Vladimuliecins A and B: Cytotoxic Pentacyclic Pregnanols from *Vladimiria muliensis*

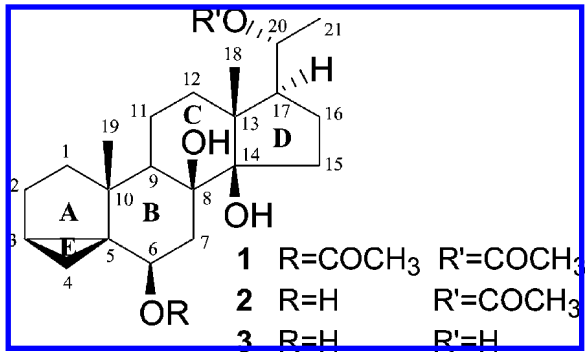
Jian-Jun Chen, Zheng-Ming Li, Kun Gao,* Jin Chang, and Xiao-Jun Yao*

State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, People's Republic of China

Received February 23, 2009

Two new steroids, vladimuliecins A (**1**) and B (**2**), were isolated by bioassay-guided fractionation from the rhizome of *Vladimiria muliensis*. Compounds **1** and **2** are the first examples possessing a pentacyclic 3α,5α-cyclopregnane-type framework. The structures of vladimuliecins A (**1**) and B (**2**) and their deacetylated derivative (**3**) were determined on the basis of IR, MS, 1D NMR, 2D NMR, and X-ray data analyses. The absolute configuration of the 10 stereogenic centers of compounds **1** and **2** was determined to be 3*R*,5*R*,6*R*,8*S*,9*R*,10*R*,13*R*,14*R*,17*S*,20*R* by means of auxiliary chiral MTPA derivatives and optical rotation calculation. A probable biosynthesis pathway to **1** and **2** is also proposed and discussed. In addition, the cytotoxicity of compounds **1**, **2**, and **3** was evaluated against selected cancer cell lines, including human leukemia cell (HL-60), human hepatoma cell (SMMC-7721), and human cervical carcinoma cell (HeLa) lines.

Natural products continue to play a highly significant role today in the discovery and development of new drugs, leads, and chemical entities. This fact is particularly evident in the areas of cancer and infectious diseases, where more than 60% and 75% of drugs, respectively, are of natural origin.[1,2] In the interest of identifying new antimicrobial and anticancer drugs or leads from traditional Chinese herbal medicine, we previously reported eremophilane-type sesquiterpene,[3] bisabolane-type sesquiterpenes,[4] and oleanane-type triterpenes.[5] Herein we describe three new cytotoxic pregnanols, vladimuliecins A (**1**) and B (**2**) and their deacetylated derivative (**3**), with modest inhibitory activity against human leukemia (HL-60), human hepatoma (SMMC-7721), and human cervical carcinoma (HeLa) cell lines. Vladimuliecins A (**1**) and B (**2**) were isolated by bioassay-guided fractionation from the dried rhizome of *Vladimiria muliensis* (H.-M.) Ling (Compositae), from which we recently reported six antimicrobial triterpenoids.[6] A literature survey indicates that vladimuliecins A (**1**) and B (**2**) possess a pentacyclic 3α,5α-cyclopregnane-type framework, a new structural feature in natural products. This report deals with the isolation, structure elucidation, absolute configuration, cytotoxicity, and possible biosynthesis pathways to vladimuliecins A (**1**) and B (**2**).



Results and Discussion

Vladimuliecin A (**1**) was obtained as an optically active, $[\alpha]^{20}_{D}$ +31 (*c* 0.8, $CHCl_3$), amorphous solid. The molecular formula was determined as $C_{25}H_{38}O_6$ by HRESIMS (*m/z* 457.2560 $[M + Na]^+$, calc 457.2561) and NMR data (Table 1) and indicated seven degrees of unsaturation.

