Supporting information

leodoglucomides A and B from a Marine-Derived Bacterium *Bacillus licheniformis*

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S1. Experimental Details

General Experimental Procedures. Optical rotations were measured with a JASCO (DIP-1000) digital polarimeter. IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. 1D and 2D NMR spectra were acquired on a Varian Unity 500 spectrometer. Low resolution LC/ESI-MS data were measured using Thermo Finnigan Surveyor LC/MS system with a reversed-phase C18 column at a flow rate of 0.4 mL/min. High-resolution mass spectra were recorded on a hybrid ion-trap time-of-flight mass spectrometer (Shimadzu LC/MS-IT-TOF). Analytical HPLC was conducted with a PrimeLine Binary pump with RI-101(Shodex) and Variable UV Detector (M 525). Semi-preparative HPLC was performed using ODS (YMC-Pack-ODS-A, 250 × 10 mm i.d, 5 μ m. All solvents used were either spectral grade or distilled prior to use.

Isolation and Taxonomy of the Strain 09IDYM23. The sediment sample was collected from Jeodo, Republic of Korea's southern reef, during an expedition in 2009. About one gram (wet) of the sediment sample was diluted in sterilized sea water (10^{-1} , 10^{-2} , 10^{-3} and 10^{-4}) aseptically and 100 μ L from each dilution was spread onto modified Bennett's agar medium plate (1% dextrose, 0.2% tryptone, 0.1% yeast extract, 0.1% beef extract, 1.8% agar and pH 7.02 for 1 L Sea water with salt concentration 18.3 g/L). The plates were incubated for 5 days at 32 °C, and the resulting colony of the strain 09IDYM23 was isolated and stocked into glycerol: Sea water (v/v, 2:3) solution. Later the strain was identified as *Bacillus licheniformis* on the basis of 16S rRNA sequencing (GenBank Accession no. JQ349055). This strain is currently stocked in the Microbial Culture Collection, KORDI, with the ID of 09IDYM23 under the curatorship of Dr. Hee Jae Shin.

Seed and Large-scale Cultures of the Strain 09IDYM23. A single colony of the strain from the agar plate was inoculated aseptically into three flasks (100 ml) containing 50 mL Bennett's medium (composition same as above) and incubated at 32 °C for 2 days on a rotary shaker at 110 rpm for seed culture. An aliquot (0.2%, v/v) from the seed culture was inoculated into 2 L flasks (total 42 flasks) containing 1.2 L sterilized culture medium for large scale culture. The production culture was incubated maintaining the same conditions as the seed culture for 10 days and then harvested.

Extraction and Isolation. The culture broth (50 L) was centrifuged and the supernatant was extracted with EtOAc (2 × 50 L). The EtOAc layer was evaporated under reduced pressure at 40 °C. The crude extract (5.6 g) was subjected to an ODS open column chromatography followed by stepwise gradient elution with MeOH: H₂O (v/v) (1:4, 2:3, 3:2, 4:1 and 5:0) as eluent. The fraction eluted with MeOH: H₂O (3:2, v/v) again subjected to a further purification by reversed-phase MPLC and HPLC respectively to yield pure compounds **1** (6.0 mg) and **2** (3.1 mg).

Ieodoglucomide A (1): yellowish oil; $[\alpha]_D^{23} +22$ (c 0.2, MeOH); IR (MeOH) $\nu_{\max}$ 3349 cm^{-1} (brd), 1660 cm^{-1} ; ¹H and ¹³C NMR data (CD₃OD), see Table 1; HRESIMS [M-H]⁻ at *m/z* 618.3497 (Calcd, 619.7413 for C₃₀H₅₃NO₁₂).

Ieodoglucomide B (2): yellowish oil; $[\alpha]_D^{23} +24$ (c 0.2, MeOH); IR (MeOH) $\nu_{\max}$ 3349 cm^{-1} (brd), 1660 cm^{-1} , ¹H and ¹³C NMR data (CD₃OD), see Table 1; HRESIMS [M-H]⁻ at *m/z* 604.3337 (calcd . 605.7147 for C₂₉H₅₁NO₁₂).

Absolute Stereochemistry of Compounds 1 and 2. Marfey's method was performed to persuade the absolute configuration of Ala in **1**. Stereochemistry at C-14 of **1** and **2** was determined by Mosher's method. β -D configuration of Glu was confirmed by TLC comparison with authentic sample and literature review for compounds **1** and **2**.

