Five New Polyoxygenated Cholestane Bisdesmosides from the Bulbs of *Galtonia candicans*

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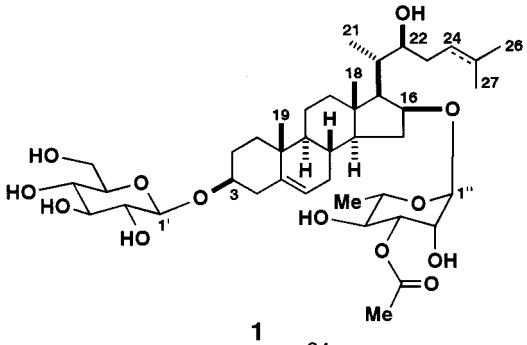
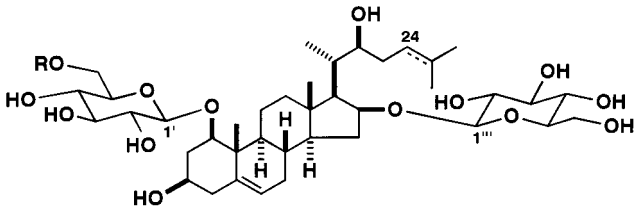
Two new cholestane bisdesmosides (**1**, **2**) based upon (22*S*)-cholest-5-ene-3β,16β,22-triol with an acetyl group at the sugar moiety and three new ones (**3**−**5**) based upon (22*S*)-cholest-5-ene-1β,3β,16β,22-tetrol, along with a known cholestane glycoside, were isolated from the bulbs of *Galtonia candicans*. The structures of the new compounds were determined by spectroscopic analysis and chemical transformations.

Galtonia candicans (Bak.) Dence. (Liliaceae) is a perennial plant native to South Africa and is taxonomically related to plants of the genus *Ornithogalum*, some of which were known to be poisonous plants. Although *G. candicans* has no medicinal folkloric background and is now cultivated for ornamental purposes, a MeOH extract of the bulbs showed considerable cytotoxic activity against HL-60 human promyelocytic leukemia cells.[1] We have reported the structural determination of a novel polyoxygenated 5β-cholestane diglycoside (galtonioside A) and a hexacyclic rearranged cholestane diglycoside (candicanoside A), isolated from *G. candicans* bulbs as the cytotoxic ingredients.[1] Further phytochemical analysis of the bulb extract led to the isolation of five new cholestane bisdesmosides (**1**−**5**), along with a known cholestane glycoside (**6**). This paper deals with the structural elucidation of the new compounds by spectroscopic analysis and chemical transformations.



1
2 Δ^{24}



	R	
3	H	Δ^{24}
4	β-D-Glc*p*	Δ^{24}
5	β-D-Glc*p*	
6	H	

Results and Discussion

The concentrated MeOH extract of the bulbs of *G. candicans* was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble phase was subjected to column chromatography on silica gel and octadecylsilanized (ODS) silica gel, as well as to preparative HPLC to give compounds **1**−**6**.

Compound **1** was obtained as an amorphous solid, $[\alpha]_D$ −72.0° (MeOH). The HRFABMS showed an $[M + Na]^+$ ion peak in accordance with the empirical molecular formula $C_{41}H_{68}O_{13}$, which was supported by the ^{13}C NMR spectrum and DEPT data. The ^1H NMR spectrum of **1** showed signals for five typical steroid methyl groups and two anomeric protons. A three-proton singlet signal at δ 1.94, which was correlated to the δ 21.1 resonance in the HMQC spectrum and to δ 176.6 in the HMBC spectrum, indicated the presence of an acetyl group in **1**. Treatment of **1** with 3% NaOMe in MeOH afforded a deacyl derivative (**1a**), and subsequent acid hydrolysis of **1a** with 1 M HCl in dioxane−H_2O (1:1) yielded (22*S*)-cholest-5-ene-3β,16β,22-triol as the aglycon,[2] and D-glucose and L-rhamnose as the carbohydrate moieties. These data implied that **1** was a cholestane diglycoside bearing an acetyl group. Sequential assignments from H-1 to H_2-6 and H-1 to Me-6 of the two monosaccharides, including their signal multiplet patterns and coupling constants, in the ^1H NMR spectrum of **1a**, were established by analysis of the $^1H-^1H$ COSY spectrum. The HMQC spectrum correlated these proton resonances with those of the corresponding one-bond coupled carbons. The assigned ^1H and ^{13}C NMR signals allowed the identification of a terminal β-D-glucopyranosyl group ($^4C_1$ form) and a terminal α-L-rhamnopyranosyl group ($^1C_4$ form) in **1a**. A multiplet proton signal centered at δ 3.94 ($W_{1/2}$ = 20.1 Hz) was shown to be coupled to the protons of two methylene groups and was assigned to H-3. The resonance at δ 4.40 (ddd, J = 7.5, 7.5, 4.1 Hz) was attributable to H-16, which had a long-range coupling with C-13 at δ 42.3. By the HMQC spectrum, the signals thus assigned to H-3 and H-16 were associated with the carbons resonating at δ 78.4 (C-3) and 82.5 (C-16), respectively, to which the sugars were linked. In the HMBC spectrum of **1a**, the anomeric proton of the glucosyl group at δ 5.11 showed a correlation peak with C-3, while that of the rhamnosyl group at δ 5.27 was correlated to C-16. The acetyl group was involved in an ester linkage at C-3 of the rhamnosyl group, since downfield shifts due to *O*-acylation could be recognized at the H-3 (+1.32 ppm) and C-3 (+3.7 ppm) signals in **1**, when the ^1H and ^{13}C NMR spectra of **1** were compared with those of **1a**. Thus, **1** was characterized as