Compound **1** had absorption bands at 3438 (hydroxy) and 1724 cm^{-1} (carbonyl) in the IR spectrum. The ^{13}C NMR spectrum ($CDCl_3$) showed two ester carbonyl signals at δ 169.3 and 170.5 taking into account two unsaturations. Hence, the five remaining degrees of unsaturation were attributed to five saturated rings. The ^1H and ^{13}C NMR and HMQC spectra also indicated the presence of four tertiary methyl, one secondary methyl, eight methylene, five methine (two oxygenated), and seven quaternary carbons (two oxygenated). The $^1H-^1H$ COSY spectrum and proton chemical shifts revealed the spin systems of four structural fragments: **a**, **b**, **c**, and **d** (Figure 1). The skeleton was established by analyzed long-range HMBC correlations of some quaternary carbons with hydrogen of the above fragments (Figure 2). The cross-peaks of C-10 (δ 43.1) with H-1a, H-1b, H-2, H-9, and H-11 attested that fragments **a** and **c** were connected through C-10 with C-1 and C-9. The quaternary carbon at δ 35.5 (C-5) showed correlations with H-1a,b, H4-a,b, and H-3 of fragment **a**, as well as H-6 and H-7a,b of fragment **b**; thus, C-5 should be connected with C-10, C-4, and C-6. Furthermore, HMBC correlations from H-3 (δ 1.20) to oxygenated methine carbon C-6 (δ 77.2) and quaternary carbon C-10 suggested that C-5 connected with C-3 directly; thus rings A and E were defined. This conclusion was further confirmed by the similarity between the chemical shifts of CH_2 at C-4 (δ_H 0.59, 0.64 and δ_C 12.5) and those of the cyclopropyl ring (δ_H 0.50 and δ_C 10.1). The oxygenated quaternary carbon C-8 at δ 78.0 showed correlations with H-6, H7-a,b, H-9, and H-11, indicating that C-8 is connected to C-7 and C-9, forming ring B. The quaternary carbon C-13 at δ 48.0 showed correlations with H-11, H-12, H-15, H-16, H-17, and H-20, indicating that C-13 is connected to C-12 of fragment **c** and C-17 of fragment **d**. The last oxygenated quaternary carbon C-14 at δ 84.7, showing HMBC correlations with H-9, H-12, H-15, H-16, and H-17, was unambiguously connected to C-8, C-13, and C-15. At the same time, rings C and D were defined. The HMBC correlations of the C-19 methyl protons (δ 1.12) with C-1, C-5, C-9, and C-10 allowed the connection of this methyl to C-10. Similarly, the C-18 methyl protons (δ 1.15) had HMBC correlations with C-12, C-13, and C-17, hence locating it at C-13. Thus, the pentacyclic skeleton was established as 3,5-cyclopregnane with CH_3-18 (δ 16.3) fixed to C-13 and CH_3-19 (δ 20.8) to C-10 and having oxygenation at C-6, C-8, C-14, and C-20. The remaining four carbons belonged to the two acetoxy units, which were concluded from HMBC analysis (Figure 2). The carbonyl carbons at δ 169.3 and 170.5 were correlated to H-6 of fragment **b** and

* To whom correspondence should be addressed. Tel: +86-0931-8912592. Fax: +86-931-8912582. E-mail: npchem@lzu.edu.cn, xjyao@lzu.edu.cn.