Acid Hydrolysis. 3.5 mg of compound **1** was taken in a 8 mL glass vial and hydrolyzed by heating with 600 μ L 1N HCL at 110 °C for 12 h. Upon removal of excess HCL under reduced pressure the reaction mixer was extracted sequentially using hexane, ethyl acetate and methanol. HMA (1.8 mg), Ala (0.5 mg) and Glu (0.6 mg) were obtained from hexane, ethyl acetate and aqueous methanol fractions, respectively which were confirmed from ¹H NMR spectrum and ESI-MS results *m/z* at 286.28, 88.08, and 180.06, respectively. Compound **2** was also hydrolyzed following the same procedure as for **1** to obtain HMA (1.6 mg) and Glu (0.7 mg).

Derivatization using Marfey's Reagent. Ala of compound **1** was taken into a 4 mL glass vial and 0.1% solution (100 μ L) of 1-fluoro-2,4- dinitrophenyl-5-L-alanine amide (FDAA, Marfey's reagent) in acetone, followed by 1.0N NaHCO₃ (20 μ L) was added, heated at 40 °C for 1 h, cooled to room temperature (rt), and then neutralized with 1N HCl. In a similar fashion, standard L-Ala and D-Ala were derivatized separately with FDAA reagent. The derivatives of the acid hydrolysis and the standard amino acids were subjected to RP-HPLC analysis (YMC ODS-A column, 50 mm, 2.0 mm) at 25 °C using the following gradient program: solvent A, MeOH with 0.1% TFA; solvent B, CH₃CN with 0.1% TFA; linear gradient, 10–40% of A for 40 min with UV detection at 340 nm; flow rate: 0.5 mL/ min. The retention times for the FDAA derivatives of L-Ala and D-Ala were 27.4 and 28.8 min, respectively, whereas that for the FDAA derivatives of Ala of compound **1** was 27.4 min.

Esterification using Mosher's Reagent. (S)-MTPA esters (1a and 1b) of 1. HMA (0.9 mg) obtained from compound **1** was dissolved in 150 μ L pyridine and stirred at rt for 10 min. 20 μ L (*R*)-(–)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) was added to the reaction vial and the mixture was stirred at rt for 16 h for the preparation of (*S*)-MTPA esters (**1a**). Completion of the reaction was monitored by ESI-MS. The reaction mixture was dried *in vacuo* and redissolved in EtOAc, washed with H₂O, and purified on a C18 HPLC using 30% H₂O in MeOH as eluent to obtain **1a** (0.5 mg). **1a**: yellowish oil; ¹H NMR (CD₃OD) δ 0.88 (H-16 and H-17), 1.283 (brs., H-12), 1.43 (H-13), 1.98 (m, H-15), 3.53 (OCH₃, s), 7.35–7.60 (15H, m), ESIMS *m/z* 546.1234 [M + Na]⁺.

(R)-MTPA Esters (1b). In an entirely analogous way, the (*R*)-MTPA esters (**1b**) was prepared using (*S*)-(+)-MTPA-Cl. **1b**: yellowish oil (0.6 mg); ¹H NMR (CD₃OD) 0.89 (d, H-16, H-17), δ 1.278 (brs. H-12), 1.41 (H-13), 2.02 (m, H-15), 3.51 (OCH₃, s), 7.31–7.50 (15H, m), ESIMS *m/z* 546.1223 [M + Na]⁺.

(S)- and (R)-MTPA esters (2a and 2b) of 2. (*S*)- and (*R*)-MTPA ester derivatives of **2** were prepared from (*R*)-(–)-and (*S*)-(+)-MTPA-Cl, respectively, according to the procedure described above. All ¹H signals were assigned by ¹H–¹H COSY experiment. **2a** (0.6 mg): ¹H NMR (CD₃OD) 0.89 (d, H-16, H-17), δ 1.287 (brs. H-12), 1.42 (H-13), 1.99 (m, H-15), 3.55 (OCH₃, s), 7.30–7.50 (15H, m), ESIMS *m/z* 546.1323 [M + Na]⁺. **2b** (0.5 mg): ¹H NMR (CD₃OD) 0.90 (d, H-16, H-17), δ 1.281 (brs. H-12), 1.40 (H-13), 2.03 (m, H-15), 3.52 (OCH₃, s), 7.31–7.50 (15H, m), ESIMS *m/z* 546.1332 [M + Na]⁺.