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(22*S*)-3β-[(β-D-glucopyranosyl)oxy]-22-hydroxycholest-5-en-16β-yl 3-*O*-acetyl-α-L-rhamnopyranoside.

Compound **2** was isolated as an amorphous solid with a molecular formula of $C_{41}H_{66}O_{13}$, as determined by HRFABMS and ^{13}C NMR. Comparison of the ^1H and ^{13}C NMR spectra of **2** with those of **1** showed their considerable structural similarity. The differences consisted only in the signals of the methyl groups assignable to Me-26 and Me-27. The two doublet signals for Me-26 and Me-27 observed in the ^1H NMR spectrum of **1** were displaced by two three-proton singlet signals at δ 1.73 and 1.68 in that of **2**, suggesting that **2** was a C-24/C-25 dehydro derivative of **1**. This was confirmed by catalytic hydrogenation of **2** over Pt_2O in an H_2 atmosphere, resulting in the production of **1a**. The structure of **2** was formulated as (22*S*)-3β-[(β-D-glucopyranosyl)oxy]-22-hydroxycholesta-5,24-dien-16β-yl 3-*O*-acetyl-α-L-rhamnopyranoside.

Compound **3** was shown to have the molecular formula $C_{39}H_{64}O_{14}$ on the basis of HRFABMS. The spectral properties of **3** were very similar to those of the known compound **6** and were suggestive of a cholestane bisdesmoside structurally related to **6**. The ^1H NMR spectrum of **3** showed two three-proton singlet signals at δ 1.74 and 1.67 attributable to methyl groups on a double bond, and catalytic hydrogenation of **3** over Pt_2O in an H_2 atmosphere yielded **6**. Thus, **3** was defined as (22*S*)-1β-[(β-D-glucopyranosyl)-oxy]-3β,22-dihydroxycholesta-5,24-dien-16β-yl β-D-glucopyranoside.

The molecular formula of compound **4** was deduced as $C_{45}H_{74}O_{19}$ (HRFABMS). The ^1H and ^{13}C NMR spectral data of **4** identified that the aglycon was identical with that of **3**. The ^1H NMR spectrum of **4** displayed signals for three anomeric protons at δ 5.33 (d, J = 7.7 Hz), 4.99 (d, J = 7.7 Hz), and 4.65 (d, J = 7.7 Hz), as well as signals for five steroid methyl groups. Acid hydrolysis of **4** with 1 M HCl in dioxane$-H_2O$ (1:1) gave D-glucose. Analysis of the $^1H-^1H$ COSY spectrum, starting from each anomeric proton, and of the HMQC spectrum of **4** led to the assignment of all the proton and carbon signals due to the sugar moieties. The ^1H and ^{13}C NMR shifts thus assigned were indicative of two terminal β-D-glucopyranosyl units and a nodal β-D-glucopyranosyl residue glycosylated at C-6. In the HMBC spectrum, the signal at δ 4.04 (br d, J = 12.0 Hz) showed long-range correlations with C-9 (δ 50.3), C-10 (δ 42.7), and C-19 (δ 14.7) and was assigned to H-1. The resonance at δ 4.63 (ddd, J = 7.5, 7.5, 4.2 Hz) was assigned to H-16, which had a long-range coupling with C-13 at δ 42.3. The signals due to H-1 and H-16 were correlated to the respective carbons resonating at δ 83.3 (C-1) and 82.7 (C-16) in the HMQC spectrum, to which the sugars were linked. The anomeric proton signal of one terminal glucosyl moiety at δ 4.90 showed a long-range correlation with C-16 of the aglycon, whereas that of another terminal glucosyl residue at δ 5.33 gave an HMBC correlation with the δ 70.4 resonance (CH_2) assignable to C-6 of the substituted glucosyl group, whose anomeric proton, in turn, was correlated to the aglycon C-1. All of these data were consistent with the structure (22*S*)-1β-[(*O*-β-D-glucopyranosyl-(1$\rightarrow$6)-β-D-glucopyranosyl)oxy]-3β,22-dihydroxycholesta-5,24-dien-16β-yl β-D-glucopyranoside, which was given to **4**.