10.1021/np900120q CCC: $40.75
Published on Web 05/08/2009

Table 1. NMR Data of Compounds **1**−**3** in $CDCl_3$

	1[a]		**2**[b]		**3**[b]	
no.	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1a	0.94 ddd (13.8, 7.8, 3.6)	36.3	0.92 ddd (12.0, 8.0, 3.6)	37.9	0.90 ddd (12.8, 7.6, 3.6)	37.9
1b	0.92 ddd (13.8, 6.0, 5.4)		0.86 ddd (12.0, 6.0, 4.8)		0.93 ddd (12.8, 5.6, 4.0)	
2	1.62 dm (7.8)	24.8	1.62 dm (8.0)	24.8	1.62 dm (7.6)	24.8
3	1.20 dddd (7.8, 4.8, 4.2, 3.6)	25.2	1.11 ddt (8.0, 4.8, 4.0)	24.9	1.08 ddt (8.0, 4.8, 4.0)	24.8
4a	0.59 t (4.8)	12.5	0.44 dd (8.0, 4.8)	12.1	0.46 dd (8.0, 4.8)	12.2
4b	0.64 dd (7.8, 4.8)		0.65 t (4.8)		0.64 t (4.8)	
5		35.5		36.4		36.4
6	4.82 t (3.0)	77.2	3.50 br s	76.0	3.46 br s	75.8
7a	2.50 dd (15.0, 3.0)	35.4	2.34 dd (14.4, 3.0)	36.0	2.34 dd (14.4, 2.8)	36.1
7b	1.68 dd (15.0, 3.0)		1.52 dd (14.4, 3.2)		1.53 dd (14.4, 3.2)	
8		78.0		78.3		78.5
9	1.30 m	44.4	1.29 m	44.7	1.30 m	44.3
10		43.1		43.3		43.3
11	1.31 m	19.7	1.33 m	19.7	1.32 m	21.3
12a	1.32 m	42.6	1.33 m	42.6	1.33 m	41.9
12b	1.69 m		1.68 m		1.68 m	
13		48.0		47.9		49.1
14		84.7		84.6		84.9
15	1.56 dm (10.2)	33.1	1.60 dm (10.2)	33.1	1.64 dm (10.0)	33.5
16	1.80 m	25.5	1.80 m	25.5	1.78 m	29.7
17	1.83 dm (9.0)	54.8	1.74 dm (9.2)	54.8	1.78 dm (9.0)	57.4
18	1.15 s	16.3	1.18 s	16.5	1.38 s	17.8
19	1.12 s	20.8	1.17 s	21.4	1.19 s	23.2
20	4.97 dq (9.0, 6.0)	73.8	4.97 dq (9.2, 6.4)	73.9	3.87 dq (9.0, 6.0)	71.9
21	1.16 d (6.0)	19.0	1.16 d (7.2)	19.0	1.26 d (6.4)	19.4
6-OAc	2.09 s	21.5				
		169.3				
20-OAc	2.00 s	21.6	2.00 s	21.6		
		170.5		170.4		

[a] Recorded at 600 and 150 MHz for ^1H and ^{13}C, respectively. [b] Recorded at 400 and 100 MHz for ^1H and ^{13}C, respectively.

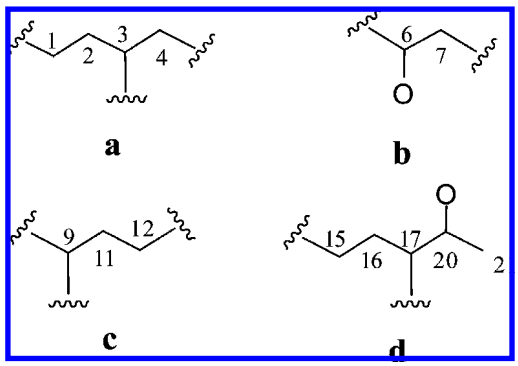


Figure 1. Structural fragments of **1**.

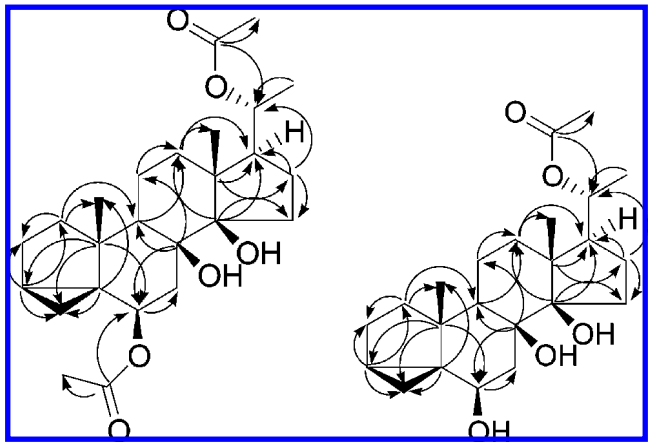


Figure 2. Important HMBC correlations of vladimuliecins A (**1**) (C⇀H).

H-20 of fragment **d**, respectively, indicating that one acetoxy group was attached to C-6 and the other to C-20. Finally, the structure of vladimuliecin A (**1**) was assigned as 6,20-diacetoxy-3,5-cyclopregnane-8,14-diol.