TLC comparison of glucopyranose of 1 and 2. MeOH : Ethyl acetate (1:4) solvent system was developed for TLC analysis of glucopyranose. Both authentic α and β -D-glucopyranose were run using the solvent system and *rf* value was recorded. Similarly, glucopyranoses of compound **1** and **2** were run using the same solvent system.

Antimicrobial test. Broth dilution assay was employed to evaluate minimum inhibitory concentrations (MICs) of compounds **1** and **2** against bacteria and fungi (Table 3). Antibacterial and antifungal tests were performed in nutrient broth and yeast maltose broth, respectively. Over the range of 0.5–256 μ g/mL, a serial double dilution of each compound was prepared in 96-microtiter plates. Each strain was allowed to grow overnight and final concentration of organisms in each culture was adjusted to 1.5×10^8 cfu/mL by comparing the culture turbidity with the 0.5 McFarland Standard. Culture broth (30 μ L) was added to each dilution of compounds **1** and **2** and the final volume of each well was adjusted to 200 μ L using the respective culture medium, and the plates were incubated 24 h at 37 °C for bacteria and 48 h at 30 °C for fungi. The minimum inhibitory concentration (MICs) is the lowest concentration of a sample at which the microorganism did not demonstrate visible growth, as indicated by the presence of turbidity.

Cytotoxicity test. Cancer cell growth inhibitory activity of compound **1** and **2** were performed according to a sulforhodamine B (SRB) assay. In brief, 96-well plate was loaded with selected cell lines (MDA-MB-231, HCT-15, PC-3, NCI-H23, NUGC-3, and ACHN) and tested samples **1** and **2** of concentration 30, 10, and 3 μ g/mL were added. After incubation of 48 hr. cell monolayer's are fixed with 50% (wt/vol) trichloroacetic acid and stained for 60 min. Access dye was washed with SRB solution (0.4% sulforhodamine B in 1% acetic acid). The protein-bound dye is dissolved in 10 mM Tris base solution and absorbance was measured at 510 nm using a microplate reader. GI₅₀ values of compound **1** and **2** were then calculated using graphed prism software.

Table 2. ¹H and ¹³C NMR data for compounds **1** and **2** in CD₃OD

Position	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
HMA				
1		176.3 173.2 ^a		176.9 173.3 ^a
2	2.22, t (7.5)	36.9	2.25, t (8.0)	36.9
3	1.61, m	27.0	1.62, m	26.9
4-11	1.29, brs.	29.9-30.9	1.29, brs.	29.0-30.9
12	1.29, brs.	26.6	1.29, brs.	26.6
13	1.46, m	32.2	1.45, m	32.2
14	3.42, m	85.6	3.43, m	85.6
15	1.87, m	32.3	1.88, m	32.3
16	0.93, d (6.5)	18.3	0.93, d (7.0)	18.3
17	0.93, d (6.5)	18.7	0.93, d (7.0)	18.7
Ala			Gly	
1'	12.4 brd. ^a	176.3 174.3 ^a	12.2, brd. ^a	173.2 171.5 ^a
2'	4.37, dd (7.0, 14.5)	49.3 47.3 ^a	3.89, s	41.9
3'	1.38, d (7.5)	17.8		
NH	8.05, d ^a		8.02, d ^a	
SA				
1''		174.0 171.8 ^a		174.1 171.5 ^a
2''	2.61, m	30.2	2.62, m	26.64
3''	2.61, m	29.9	2.61, m	26.99
4''	12.4, brd ^a	175.9 173.2 ^a	12.2, brd ^a	175.9 172.6 ^a
Glu				
1'''	4.29, d (8.0)	104.2	4.29, d (8.0)	104.2
2'''	3.18, dd (8.5, 12.7)	75.5	3.19 dd (8.0, 13.0)	75.5
3'''	3.33, m	78.1	3.33, m	78.2
4'''	3.28, m	71.9	3.28, m	72.0
5'''	3.40, m	75.1	3.40, m	75.2
6a'''	4.18, dd (6.0, 12.5)	65.2	4.18, dd (6.5, 12.0)	
6b'''	4.44 d (11.5)		4.44, d (10.0)	65.3

^aChemical shifts and coupling constant were determined in DMSO-*d*₆.The assignments were aided by ¹H-¹H COSY, ROESY, HSQC, and HMBC NMR spectra.SI 2. NMR data for Ieodoglucomides A (**1**) and Ieodoglucomide B (**2**).