Compound **5** exhibited a molecular formula of $C_{45}H_{76}O_{19}$ based on HRFABMS. The ^1H NMR, which showed signals for five steroid methyls and three anomeric protons, and ^{13}C NMR spectra of **5** suggested that it was a cholestane bisdesmoside closely related to **4**. Acid hydrolysis of **5** with 1 M HCl in dioxane$-H_2O$ (1:1) yielded a known polyhydroxylated cholestane derivative, identified as (22*S*)-cholest-5-ene-1β,3β,16β,22-tetrol,[3] and D-glucose. Catalytic hydrogenation of **4** over Pt_2O in an H_2 atmosphere gave **5**. Thus, the structure of **5** was assigned as (22*S*)-1β-[(*O*-β-D-glucopyranosyl-(1$\rightarrow$6)-β-D-glucopyranosyl)oxy]-3β,22-dihydroxycholest-5-en-16β-yl β-D-glucopyranoside.

Compound **6** was identified as (22*S*)-1β-[(β-D-glucopyranosyl)oxy]-3β,22-dihydroxycholest-5-en-16β-yl β-D-glucopyranoside, which was isolated by us from *Nolina recurvata* (Agavaceae).[3]

Compounds **1**$-$**6** were evaluated for their cytotoxic activities against HL-60 human promyelocytic leukemia cells. Only **2** showed moderate cytotoxicity with an IC_{50} value of 6.8 μg/mL. Etoposide used as a positive control gave an IC_{50} of 0.25 μg/mL.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^1H NMR, Karlsruhe, Germany) spectrometer using standard Bruker pulse programs. MS were recorded on a Finnigan MAT TSQ-700 (San Jose, CA) mass spectrometer, using a dithiothreitol and dithioerythritol (3:1) matrix. Sephadex LH-20 (Pharmacia, Uppsala, Sweden), silica gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. HPLC was performed using a system comprised of a Tosoh CCPM pump (Tokyo, Japan), a Tosoh CCP PX-8010 controller, a Tosoh UV-8000 or an Tosoh RI-8010 detector, and Rheodyne injection port with a 2 mL sample loop for preparative HPLC and a 20 μL sample loop for analytical HPLC. A Capcell Pak C_{18} column (10 mm i.d. $\times$ 250 mm, 5 μm, Shiseido, Tokyo, Japan) was used for preparative HPLC, and a Capcell Pak C_{18} column (4.6 mm i.d. $\times$ 250 mm, 5 μm, Shiseido) was employed for analytical HPLC. The following materials and reagents were used for bioassays: microplate reader, Inter Med Immuno-Mini NJ-2300 (Tokyo, Japan); 96-well flat-bottom plate, Iwaki Glass (Chiba, Japan); HL-60 cells, ICN Biomedicals (Costa Mesa, CA); RPMI 1640 medium, GIBCO BRL (Rockville, MD); MTT, Sigma (St. Louis, MO).

Plant Material. The bulbs of *G. candicans* were purchased from a nursery in Heiwaen, Nara, Japan. The bulbs were cultivated, and the flowered plant was identified by one of the authors (Y.S.). A voucher specimen of the plant is on file in our laboratory (97-7-GC-F).

