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Ieodomycins A−D, Antimicrobial Fatty Acids from a Marine *Bacillus* sp.

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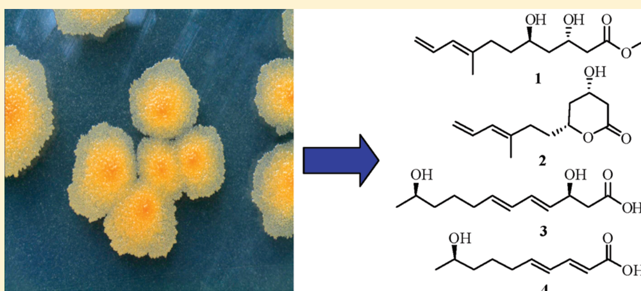
S Supporting Information

ABSTRACT: Bioassay-guided isolation of the EtOAc extract of a marine *Bacillus* sp., cultured in modified Bennett's broth medium, yielded four new antimicrobial fatty acids, named ieodomycins A−D (**1**−**4**). The planar structures of these new compounds were determined by extensive 1D and 2D NMR and HRESIMS spectroscopic data analysis. Their absolute configurations were elucidated by modified Mosher's method and literature data review. All four new compounds (**1**−**4**) demonstrated antimicrobial activities *in vitro*.

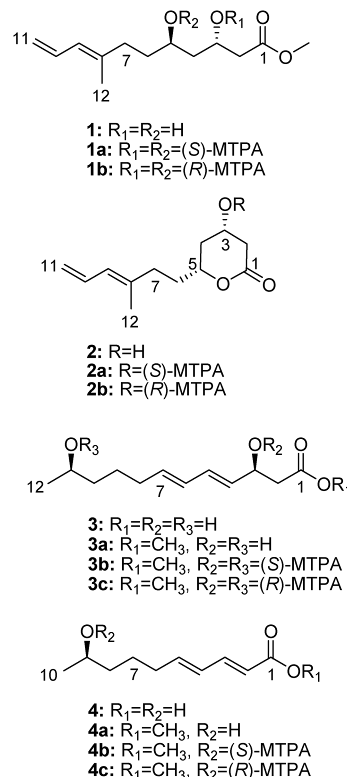


Marine organisms represent a promising source for natural products in the future due to the incredible diversity of chemical compounds and bioactivities.[1] Marine *Bacillus* species, ubiquitous and diverse in marine ecosystems,[2] are well known for producing antimicrobial[3] and anticancer compounds,[4] biosurfactants,[5] and so on. There is an urgent need for new antimicrobial agents to combat the threats imposed by antibiotic-resistant pathogenic bacteria, limited efficacy of antifungal drugs, and the recent advent of bioterrorism.[6] Marine bacteria isolated from the sediments and the surface of marine algae and invertebrates have been shown to produce secondary metabolites that display antibacterial properties.[7]

As a part of our continuing program on the discovery of bioactive secondary metabolites from marine microorganisms, we isolated a marine bacterium from a sediment sample collected from Ieodo, Republic of Korea's southern reef. This strain, designated as 09ID194, was identified as *Bacillus* sp. based on 16S rDNA sequencing. The EtOAc extract of the culture broth of strain 09ID194 showed potent antimicrobial activity. Bioassay-guided isolation and repeated chromatographic steps led to the isolation of four new antimicrobial compounds, ieodomycins A−D (**1**−**4**). This paper reports the isolation, structure elucidation, and antimicrobial activities of these compounds (**1**−**4**).