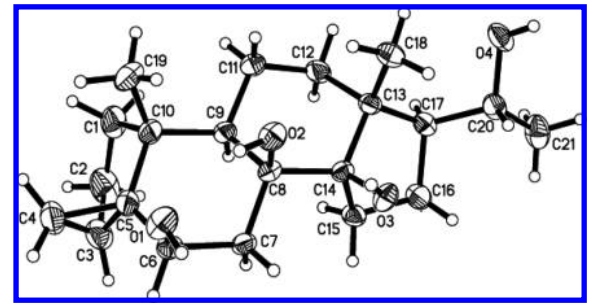


Figure 3. ORTEP drawing of **3**.

To confirm the structure of vladimuliecin A (**1**), 6 mg of **1** was deacetylated in K_2CO_3−aqueous CH_3OH (5%),[7] and 5 mg of **3** was obtained. Compound **3** was obtained as prisms from acetone and subjected to X-ray diffraction analysis (Figure 3). Thus, the relative configuration of vladimuliecin A (**1**) was assigned from the X-ray diffraction data of **3**.

The absolute configuration of compound **1** could also be determined from that of compound **3** by means of the auxiliary chiral anisotropic reagent MTPA.[8] Compound **3** was treated with (*R*)- and (*S*)-MTPA-Cl, and the (*S*)- and (*R*)-MTPA esters at C-20 of **3** were obtained, respectively. Comparison of the ^1H NMR chemical shifts between the (*S*)- and (*R*)-MTPA esters of **3** (Δ values shown in Figure 4) led to the assignment of an *R*-configuration at C-20. Furthermore optical rotation calculation was used as another method to confirm this conclusion. The B3LYP/6-31G* method was used to predict the optical rotation of compound **3** and its enantiomer **3a** (Figure 5).[9] The sum of optical rotations for compound **3** is +20.89 and for its enantiomer **3a** is −4.95. The former value was close to the experimental value of +12, which strongly suggested the configuration of compound **3** given in Figure 3. Hence, the absolute configuration of vladimuliecin

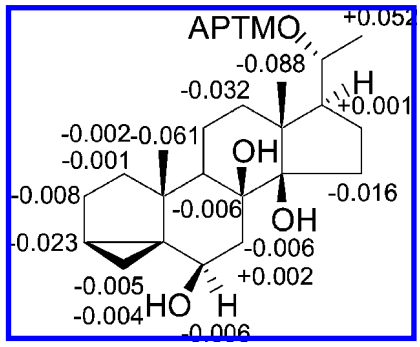


Figure 4. ^{1}H NMR chemical shift differences $\Delta\delta$ ($\delta_S - \delta_R$) in ppm for the MTPA esters of **3**.

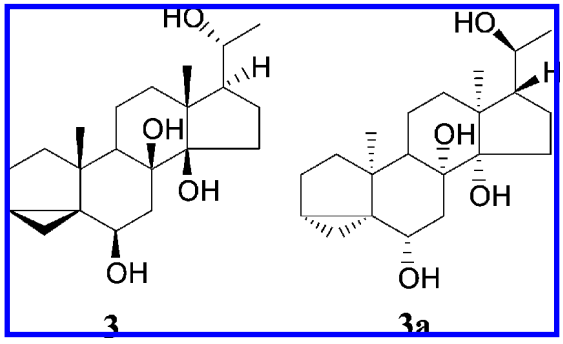


Figure 5. Structure of enantiomers **3** and **3a**.

A (**1**) was determined as (3*R*,5*R*,6*R*,8*S*,9*R*,10*R*,13*R*,14*R*,17*S*,20*R*)-6,20-diacetoxy-3,5-cyclopregnane-8,14-diol.

Vladimuliecin B (**2**) was an optically active, $[\alpha]^{20}_D$ +9 (*c* 0.2, $CHCl_3$), amorphous solid. The molecular formula was determined as $C_{23}H_{36}O_5$ by HRESIMS (*m/z* 415.2450 [M + Na]$^+$, calcd 415.2455) and NMR data (Table 1) and indicated six degrees of unsaturation.