Extraction and Isolation. The plant material (fresh weight, 5.5 kg) was extracted twice with hot MeOH. The MeOH extract was concentrated under reduced pressure, and the viscous concentrate was partitioned between *n*-BuOH and H_2O. Column chromatography of the *n*-BuOH phase on silica gel and elution with a stepwise gradient mixture of $CHCl_3-$MeOH (9:1; 4:1; 2:1) and finally with MeOH alone gave four fractions (I$-$IV). Fraction II was chromatographed on silica gel eluting with $CHCl_3-$MeOH (9:2) and ODS silica gel with MeOH$-H_2O$ (3:1) to give a mixture of **1** and **2**, which were separated by preparative HPLC using MeCN$-H_2O$ (9:11) to furnish **1** (15.0 mg) and **2** (75.0 mg). Fraction III was subjected to column chromatography on silica gel using $CHCl_3-$MeOH$-H_2O$ (20:10:1) and was further divided into four fractions (IIIa$-$IIId). Fraction IIIc was chromatographed on silica gel eluting with $CHCl_3-$MeOH$-H_2O$ (40:10:1), ODS silica gel with MeOH$-H_2O$ (7:3; 13:7; 3:2), and Sephadex LH-20 with MeOH to give a mixture of **3** and **6**, separation of which was carried out by preparative HPLC using MeOH$-H_2O$ (3:2) to afford **3** (47.8 mg) and **6** (12.4 mg). Compounds **4** (36.0 mg) and **5** (13.5 mg) were isolated from fraction IIId by subjecting it to silica gel column chromatography eluting with $CHCl_3-$MeOH$-H_2O$ (20:10:1), ODS silica gel column chromatography with MeOH$-H_2O$ (7:3), and preparative HPLC using MeOH$-H_2O$ (3:2).

Compound 1: amorphous solid; $[\alpha]^{25}_D$ $-72.0°$ (*c* 0.10, MeOH); IR (KBr) ν_{max} 3420 (OH), 2940 and 2860 (CH), 1720

(C=O), 1460, 1375, 1365, 1255, 1060, 1045, 975, 825 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.76 (1H, dd, J = 9.6, 3.3 Hz, H-3″), 5.34 (1H, br d, J = 4.6 Hz, H-6), 5.24 (1H, br s, H-1″), 5.05 (1H, d, J = 7.7 Hz, H-1′), 1.94 (3H, s, Ac), 1.70 (3H, d, J = 6.1 Hz, Me-6″), 1.22 (3H, d, J = 6.9 Hz, Me-21), 0.96 (3H, s, Me-19), 0.93 (3H, s, Me-18), 0.92 (3H, d, J = 6.3 Hz, Me-26), 0.91 (3H, d, J = 6.3 Hz, Me-27); ^{13}C NMR (C_5D_5N) δ 37.5 (C-1), 30.3 (C-2), 78.5 (C-3), 39.4 (C-4), 141.1 (C-5), 121.8 (C-6), 32.1 (C-7), 31.9 (C-8), 50.4 (C-9), 37.0 (C-10), 21.2 (C-11), 40.1 (C-12), 42.3 (C-13), 55.0 (C-14), 35.6 (C-15), 82.5 (C-16), 57.9 (C-17), 13.2 (C-18), 19.5 (C-19), 36.2 (C-20), 12.1 (C-21), 72.8 (C-22), 34.4 (C-23), 37.0 (C-24), 28.8 (C-25), 22.8 (C-26), 22.9 (C-27), 102.6 (C-1′), 75.4 (C-2′), 78.7 (C-3′), 71.9 (C-4′), 78.2 (C-5′), 63.0 (C-6′), 104.8 (C-1″), 71.0 (C-2″), 76.3 (C-3″), 71.2 (C-4″), 70.5 (C-5″), 18.5 (C-6″), 170.6 and 21.1 (Ac); FABMS (negative mode) m/z 767 $[M - H]^-$, 725 $[M -$ acetyl$]^-$, 606 $[M - H -$ glucosyl$]^-$; HRFABMS (positive mode) m/z 791.4568 $[M + Na]^+$ (calcd for $C_{41}H_{68}O_{13}Na$, 791.4558).

Alkaline Methanolysis of 1. Compound **1** (10.0 mg) was treated with 3% NaOMe in MeOH (2 mL) at room temperature for 1 h. The reaction mixture was passed through an Amberlite IR-120B (Organo, Tokyo, Japan) column and fractionated using silica gel column chromatography eluting with $CHCl_3$–MeOH (4:1) to give a deacyl derivative (**1a**) (8.0 mg).