RESULTS AND DISCUSSION

Ieodomycin A (**1**) was isolated as a yellowish, amorphous solid. The molecular formula of **1** was determined to be $C_{13}H_{22}O_4$ by extensive analysis of its combined HRESIMS and 1D and 2D NMR data, which required three degrees of unsaturation. The UV spectrum of **1** exhibited an absorption band at λ_{max} 232 nm, which was assigned to a conjugated diene. The IR absorptions at 3400 and 1715 cm^{-1} indicated the presence of



hydroxy (OH) and ester carbonyl (C=O) groups, respectively. The ^{13}C NMR and HSQC spectra displayed four olefinic carbon

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Table 1. NMR Data for Compounds 1 and 2 in CD_3OD

position	1			2		
	δ_C[a]	δ_H, mult. (J in Hz)[b]	HMBC[c]	δ_C[a]	δ_H, mult. (J in Hz)[b]	HMBC[c]
1	174.0			174.1		
2	43.9	2.47, dd (10.0, 5.0)	1, 3, 4	40.2	2.36, dd (16.8, 7.3, H-2ax)	3, 4
					2.86, dd (16.8, 1.3, H-2eq)	
3	66.6	4.25, m		64.4	4.19, dddd (10.4, 7.3, 3.5, 1.3, H-3ax)	1
4	45.3	1.52, m	5	38.7	2.24, ddd (13.5, 10.4, 7.3, H-4ax)	2, 3
					2.27, ddd (13.5, 4.2, 3.5, H-4eq)	
5	68.8	3.76, m	3	78.5	4.24, dddd (10.4, 7.3, 4.2, 3.5, H-5ax)	4
6	37.5	1.56, m	4, 5, 7, 8	34.9	1.80, m	5, 7
7	37.0	2.10, m	5, 6, 8, 9, 12	36.1	2.18, m	5, 6, 8, 9, 12
		2.19, m			2.25, m	
8	140.1			139.0		
9	127.0	5.86, d (10.5)	7, 10, 11, 12	127.6	5.88, d (10.6)	7, 12
10	134.7	6.57, ddd (16.7, 10.5, 10.5)	8, 9	134.5	6.58, ddd (16.6, 10.6, 10.6)	
11	115.1	4.93, d (10.5, H-11b)	9, 10	115.6	4.96, dd (10.6, 2.2, H-11b)	9, 10
		5.04, d (16.7, H-11a)			5.07, dd (16.6, 2.2, H-11a)	
12	16.8	1.75, s	7, 8, 9	16.6	1.77, s	7, 8, 9
OCH_3	52.1	3.67, s	1			

[a] Measured at 125 MHz. [b] Measured at 500 MHz. [c] HMBC correlations are from proton(s) stated to the indicated carbon(s).

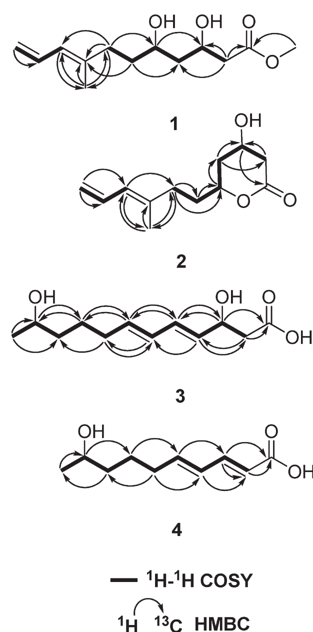

Figure 1. $^1H-^1H$ COSY and HMBC correlations for compounds 1−4.

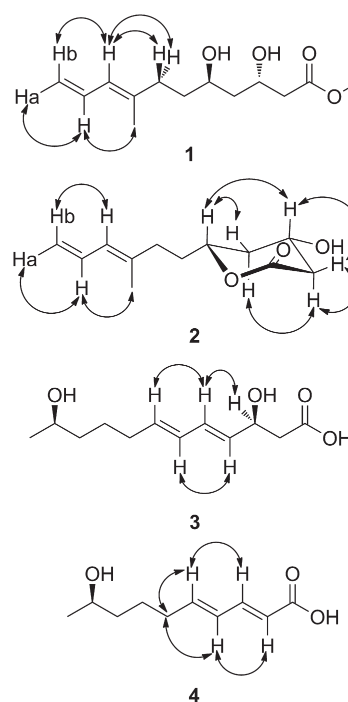

Figure 2. Key ROESY correlations for compounds 1−4.