The ^{1}H and ^{13}C NMR spectra ($CDCl_3$) of vladimuliecin B (**2**) presented similar signals to those of compound **1**. The only significant differences included the lack of an acetate group, and the proton signal of H-6 was upfield-shifted from δ 4.82 (t, J = 3.0) to δ 3.50 (br s). These observations strongly suggested that compound **2** differed from **1** in that the C-6 *O*-acetyl moiety was replaced by a hydroxy group. Analysis of ^{1}H−^{1}H COSY, HMQC, and HMBC data affirmed this assignment. The structure of **2** was further confirmed by acetylation of **3** to give **2**.[10] Thus, the structure of vladimuliecin B (**2**) was assigned as (3*R*,5*R*,6*R*,8*S*,9*R*,10*R*,13*R*,14*R*,17*S*,20*R*)-20-acetoxy-3,5-cyclopregnane-6,8,14-triol.

Vladimuliecins A (**1**) and B (**2**) are the first examples of the natural occurrence of pentacyclic 3α,5α-cyclosteroid types, which were previously synthesized from corresponding 3β-hydroxy steroids, such as cholesterol,[11] dehydroisoandrosterone,[12] ecdysteroid,[13,14]etc. The biosyntheses of **1** and **2** could proceed through oxidation of pregnenolone **4**, which was also obtained from *V. muliensis*, and followed by reduction, intramolecular cycloaddition, and acetification (Scheme 1).

The cytotoxicities of compounds **1**, **2**, and **3** were evaluated against human leukemia (HL-60), human hepatoma (SMMC-7721), and human cervical carcinoma (HeLa) cell lines using the sulforhodamine B (SRB) method as previously reported.[15] The results are shown in Table 2. The three compounds exhibited moderate activity against the three lines ($IC_{50} \approx 100$ μM/L). Mitomycin was used as positive control with an $IC_{50} \approx 3$ μM/L. The *O*-acetyl moiety at C-6 or C-20 in the three compounds has no effect on their cytotoxicities.

Experimental Section

General Experimental Procedures. Melting points were determined on an X-4 digital display micromelting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Nicolet NEXUS 670 FT-IR spectrometer. NMR spectra were recorded on Varian Mercury-600BB NMR (600 MHz) and Varian Mercury plus-400 (400 MHz) spectrometers with TMS as internal standard. EIMS data were obtained on an HP5988AGCMS spectrometer. HRESIMS data were measured on a Bruker Daltonics APEX II 47e spectrometer. The X-ray crystallographic data were collected on a Bruker Smart CCD diffractometer using graphite-monochromated Mo Kα radiation. Silica gel (200−300 mesh) used for CC and silica gel GF_{254} (10−40 μ) used for TLC were supplied by Qingdao Marine Chemical Factory, Qingdao, P. R. China. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in EtOH (v/v).

Plant Material. The rhizome of *V. muliensis* was collected from Muli Autonomic County of Sichuan, China, in August 2004, and authenticated by Prof. Guoliang Zhang from the School of Life Science, Lanzhou University. A specimen (No. 20040814) was deposited at the Natural Product Laboratory of the College of Chemistry and Chemical Engineering, Lanzhou University.

Extraction and Isolation. The air-dried rhizome of *V. muliensis* (6.25 kg) was pulverized and extracted with MeOH (×3) for seven days each time at room temperature. The solvent was evaporated under reduced pressure to obtain an extract (618 g), which was suspended in hot H_2O (60 °C, 1.5 L). This suspension was extracted successively with petrol, EtOAc, and *n*-BuOH. The petrol-soluble fraction was concentrated under reduced pressure to afford a residue (190 g), which was subjected to silica gel column chromatography (200−300 mesh, 1200 g) with a gradient of petrol−EtOAc (100:0, 50:1, 30:1, 10:1, 5:1, 2:1) as eluent, and six fractions (A, B, C, D, E, and F) were collected according to TLC analysis. Fraction B was further separated by bioassay-guided fractionation on a silica gel column eluting again with petroleum ether−acetone (15:1), followed by Sephadex-LH20 eluted with MeOH−$CHCl_3$ (1:1) to afford, respectively, new compounds **1** (8 mg) and **2** (3 mg).