Compound 1a: amorphous solid; $[\alpha]^{25}_D$ −60.0° (c 0.10, MeOH); IR (KBr) ν_{max} 3400 (OH), 2940 (CH), 1450, 1375, 1245, 1120, 1040, 1010, 975, 905 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.34 (1H, br d, J = 4.6 Hz, H-6), 5.27 (1H, br s, H-1″), 5.11 (1H, d, J = 7.7 Hz, H-1′), 4.85 (1H, dq, J = 8.5, 6.1 Hz, H-5″), 4.62 (1H, dd, J = 11.8, 2.4 Hz, H-6′a), 4.49 (1H, br d, J = 3.1 Hz, H-2″), 4.47 (1H, dd, J = 11.8, 5.3 Hz, H-6′b), 4.44 (1H, dd, J = 8.5, 3.1 Hz, H-3″), 4.40 (1H, ddd, J = 7.5, 7.5, 4.1 Hz, H-16), 4.35 (1H, dd, J = 8.8, 8.8 Hz, H-3′), 4.35 (1H, dd, J = 8.5, 8.5 Hz, H-4″), 4.32 (1H, dd, J = 8.8, 8.8 Hz, H-4′), 4.16 (1H, br dd, J = 8.1, 5.0 Hz, H-22), 4.11 (1H, dd, J = 8.8, 7.7 Hz, H-2′), 4.04 (1H, ddd, J = 8.8, 5.3, 2.4 Hz, H-5′), 3.94 (1H, br m, $W_{1/2}$ = 20.1 Hz, H-3), 1.70 (3H, d, J = 6.1 Hz, Me-6″), 1.22 (3H, d, J = 6.9 Hz, Me-21), 0.96 (3H, s, Me-19), 0.93 (3H, s, Me-18), 0.92 (3H, d, J = 6.3 Hz, Me-26), 0.91 (3H, d, J = 6.3 Hz, Me-27); ^{13}C NMR (C_5D_5N) δ 37.5 (C-1), 30.3 (C-2), 78.4 (C-3), 39.4 (C-4), 141.1 (C-5), 121.8 (C-6), 32.1 (C-7), 31.9 (C-8), 50.4 (C-9), 37.0 (C-10), 21.2 (C-11), 40.1 (C-12), 42.3 (C-13), 55.1 (C-14), 35.6 (C-15), 82.5 (C-16), 57.9 (C-17), 13.2 (C-18), 19.5 (C-19), 36.2 (C-20), 12.2 (C-21), 72.8 (C-22), 34.4 (C-23), 37.1 (C-24), 28.8 (C-25), 22.8 (C-26), 22.9 (C-27), 102.6 (C-1′), 75.4 (C-2′), 78.7 (C-3′), 71.9 (C-4′), 78.2 (C-5′), 63.0 (C-6′), 104.9 (C-1″), 72.0 (C-2″), 72.6 (C-3″), 74.0 (C-4″), 70.9 (C-5″), 18.4 (C-6″); FABMS (negative mode) m/z 725 $[M - H]^-$.

Acid Hydrolysis of 1. A solution of **1** (6.5 mg) in 1 M HCl (dioxane–H_2O, 1:1, 2 mL) was heated at 95 °C for 2 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized using an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA), eluting with H_2O–MeOH (4:1, 10 mL) followed by MeOH (10 mL), to give a sugar fraction (1.5 mg) and an aglycon fraction. The aglycon fraction was chromatographed on silica gel eluting with $CHCl_3$–MeOH (19:1) to give an aglycon (**1b**) (2.5 mg). The sugar fraction was dissolved in H_2O (1 mL), to which (−)-α-methylbenzylamine (5 mg) and Na[BH_3CN] (8 mg) in EtOH (1 mL) were added. After being set aside at 40 °C for 4 h followed by addition of AcOH (0.2 mL) and evaporation to dryness, the reaction mixture was acetylated with Ac_2O (0.3 mL) in pyridine (0.3 mL) at 40 °C for 12 h. The crude mixture was passed through a Sep-Pak C_{18} cartridge with H_2O–MeCN (4:1; 1:1, each 10 mL) mixtures as solvents. The H_2O–MeCN (1:1) eluate was further passed through a Toyopak IC-SP M cartridge (Tosoh, Tokyo, Japan) with EtOH (10 mL) to give a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides,[4] which was then analyzed by HPLC under the following conditions: solvent, MeCN–H_2O (2:3); flow rate, 0.8 mL/min; detection, UV 230 nm. The derivatives of L-rhamnose and D-glucose were detected as follows: t_R (min) 18.52 (derivative of D-glucose); 20.56 (derivative of L-rhamnose).

