls and spatially close protons in the ROESY experiment. Weak NOE signals of H-12 and H-12′ of the two tropolone rings

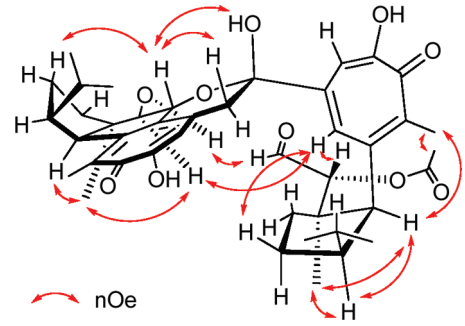
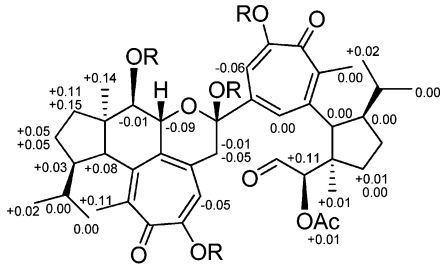


Figure 1. Key NOE correlations of **1**.

with acetylated methyl protons suggested that the hydroxy groups should be placed at C-11 and C-11′.

The relative configuration of the stereocenters of **1** was assigned by ROESY analysis (Figure 1). Me-15 exhibited NOE correlations with H-2 and H-7, indicating that the A/B ring junction is *cis*-configured. NOE correlations of H-1 with H-4b, H-5a, H-16, H-20a, and OH-21 revealed that all of these protons were oriented on the



1a R = (*S*)-MTPA
1b R = (*R*)-MTPA

Figure 2. ^{1}H NMR chemical shift differences ($\Delta\delta^{S-R}$) in ppm for tetrakis-(*S*/*R*)-MTPA esters of **1** in $CDCl_3$.

same face of rings A−D. Similarly, on the basis of the NOE correlations of Me-15′ with H-6′ and H-7′, both 1-acetoxy-1-formylmethyl and isopropyl groups were placed on the same face of ring F. Furthermore, the orientation of ring F relative to ring E could be determined by the NOE correlations between H-7′ and Me-19′, along with NOE peaks of H-14′ with H-2′, H-4′b, H-5′a, and H-16′. However, these correlations were also satisfied irrespective of the specific configuration of C-7′. The configuration of C-7′ was determined by a weak NOE correlation between OH-2 on ring B and H-1′, indicating *R** configuration. Finally, the configuration of C-2′ was established on the basis of a preferred conformation with the formyl group oriented to the A−D substructure and H-2′ arranged toward the plane of ring E. The free rotation around the C-2′−C3′ bond in ring F was hindered by the steric interaction of acetyl group with ring E, explaining all the related NOE correlations. The dimeric characterization of **1** also supported the configuration of C-2′ being the same as that of C-2 in ring B.

The absolute configuration of C-2 was determined using the modified Mosher's method.[15,16] Esterification of **1** with *R*-(−)- and *S*-(+)-MTPA chlorides led to the tetrakis-(*S*)-MTPA ester of **1** and the tetrakis-(*R*)-MTPA ester of **1**, respectively. All proton signals in the two ester derivatives were assigned, and the difference in chemical shifts between the corresponding protons in the ^{1}H NMR spectra of the tetrakis-(*S*/*R*)-MTPA esters was calculated (Figure 2). This suggested that the absolute configuration of C-2 was *R*, which allowed the full assignment of the absolute configuration of **1**.

Gukulenin B (**2**) had the molecular formula $C_{42}H_{56}O_{10}$ on the basis of HRFABMS data ([M + H]$^+$ peak at m/z = 721.3956), indicating 15 degrees of unsaturation. Compared with **1**, the main difference is that the signal corresponding to a formyl group was absent in the ^{1}H and ^{13}C NMR spectra of **2**. From the analysis of 1D and 2D NMR spectra, **2** had the same carbon skeleton as **1** except for a difference in the functional group connected to C-2′. Two new protons (δ_H 3.41 and 3.30) in a hydroxymethylene group showed coupling with an oxymethine proton at δ_H 4.20 (H-2′) in the COSY spectrum of **2**. This infers that **2** was produced by the reduction of the formyl group in **1**.