Vladimuliecin A (1) [(3*R*,5*R*,6*R*,8*S*,9*R*,10*R*,13*R*,14*R*,17*S*,20*R*)-6,20-diacetoxy-3,5-cyclopregnane-8,14-diol]: amorphous solid; $[\alpha]^{20}_D$ +31 (*c* 0.8, $CHCl_3$); IR (KBr) ν_{max} 3438, 2926, 1724, 1632, 1418, 1378, 1256, 1155, 1107, 1066, 704 cm^{-1}; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) see Table 1; EIMS *m/z* 434 [M]$^+$ (2), 374 (85), 359 (35), 314 (100), 299 (95), 296 (45), 273 (73), 175 (66), 161 (77), 147 (74), 121 (91), 93 (57), 91 (48), 79 (40), 55 (38); HRESIMS *m/z* 457.2560 [M + Na]$^+$ (457.2561 calcd for $C_{25}H_{38}O_6Na$).

Vladimuliecin B (2) [(3*R*,5*R*,6*R*,8*S*,9*R*,10*R*,13*R*,14*R*,17*S*,20R)-20-acetoxy-3,5-cyclopregnane-6,8,14-triol]: amorphous solid; $[\alpha]^{20}_D$ +9 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3435, 2927, 1720, 1631, 1419, 1376, 1250, 1155, 1105, 1066, 840, 550 cm^{-1}; ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR see Table 1; EIMS *m/z* 374 [M − H_2O]$^+$ (1), 352 (15), 338 (23), 324 (11), 299 (11), 295 (12), 281 (20), 265 (42), 238 (78), 135 (80), 121 (81), 93 (57), 71 (84), 43 (100); HRESMS *m/z* 415.2450 [M + Na]$^+$ (415.2455 calcd for $C_{23}H_{36}O_5Na$).

(3*R*,5*R*,6*R*,8*S*,9*R*,10*R*,13*R*,14*R*,17*S*,20*R*)-3,5-Cyclopregnane-6,8,14,20-tetraol (3): colorless crystals; mp 250−251 °C (acetone); $[\alpha]^{20}_D$ +12 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3437, 2927, 1640, 1449, 1382, 1289, 1242, 1065, 840, 554 cm^{-1}; ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR see Table 1; EIMS *m/z* 350 [M]$^+$ (28), 332 (37), 317 (16), 299 (30), 288 (25), 281 (11), 212 (27), 191 (32), 121 (78), 97 (79), 55 (84), 45 (100); HRESMS *m/z* 373.2342 [M + Na]$^+$ (373.2349 calcd for $C_{21}H_{34}O_4Na$).

3-*O*-MTPA Ester Derivatives. (*R*)-MTPA chloride (10 μL) was added to a solution of **1** (3 mg) in anhydrous pyridine (100 μL). After 6 h of reaction at room temperature, the mixture was diluted with 0.5 mL of 1 M $NaHCO_3$ and extracted with CH_2Cl_2. The residue of the extract afforded 3-*O*-(*S*)-MTPA ester by purification on TLC ($CHCl_3$/EtOAc = 5:1).

3-*O*-(*S*)-MTPA ester: amorphous solid; $[\alpha]^{20}_D$ −31 (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.557−7.383 (5H, m, ArH), 5.263 (1H, dq, J = 7.5, 6.0 Hz, H-20), 3.493 (1H, br s, H-6), 3.567 (1H, s, OMe), 2.338 (1H, dd, J = 14.4, 2.8 Hz, H-7a), 1.830 (2H, m, H-16), 1.832 (2H, m, H-17), 1.676 (1H, m, H-12b), 1.619 (2H, dm, J = 8.0 Hz, H-2), 1.570 (2H, dm, J = 10.0 Hz, H-15), 1.498 (1H, dd, J = 14.4, 3.2 Hz, H-7b), 1.331 (1H, m, H-12a), 1.318 (2H, m, H-11), 1.321 (3H,