Compound 2: amorphous solid; $[\alpha]^{25}_D$ −78.0° (c 0.10, MeOH); IR (KBr) ν_{max} 3410 (OH), 2935 and 2860 (CH), 1725 (C=O), 1460, 1440, 1380, 1365, 1245, 1110, 1040, 1010, 970, 880 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.78 (1H, dd, J = 9.7, 3.0 Hz, H-3″), 5.59 (1H, br t, J = 7.0 Hz, H-24), 5.38 (1H, br d, J = 4.8 Hz, H-6), 5.22 (1H, br s, H-1″), 5.06 (1H, d, J = 7.7 Hz, H-1′), 1.94 (3H, s, Ac), 1.73 (3H, s, Me-26), 1.70 (3H, d, J = 6.1 Hz, Me-6″), 1.68 (3H, s, Me-27), 1.25 (3H, d, J = 6.9 Hz, Me-21), 0.96 (3H, s, Me-19), 0.91 (3H, s, Me-18); ^{13}C NMR (C_5D_5N) δ 37.5 (C-1), 30.3 (C-2), 78.4 (C-3), 39.4 (C-4), 141.1 (C-5), 121.8 (C-6), 32.1 (C-7), 31.9 (C-8), 50.4 (C-9), 37.0 (C-10), 21.1 (C-11), 40.1 (C-12), 42.2 (C-13), 54.8 (C-14), 35.6 (C-15), 82.3 (C-16), 57.9 (C-17), 13.1 (C-18), 19.5 (C-19), 35.4 (C-20), 12.0 (C-21), 72.2 (C-22), 35.4 (C-23), 123.5 (C-24), 132.3 (C-25), 25.9 (C-26), 18.1 (C-27), 102.6 (C-1′), 75.4 (C-2′), 78.6 (C-3′), 71.8 (C-4′), 78.1 (C-5′), 62.8 (C-6′), 104.9 (C-1″), 71.0 (C-2″), 76.3 (C-3″), 71.2 (C-4″), 70.4 (C-5″), 18.4 (C-6″), 170.7 and 21.1 (Ac); FABMS (negative mode) m/z 765 $[M - H]^-$, 723 $[M -$ acetyl$]^-$, 603 $[M - H -$ glucosyl$]^-$; HRFABMS (positive mode) m/z 789.4412 $[M + Na]^+$ (calcd for $C_{41}H_{66}O_{13}Na$, 789.4401).

Catalytic Hydrogenation of 2. A mixture of **2** (5.0 mg) and PtO_2 (5.0 mg) was stirred under an H_2 atmosphere at room temperature for 12 h. The reaction mixture, after removal of the catalyst by filtration, was subjected to a silica gel column eluting with $CHCl_3$–MeOH (4:1) to give **1a** (3.2 mg).

Compound 3: amorphous solid; $[\alpha]^{25}_D$ −38.0° (c 0.10, MeOH); IR (KBr) ν_{max} 3380 (OH), 2910 (CH), 1725 (C=O), 1435, 1370, 1340, 1250, 1150, 1060, 1000, 970 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.50 (1H, br d, J = 5.1 Hz, H-6), 4.98 (1H, d, J = 7.7 Hz, H-1′), 4.75 (1H, d, J = 7.6 Hz, H-1″), 1.74 (3H, s, Me-26), 1.67 (3H, s, Me-27), 1.24 (3H, s, Me-19), 1.19 (3H, d, J = 7.0 Hz, Me-21), 1.02 (3H, s, Me-18); ^{13}C NMR (C_5D_5N) δ 82.8 (C-1), 37.6 (C-2), 68.0 (C-3), 43.7 (C-4), 139.5 (C-5), 124.8 (C-6), 31.8 (C-7), 33.1 (C-8), 50.3 (C-9), 42.8 (C-10), 23.8 (C-11), 40.5 (C-12), 42.1 (C-13), 55.2 (C-14), 37.1 (C-15), 82.6 (C-16), 58.1 (C-17), 13.8 (C-18), 14.8 (C-19), 35.5 (C-20), 12.5 (C-21), 72.8 (C-22), 34.8 (C-23), 124.7 (C-24), 131.4 (C-25), 26.0 (C-26), 18.3 (C-27), 101.3 (C-1′), 75.4 (C-2′), 78.8 (C-3′), 72.4 (C-4′), 78.2 (C-5′), 63.6 (C-6′), 106.8 (C-1″), 75.7 (C-2″), 78.6 (C-3″), 71.7 (C-4″), 78.1 (C-5″), 62.9 (C-6″); FABMS (negative mode) m/z 755 $[M - H]^-$, 594 $[M - H -$ glucosyl$]^-$, 431 $[M - H -$ glucosyl × 2$]^-$; HRFABMS (positive mode) m/z 779.4188 $[M + Na]^+$ (calcd for $C_{39}H_{64}O_{14}Na$, 779.4194).