Gukulenins A (**1**) and B (**2**) are unusual tetraterpenoids that include two methyl tropolone groups. At first glance, the structure of rings A−C in gukulenins could be considered as a variant derived from the polyoxgenated 7,6,5-tricyclic moiety that is the skeleton of gagunins isolated from the same organisms.[9,10] Although the tricyclic structures in both gagunins and gukulenins commonly stem from geranylgeranyl pyrophosphate, careful examination shows that cyclization from the precursor to the compounds is not identical. This is evident from the different position of the methyl groups on the seven-membered ring system of the two compounds. Accordingly, gukulenins A (**1**) and B (**2**) are not simple dimeric variants of the gagunins, but modified dimers derived from the skeleton of a new monomer.

The cytotoxicities of gukulenins A (**1**) and B (**2**) were evaluated against four human cancer cell lines using the colorimetric methylthiazole tetrazolium bromide (MTT) assay. Gukulenins A and B exhibited potent activities against human pharynx cancer FaDu (IC_{50} 57 nM and 0.63 μM), colon cancer HCT-116 (IC_{50} 62 nM and 0.55 μM), renal cancer SN12C (IC_{50} 92 nM and 0.61 μM), and stomach cancer MKN45 (IC_{50} 0.13 μM and 0.72 μM) cell lines, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1010 polarimeter in a 5 cm cell. UV spectra were obtained in MeOH using a Varian Cary 50, and IR spectra were measured on a JASCO FT/IR 4100 spectrometer. All NMR spectra were recorded on a Varian VNMRS 500 spectrometer in DMSO-d_6 and $CDCl_3$ solutions. Chemical shifts of the proton and carbon spectra measured in DMSO-d_6 solution are reported with reference to residual solvent peaks at 2.50 and 39.5 ppm, respectively. Positive HRFAB mass spectra were obtained on a JEOL JMS-700 spectrometer at Korea Basic Science Research Institute, Daegu, South Korea. HPLC was carried out on a Varian system (Prostar 210 pump and Prostar 355 refractive index detector).

Material. The specimen of *Phorbas gukulensis* was collected by hand using scuba at a depth of 20−25 m in 2007 off shore of Gageo Island at the West Sea of South Korea. The sponge had a thick mass; it measured 110 × 95 mm and was 20 mm thick. Oscules were rare and the texture was soft. The life color was red. In the skeleton, the megascleres were tornotes (340−420 × 6−10 μm), small acanthostyles (160−195 × 5−10 μm), and large acanthostyles (390−470 × 8−11 μm), and microscleres were isochelas (25−30 μm). A voucher specimen (No. 07G-6) is deposited at the Natural History Museum, Hannam University, South Korea.

Extraction and Isolation. The collected specimen was frozen on site, delivered to the laboratory under dry ice, and then kept in a refrigerator at −25 °C. Freshly thawed sponge was cut into small pieces and extracted twice with MeOH at room temperature. The MeOH extract (ca. 10 g) was partitioned between CH_2Cl_2 and H_2O. The organic layer was evaporated under reduced pressure and repartitioned between *n*-hexane and 15% aqueous MeOH for defatting. Then, the aqueous MeOH fraction (ca. 2 g) was subjected to reversed-phase flash column chromatography eluting with solvents of decreasing polarity (MeOH/H_2O, 50/50; 60/40; 70/30; 80/20; 90/10; 100% MeOH; 100% acetone) to give seven fractions. The 10% aqueous MeOH fraction (ca. 400 mg) directed by the cytotoxicity assay and ^{1}H NMR monitoring was separated by reversed-phase HPLC (flow: isocratic 2 mL/min, column: YMC ODS-A, 5 μm, 250 mm × 10 mm i.d.) eluting with 15% aqueous MeOH solvent. Most of the isolated peaks corresponded to gagunin-like compounds except for a mixture at a retention time of 30 min. The mixture was purified on reversed-phase HPLC (flow: isocratic 1 mL/min, column: YMC ODS-A, 5 μm, 250 mm × 4.6 mm i.d.) using 20% aqueous MeCN solvent to obtain gukulenins A (**1**, 25 mg) and B (**2**, 8 mg), respectively.

Gukulenin A (1): yellowish, amorphorous solid; $[\alpha]^{25}_D$ −64.7 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 256 (4.91), 340 (4.01), 360 (4.11), 373 (4.20) nm; IR (film) ν_{max} 3375, 1723, 1616, 1240 cm^{-1}; ^{1}H and ^{13}C NMR data are given in Table 1; HRFABMS *m*/*z* 719.3792 (calcd 719.3795 for $C_{42}H_{55}O_{10}$).