Scheme 1. Possible Biosynthesis Pathway of **1** and **2**

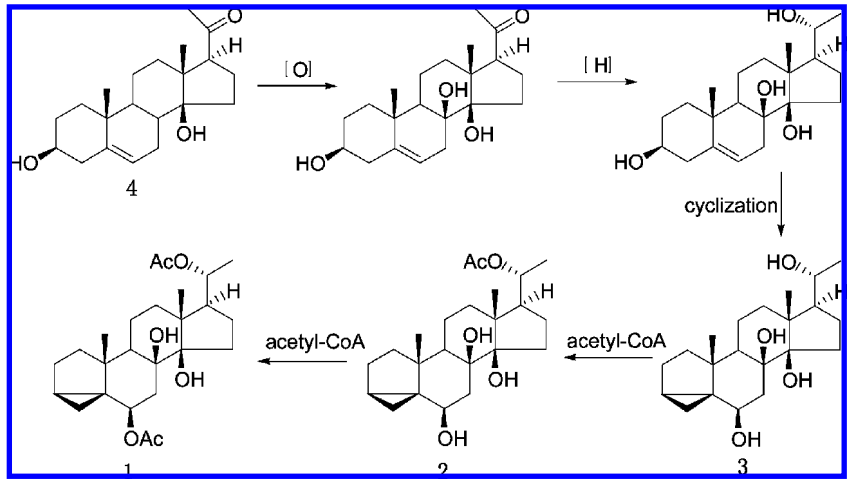


Table 2. IC_{50} Values for Cytotoxicity of Compounds **1**, **2**, and **3**

	IC_{50} (μM/L)		
compound	HL-60 cell	SMMC-7721 cell	HeLa cell
1	99.0 ± 11.0	98.6 ± 14.7	166.8 ± 21.6
2	88.0 ± 16.3	104.3 ± 15.0	120.9 ± 15.6
3	138.5 ± 23.1	100.6 ± 8.2	165.4 ± 23.4
mitomycin	1.5 ± 0.6	5.4 ± 1.4	3.3 ± 1.7

d, J = 6.6 Hz, H-21), 1.209 (1H, m, H-9), 1.103 (3H, s, H-18), 1.009 (3H, s, H-19), 1.074 (1H, m, H-3), 0.879 (1H, m, H-1a), 0.830 (1H, m, H-1b), 0.627 (1H, t, J = 4.8 Hz, H-4a), 0.418 (1H, dd, J = 7.8, 4.8 Hz, H-4b).

3-*O*-(*R*)-MTPA ester: prepared from **1** and (*S*)-MTPA chloride; amorphous solid; $[\alpha]^{20}_D$ +22 (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.524−7.399 (5H, m, ArH), 5.261 (1H, dq, J = 7.5, 6.3 Hz, H-20), 3.499 (4H, br s, H-6, OMe), 2.344 (1H, dd, J = 14.4, 3.0 Hz, H-7a), 1.846 (2H, m, H-16), 1.831 (2H, m, H-17), 1.680 (1H, m, H-12b), 1.627 (2H, dm, J = 8.0 Hz, H-2), 1.602 (2H, dm, J = 10.0 Hz, H-15), 1.496 (1H, dd, J = 14.4, 3.2 Hz, H-7b), 1.339 (1H, m, H-12a), 1.326 (2H, m, H-11), 1.269 (3H, d, J = 7.2 Hz, H-21), 1.215 (1H, m, H-9), 1.164 (3H, s, H-18), 1.097 (3H, s, H-19), 1.098 (1H, m, H-3), 0.881 (1H, m, H-1a), 0.831 (1H, m, H-1b), 0.632 (1H, t, J = 4.8 Hz, H-4a), 0.422 (1H, dd, J = 8.0, 4.8 Hz, H-4b).

X-ray Crystal Structure Analysis of 3. A crystal of **3** (CCDC710696) with dimensions 0.28 × 0.25 × 0.24 mm was selected for X-ray analysis. Structure analysis was performed using the SHELEXTL-97 program on a PC. Data were collected over a hemisphere of reciprocal space, by a combination of three sets of exposures. At T = 296(2) K the crystals were found to belong to the monoclinic space group $C2$, with a = 17.854(10) Å, b = 7.164(4) Å, c = 16.135(8) Å, β = 107.171(13)°, V = 1971.8(17) Å^3, and Z = 4. Multiscans as programmed in the Bruker SMART program were used to make data corrections. A total of 5159 reflections, collected in the range 2.36° ≤ θ ≤ 25.50°, yielded 3385 unique reflections. The structure was solved using direct methods and was refined by full-matrix least-squares on F^2 values for 3128 $I > 2\sigma(I)$. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were R = 0.0352, R_w = 0.1029 with goodness-of-fit = 0.783. Scattering factors were taken from International Tables for X-ray Crystallography.[16]