Catalytic Hydrogenation of 3. Compound **3** (5.0 mg) was subjected to catalytic hydrogenation as described for **2** to give **6** (3.5 mg).

Compound 4: amorphous solid; $[\alpha]^{25}_D$ −14.0° (c 0.10, MeOH); IR (KBr) ν_{max} 3375 (OH), 2920 and 2870 (CH), 1435, 1375, 1340, 1150, 1040, 970 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.84 (1H, br t, J = 6.9 Hz, H-24), 5.43 (1H, br d, J = 5.1 Hz, H-6), 5.33 (1H, d, J = 7.7 Hz, H-1″), 4.90 (1H, d, J = 7.7 Hz, H-1′), 4.89 (1H, br d, J = 11.8 Hz, H-6′a), 4.65 (1H, d, J = 7.7 Hz, H-1‴), 4.63 (1H, ddd, J = 7.5, 7.5, 4.2 Hz, H-16), 4.60 (1H, dd, J = 11.4, 2.4 Hz, H-6″a), 4.50 (1H, m, H-22), 4.48 (1H, dd, J = 11.5, 2.4 Hz, H-6‴a), 4.42 (1H, dd, J = 11.5, 4.7 Hz, H-6‴b), 4.39 (1H, dd, J = 11.4, 5.0 Hz, H-6″b), 4.35 (1H, dd, J = 8.8, 8.8 Hz, H-3″), 4.33 (1H, dd, J = 11.8, 4.6 Hz, H-6′b), 4.30 (1H, dd, J = 8.9, 8.9 Hz, H-4‴), 4.25 (1H, dd, J = 8.8, 8.8 Hz, H-4″), 4.19 (1H, ddd, J = 9.9, 5.0, 2.4 Hz, H-5″), 4.16 (1H, dd, J = 9.3, 9.3 Hz, H-3′), 4.16 (1H, dd, J = 8.9, 8.9 Hz, H-3‴), 4.08 (1H, dd, J = 8.8, 7.7 Hz, H-2″), 4.06 (1H, br dd, J = 9.3, 4.6 Hz, H-5′), 4.04 (1H, br d, J = 12.0 Hz, H-1), 4.03 (1H, dd, J = 9.3, 7.7 Hz, H-2′), 4.02 (1H, dd, J = 8.9, 7.7 Hz, H-2‴), 3.91 (1H, dd, J = 9.3, 9.3 Hz, H-4′), 3.90 (1H, br m, $W_{1/2}$ = 21.0 Hz, H-3), 3.76 (1H, dd, J = 8.9, 4.7, 2.4 Hz, H-5‴), 1.72 (3H, s, Me-26), 1.66 (3H, s, Me-27), 1.25 (3H, s, Me-19), 1.23 (3H, d, J = 7.0 Hz, Me-21), 1.07 (3H, s, Me-18); ^{13}C NMR (C_5D_5N) δ 83.3 (C-1), 38.0 (C-2), 68.0 (C-3), 43.9 (C-4), 139.1 (C-5), 124.6 (C-6), 31.6 (C-7), 33.3 (C-8), 50.3 (C-9), 42.7 (C-10), 24.0 (C-11), 40.6 (C-12), 42.3 (C-13), 54.9 (C-14), 37.1 (C-15), 82.7 (C-16), 58.1 (C-17), 13.9 (C-18), 14.7 (C-19), 35.6 (C-20), 12.4 (C-21), 72.9 (C-22), 34.8 (C-23), 125.2 (C-24), 131.4 (C-25), 26.0 (C-26), 18.3 (C-27), 101.5 (C-1′), 75.3 (C-2′), 78.0 (C-3′), 72.0 (C-4′), 77.7 (C-5′), 70.4 (C-6′), 105.5 (C-1″), 75.5 (C-2″), 78.4

(C-3″), 72.0 (C-4″), 78.6 (C-5″), 63.0 (C-6″), 106.7 (C-1‴), 75.7 (C-2‴), 78.4 (C-3‴), 71.8 (C-4‴), 78.7 (C-5‴), 63.0 (C-6‴); FABMS (negative mode) *m/z* 917 $[M - H]^-$; HRFABMS (positive mode) *m/z* 941.4731 $[M + Na]^+$ (calcd for $C_{45}H_{74}O_{19}Na$, 941.4722).