Gukulenin B (2): yellowish, amorphorous solid; $[\alpha]^{25}_D$ −45.3 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 256 (4.99), 340 (4.00), 360 (4.08), 373 (4.24) nm; IR (film) ν_{max} 3392, 1724, 1617, 1243 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.37 (1H, s, H-12′), 7.09 (1H, s, OH-21), 6.90 (1H, s, H-12), 6.80 (1H, s, H-14′), 4.97 (1H, d, J = 3.4 Hz, OH-2), 4.72 (1H, d, J = 9.3 Hz, H-1), 4.20 (1H, dd, J = 6.4, 4.4 Hz, H-2′), 3.67 (1H, d, J = 9.8 Hz, H-7), 3.41 (1H, dd, J = 12.2, 4.4 Hz, H-1′b), 3.37 (1H, dd, J = 9.3, 3.4 Hz, H-2), 3.30 (1H, dd, J = 12.2, 6.4 Hz, H-1′a), 3.26 (1H, d, J = 7.8 Hz, H-7′), 3.23 (1H, d, J = 15.2 Hz, H-20b), 3.07 (1H, d, J = 15.2 Hz, H-20a), 2.41 (3H, s, H-19), 2.34 (3H, s, H-19′), 2.32 (1H, m, H-6), 2.11 (1H, dt, J = 12.7, 6.4 Hz, H-4b), 2.07 (1H, m, H-6′), 1.92 (1H, m, H-4′b), 1.79 (1H, m, H-5′b), 1.76 (3H, s, AcO-2′), 1.65 (1H, dq, J = 12.7, 6.4 Hz, H-5b), 1.43 (1H, ddd, J = 13.2, 9.3, 3.4 Hz, H-4′a), 1.38 (1H, m, H-5a), 1.29 (3H, s, H-15), 1.26 (1H, m, H-4a), 1.17 (3H, s, H-15′), 1.05 (1H, m, H-5′a), 1.03 (1H, m, H-16), 0.54 (3H, d, J = 6.9 Hz, H-17), 0.52 (1H, m, H-16′), 0.38 (3H, d, J = 6.4 Hz, H-17′), 0.36 (3H, d, J = 6.4 Hz,

H-18′), 0.19 (3H, d, $J = 6.4$ Hz, H-18); ^{13}C NMR (DMSO, 125 MHz) δ 175.7 (C, C-10′), 168.8 (C, C═O), 168.1 (C, C-10), 167.8 (C, C-11), 162.0 (C, C-11′), 150.5 (C, C-8′), 147.6 (C, C-13′), 147.5 (C, C-8), 141.0 (C, C-13), 140.4 (C, C-9′), 138.9 (C, C-14), 132.8 (C, C-9), 125.5 (CH, C-14′), 123.6 (CH, C-12), 115.4 (CH, C-12′), 97.5 (C, C-21), 77.4 (CH, C-2′), 75.7 (CH, C-2), 72.4 (CH, C-1), 61.1 (CH_2, C-1′), 56.3 (CH, C-7′), 55.6 (CH, C-7), 51.5 (C, C-3′), 51.1 (CH, C-6), 50.7 (CH, C-6′), 47.4 (CH_2, C-20), 44.0 (C, C-3), 34.9 (CH_2, C-4′), 33.4 (CH_2, C-4), 30.4 (CH_3, C-15), 29.9 (CH, C-16′), 29.2 (CH_2, C-5′), 26.7 (CH_2, C-5), 26.6 (CH, C-16), 24.3 (CH_3, C-15′), 24.0 (CH_3, C-17), 22.5 (CH_3, C-18′), 21.4 (CH_3, C-17′), 21.1 (CH_3, AcO-2′), 19.0 (CH_3, C-19), 18.7 (CH_3, C-18), 18.3 (CH_3, C-19′); HRFABMS *m*/*z* 721.3956 (calcd 721.3952 for $C_{42}H_{57}O_{10}$).