Biological Activity. The cytotoxicity of compounds **1**, **2**, and **3** toward human leukemia (HL-60), human hepatoma (SMMC-7721), and human cervical carcinoma (HeLa) cell lines was determined in 96-well microtiter plates by the sulforhodamine B method. Briefly, exponentially growing HL-60, SMMC-7721, and HeLa cells were harvested and seeded in 96-well plates with the final volume 100 μL containing 4 × 10^3 cells per well. After 24 h incubation, cells were treated with various concentrations of **1**, **2**, and **3** for 48 h. The cultures were fixed at 4 °C for 1 h by addition of ice-cold 50% trichloroacetic acid (TCA) to give a final concentration of 10%. Fixed cells were rinsed five times with deionized H_2O and stained for 10 min with 0.4% sulforhodamine B dissolved in 0.1% HOAc. The wells were washed five times with 0.1% HOAc and left to dry overnight. The absorbed sulforhodamine B was dissolved in 150 μL of unbuffered 1% Tris base [tris(hydroxymethyl)aminomethane] solution in H_2O (pH 10.5). The absorbance of extracted sulforhodamine B at 515 nm was measured on a microplate reader (Bio-Rad). The experiments were carried out in triplicate. Each run entailed five or six concentrations of the compounds being tested. The percentage survival rates of cells exposed to the compounds were calculated by assuming the survival rate of untreated cells to be 100%.

Acknowledgment. Financial support of the National Basic Research Program of China (No. 2007CB108903) and the National Natural Science Foundation of China (No. 20672052 and No. 20621091) is gratefully acknowledged.

Supporting Information Available: 1D, 2D NMR and HRESMS spectra of compounds **1**, **2**, and **3**; X-ray crystallographic data and optical rotation calculation details for compound **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod.* **2003**, *66*, 1022–1037.
(2) Saklani, A.; Kutty, S. K. *Drug Discovery Today* **2008**, *13*, 161–171.
(3) Fei, D. Q.; Li, S. G.; Liu, C. M.; Wu, G.; Gao, K. *J. Nat. Prod.* **2007**, *70*, 241–245.
(4) Liu, C. M.; Fei, D. Q.; Wu, Q. H.; Gao, K. *J. Nat. Prod.* **2006**, *69*, 695–699.
(5) Liu, C. M.; Wang, H. X.; Wei, S. L.; Gao, K. *J. Nat. Prod.* **2008**, *71*, 789–792.
(6) Chen, J. J.; Fei, D. Q.; Chen, S. G.; Gao, K. *J. Nat. Prod.* **2008**, *71*, 547–550.
(7) Plattner, J.; Gless, R.; Rapoport, H. *J. Am. Chem. Soc.* **1972**, *94*, 8613–8615.
(8) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
(9) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C. ; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. A.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T. ; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98, Revision A.9*; Gaussian, Inc.: Pittsburgh, PA, 1998.
(10) Weber, H.; Khorana, H. G. *J. Mol. Biol.* **1972**, *72*, 219–249.
(11) Partridge, J. J.; Faber, S.; Uskokovic, M. R. *Helv. Chim. Acta* **1974**, *57*, 764–771.

(12) Tang, P. P.; Yu, B. *Angew. Chem., Int. Ed.* **2007**, *46*, 2527–2530.
(13) Tsubuki, M.; Iwabuchi, K.; Honda, T. *Tetrahedron: Asymmetry* **2005**, *16*, 3913–3918.
(14) Schmuff, N. R.; Trost, B. M. *J. Org. Chem.* **1983**, *48*, 1404–1412.
(15) Skehan, P.; Storeng, R.; Scudiero, D. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
(16) Ibers, J. A.; Hamilton, W. C. *International Tables for X-Ray Crystallography*; The Kynoch Press: Birmingham, U.K., 1974; *IV*.

NP900120Q