Acid Hydrolysis of 4. Compound **5** (5.0 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction (2.5 mg). The monosaccharide constituent in the fraction was converted to the corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative, which was then analyzed by HPLC. The derivative of D-glucose was detected at a t_R of 18.52 min.

Compound 5: amorphous solid; $[\alpha]^{25}_D$ −46.0° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3380 (OH), 2920 (CH), 1460, 1440, 1380, 1345, 1155, 1065, 1015 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.43 (1H, br d, J = 5.2 Hz, H-6), 5.33 (1H, d, J = 7.7 Hz, H-1″), 4.90 (1H, d, J = 7.7 Hz, H-1′), 4.65 (1H, d, J = 7.7 Hz, H-1‴), 1.26 (3H, s, Me-19), 1.20 (3H, d, J = 6.9 Hz, Me-21), 1.09 (3H, s, Me-18), 0.92 (3H, d, J = 6.2 Hz, Me-26), 0.91 (3H, d, J = 6.2 Hz, Me-27); ^{13}C NMR (C_5D_5N) δ 83.3 (C-1), 38.1 (C-2), 68.0 (C-3), 43.9 (C-4), 139.1 (C-5), 125.3 (C-6), 31.7 (C-7), 33.3 (C-8), 50.3 (C-9), 42.7 (C-10), 24.0 (C-11), 40.7 (C-12), 42.3 (C-13), 55.0 (C-14), 37.3 (C-15), 82.8 (C-16), 58.2 (C-17), 13.9 (C-18), 14.7 (C-19), 36.1 (C-20), 12.5 (C-21), 73.4 (C-22), 33.9 (C-23), 36.7-(C-24), 28.9 (C-25), 23.0 (C-26), 23.1 (C-27), 101.5 (C-1′), 75.3 (C-2′), 78.0 (C-3′), 72.1 (C-4′), 77.7 (C-5′), 70.4 (C-6′), 105.5 (C-1″), 75.5 (C-2″), 78.4 (C-3″), 72.0 (C-4″), 78.7 (C-5″), 63.0 (C-6″), 106.9 (C-1‴), 75.6 (C-2‴), 78.4 (C-3‴), 71.8 (C-4‴), 78.7 (C-5‴), 63.0 (C-6‴); FABMS (negative mode) *m/z* 919 $[M - H]^-$; HRFABMS (positive mode) *m/z* 943.4885 $[M + Na]^+$ (calcd for $C_{45}H_{76}O_{19}Na$, 941.4879).

Acid Hydrolysis of 5. Compound **5** (5.0 mg) was subjected to acid hydrolysis as described for **1** to give an aglycon (**5a**) (1.2 mg) and a sugar fraction (2.5 mg). The monosaccharide constituent in the fraction was converted to the corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative, which was then analyzed by HPLC. The derivative of D-glucose was detected at a t_R of 18.50 min.

Catalytic Hydrogenation of 4. Compound **4** (5.0 mg) was subjected to catalytic hydrogenation as described for **2** to give **5** (3.6 mg).

HL-60 Cell Culture Assay. HL-60 leukemia cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum supplemented with L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. The cells (3×10^4 cells/mL) were continuously treated with each compound for 72 h, and the cell growth was measured with an MTT reduction assay procedure.[5] A dose response curve was plotted for compound **2**, and the concentration giving 50% inhibition (IC_{50}) was calculated.

References and Notes

(1) Kuroda, M.; Mimaki, Y.; Sashida, Y.; Yamori, T.; Tsuruo, T. *Tetrahedron Lett.* **2000**, *41*, 251−255. Kuroda, M.; Mimaki, Y.; Sashida, Y.; Yamori, T.; Tsuruo, T. *Helv. Chim. Acta* **2000**, *83*, 2698−2704.
(2) Kawashima, K.; Mimaki, Y.; Sashida, Y. *Chem. Pharm. Bull.* **1991**, *39*, 2761−2763.
(3) Takaashi, Y.; Mimaki, Y.; Kameyama, A.; Kuroda, M.; Nikaido, T.; Koike, K.; Ohmoto, T. *Chem. Pharm. Bull.* **1995**, *43*, 1180−1185.
(4) Oshima, R.; Yamauchi, Y.; Kumanotani, J. *Carbohydr. Res.* **1982**, *107*, 169−176.
(5) Sargent, J. M.; Taylor, C. G. *Br. J. Cancer* **1989**, *60*, 206−210.

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