SI 3. ^1H NMR spectrum of compound **1** in CD_3OD at 500 MHz.

leodoglucomide A (1)

SI 4. ^{13}C NMR spectrum of compound **1** in CD_3OD at 125 MHz.

Compound-1-HSQC-cd3od

SI 5. HSQC spectrum of compound **1** in CD₃OD.

Compound-1-COSY-cd3od

SI 6. COSY spectrum of compound **1** in CD₃OD.

Compound-1-HMBC-cd3od

SI 7. HMBC spectrum of compound **1** in CD_3OD .

leodoglucomide A (1)

SI 8. ^1H NMR spectrum of compound **1** in $\text{DMSO}-d_6$ at 500 MHz.

leodoglucomide A (1)

SI 9. ^{13}C NMR spectrum of compound **1** in $\text{DMSO}-d_6$ at 125 MHz.

Compound-1-COSY-DMSO

SI 10. COSY spectrum of compound **1** in DMSO- d_6 .

Compound-1-HMBC-dmso

SI 11. HMBC spectrum of compound **1** in DMSO- d_6 .

Compound-1-ROESY-dmso

SI 12. ROESY spectrum of compound **1** in DMSO-*d*₆.

Data File: D:\WESI 기기지원 Data\W20110909\sample7.lcd

Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	0	300	O	2	0	20	H
C	4	0	150					
N	3	1	1					

Error Margin (ppm): 10
 HC Ratio: unlimited
 Max Isotopes: all
 MSn Iso RI (%): 75.00

DBE Range: not fixed
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: no
 Isotope Res: 10000
 Max Results: 10

Event#: 2 MS(E-) Ret. Time : 0.200 Scan#: 32

Measured region for 618.3497 m/z

C30 H53 N O12 [M-H] - : Predicted region for 618.3495 m/z

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	83.73	C30 H53 N O12	[M-H] -	618.3497	618.3495	0.2	0.32	83.73	5.0

leodoglucomide A (1)

SI 13. HRESIMS spectrum of compound 1.

Compound-2-13C-CD3OD

leodoglucomide B (2)

SI 15. ¹³C NMR spectrum of compound 2 in CD₃OD at 125 MHz.

Compound-2-gHSQC-CD3OD

SI 16. HSQC spectrum of compound 2 in CD₃OD.

Compound-2-gCOSY-CD300

SI 17. COSY spectrum of compound **2** in CD₃OD.

Compound-2-gHMBC-CD3OD

SI 18. HMBC spectrum of compound **2** in CD₃OD.

Compound-2-1H-DMSO

leodoglucomide B (2)

SI 19. ¹H NMR spectrum of compound 2 in DMSO-*d*₆ at 500 MHz.

leodoglucomide B (2)

SI 20. ^{13}C NMR spectrum of compound **2** in $\text{DMSO}-d_6$ at 125 MHz.

Compound-2-gCOSY-dmsO

SI 21. COSY spectrum of compound 2 in DMSO- d_6 .

SI 22. ROESY spectrum of compound **2** in DMSO- d_6 .

Data File: D:\WESI 기기지원 Data\20110909\sample5.lcd

Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	0	300	O	2	0	20	H
C	4	0	150					
N	3	1	1					

Error Margin (ppm): 10
 HC Ratio: unlimited
 Max Isotopes: all
 MSn Iso RI (%): 75.00

DBE Range: not fixed
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: no
 Isotope Res: 10000
 Max Results: 10

Event#: 2 MS(E-) Ret. Time : 0.280 Scan#: 44

Measured region for 604.3337 m/z

C29 H51 N O12 [M-H] - : Predicted region for 604.3338 m/z

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	63.01	C29 H51 N O12	[M-H] -	604.3337	604.3338	-0.1	-0.17	63.01	5.0

leodoglucomide B (2)

leodoglucomide A (1)

09IDYM23-2-bEA-80%M-rp5-rp1-after-hydrolysis #80 RT: 1.00 AV: 1 NL: 1.71E5
F: {0,1} - c APCI corona sid=30.00 det=1106.00 Full ms

SI 24. ESIMS spectrum of compound **1** after acid hydrolysis.

SI. 25. HPLC comparison of Marfey's derivative of Ala in **1** with standard L- and D-Ala derivatives.