MTPA Reaction of Gukulenin A (1). To a stirred solution of gukulenin A (**1**, 4 mg) and dried pyridine (20 μL) in dry CH_2Cl_2 (0.5 mL) at room temperature was added (*R*)-(−)-MTPA-Cl (150 μL). The reaction progress was monitored by TLC chromatography on silica gel (*n*-hexane/EtOAc, 4:1). After ~10 h, the reaction mixture was quenched by the addition of H_2O and Me_2O. The organic layer was concentrated *in vacuo*. The crude was eluted by silica gel SPE with *n*-hexane/EtOAc (5:1) to give tetrakis-(*S*)-MTPA ester (**1a**) as a pale yellow gum: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.67 (1H, s, H-1′), 7.16 (1H, s, H-12′), 6.80 (1H, s, H-14′), 6.67 (1H, s, H-12), 5.15 (1H, d, $J = 10.0$ Hz, H-2), 4.70 (1H, s, H-2′), 4.58 (1H, d, $J = 10.0$ Hz, H-1), 3.69 (1H, d, $J = 8.8$ Hz, H-7), 3.28 (1H, d, $J = 7.8$ Hz, H-7′), 2.90 (1H, d, $J = 15.9$ Hz, H-20), 2.84 (1H, d, $J = 15.9$ Hz, H-20), 2.46 (3H, s, H-19), 2.45 (1H, m, H-4′b), 2.43 (3H, s, H-19′), 2.25 (2H, m, H-4b, −6), 2.06 (1H, m, H-6′), 2.05 (3H, s, AcO-2′), 2.00 (1H, m, H-5′b), 1.77 (1H, m, H-4′a), 1.74 (1H, m, H-5b), 1.40 (1H, m, H-4a), 1.38 (3H, s, H-15), 1.25 (1H, m, H-5a), 1.16 (3H, s, H-15′), 1.12 (1H, m, H-16), 0.83 (1H, m, H-5′a), 0.73 (3H, d, $J = 6.6$ Hz, H-17), 0.57 (3H, br s, H-17′), 0.52 (1H, m, H-16′), 0.51 (3H, br s, H-18′), 0.34 (3H, d, $J = 6.6$ Hz, H-18). In an entirely analogous way, tetrakis-(*R*)-MTPA ester (**1b**) was obtained using (*S*)-(+)-MTPA-Cl. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.58 (1H, s, H-1′), 7.22 (1H, s, H-12′), 6.80 (1H, s, H-14′), 6.72 (1H, s, H-12), 5.16(1H, d, $J = 10.3$ Hz, H-2), 4.67 (1H, d, $J = 10.3$ Hz, H-1), 4.59 (1H, s, H-2′), 3.61 (1H, d, $J = 8.8$ Hz, H-7), 3.28 (1H, d, $J = 7.3$ Hz, H-7′), 2.91 (2H, br s, H-20), 2.45 (1H, m, H-4′b), 2.43 (3H, s, H-19′), 2.35 (3H, s, H-19), 2.22 (1H, m, H-6), 2.10 (1H, m, H-4b), 2.06 (2H, m, H-5′b, -6′), 2.04 (3H, s, AcO-2′), 1.76 (1H, m, H-4′a), 1.69 (1H, m, H-5b), 1.29 (1H, m, H-4a), 1.24 (3H, s, H-15), 1.20 (1H, m, H-5a), 1.15 (3H, s, H-15′), 1.12 (1H, m, H-16), 1.01 (1H, m, H-5′a), 0.73 (3H, d, $J = 6.6$ Hz, H-17), 0.59 (3H, d, $J = 6.4$ Hz, H-17′), 0.56 (1H, m, H-16′), 0.51 (3H, d, $J = 6.4$ Hz, H-18′), 0.32 (3H, d, $J = 6.6$ Hz, H-18).

Acetylation of Gukulenin A (1). Gukulenin A (**1**, 2 mg) was acetylated with Ac_2O/pyridine (1:4, 0.5 mL) at 70 °C for 2 h. After workup with MeOH and solvent drying procedures, the mixture was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was subjected to a silica column to furnish an acetyl derivative of **1**. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.46 (1H, s, H-1′), 5.38 (1H, d, $J = 9.3$ Hz, H-2), 7.21 (1H, s, H-12′), 7.14 (1H, s, H-14′), 6.13 (1H, s, H-12), 4.83 (1H, d, $J = 9.3$ Hz, H-1), 4.31 (1H, s, H-2′), 3.53 (1H, d, $J = 9.5$ Hz, H-7), 3.43 (1H, d, $J = 7.3$ Hz, H-7′), 2.45 (3H, s, H-19′), 2.35 (3H, s, H-19), 2.32 (3H, s, AcO-11′), 2.29 (3H, s, AcO-11), 2.19 (3H, s, AcO-20), 2.09 (3H, s, AcO-2), 2.06 (3H, s, AcO-2′), 1.27 (3H, s, H-15), 1.24 (3H, s, H-15′).

MTT Assay of Gukulenins against Four Human Cancer Cells. Cytotoxic activity was determined as described in the previous paper.[17] In the cytotoxic evaluation on four cells, both actinomycin D and doxorubicin compounds are used as positive controls.

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Supporting Information Available: ^{1}H, ^{13}C, and 2D NMR spectra of **1** and **2** are available free of charge via the Internet at http://pubs.acs.org.

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