signals (δ_C 115.1−140.1) including an unusual terminal olefinic methylene carbon (δ_C 115.1), four methylene carbons (δ_C 37.0−45.3), two oxygenated methine carbons (δ_C 66.6 and 68.8), two methyl carbons (δ_C 16.8 and 52.1), one of which was oxygenated, and an ester carbonyl carbon (δ_C 174.0) (Table 1). Analysis of the $^1H-^1H$ COSY spectrum suggested two isolated spin systems: one from H_2-2 at δ_H 2.47 to H_2-7 at δ_H 2.10/2.19 and another from H-9 at δ_H 5.86 to H_2-11 at δ_H 4.93/5.04. Their connectivity with C-8 (δ_C 140.1) was established by long-range correlations of H_2-7 with C-8 and C-9 (δ_C 127.0) (Figure 1). The connectivity of the methoxy (OCH_3) group (δ_H 3.67, s) with carbonyl carbon C-1 (δ_C 174.0) was readily determined, as its protons showed an HMBC correlation with C-1 (Figure 1). Similarly, the remaining methyl group signal (δ_H 1.75, s, H_3-12) showed HMBC cross-peaks with C-7, C-8, and C-9, and from these correlations, its connectivity with C-8 was confirmed (Figure 1). From these correlation data, the planar structure of compound 1 was established. ROESY correlations were observed between H-10 and H_3-12 and between H-9

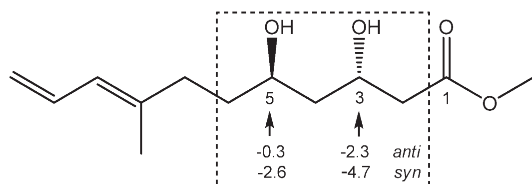

Figure 3. Assignment of the relative configuration of the 3,5-diol of **1** based on Kishi's Universal NMR Database (Database 2). $\Delta\delta_C$ values between the model system and **1** are shown. The relative configuration of the 3,5-diol is assigned as *anti* on the basis of the best fit with the model system.

and H-11b (δ_H 4.93), but no correlation was found between H-9 and H_3-12 (Figure 2). From these correlations, the geometry of the C-8/C-9 trisubstituted double bond was established as *E*. The relative configuration of the 3,5-diol of **1** was assigned by using Kishi's Universal NMR Database (Database 2).[8−10] The ^{13}C NMR resonances of C-3/C-5 were in good agreement with an *anti* arrangement of the 1,3-diol model system (Figure 3). Assignment of the absolute configuration of the 3,5-diol of **1** was carried out by the modified Mosher's ester method.[11−14] Compound **1** was treated with (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) in dry pyridine separately to yield bis-(*S*)- and (*R*)-MTPA ester derivatives **1a** and **1b**, respectively. All proton signals of the two diester derivatives were assigned by $^1H-^1H$ COSY experiments, and the ^1H chemical shift values of the bis-(*R*)-MTPA ester (**1b**) were subtracted from the corresponding values of the bis-(*S*)-MTPA ester (**1a**). The $\Delta\delta_H$ values [$\Delta\delta_H = \delta_S - \delta_R$] are shown in Figure 4. Negative $\Delta\delta_H$ values for H-3 (−0.11), H-4 (−0.12/−0.13), and H-5 (−0.17), corresponding to the $\Delta\delta_H$ pattern for diesters of *anti*-1,3-diols reported by Riguera,[13] indicated that the absolute configurations of C-3 and C-5 of **1** were *S* and *R*, respectively. The structure of **1** was therefore conclusively determined to be (3*S*,5*R*,8*E*)-methyl 3,5-dihydroxy-8-methylundeca-8,10-dienoate, and **1** was named ieodomycin A.

Ieodomycin B (**2**) was isolated as an optically active, white, amorphous solid. The molecular formula of **2** was determined to be $C_{12}H_{18}O_3$ (one carbon, four hydrogens, and one oxygen atom less than **1**) from the HRESIMS spectrum, which gave a molecular ion peak ($[M + Na]^+$) at *m*/*z* 233.1145. Its IR spectrum indicated the presence of hydroxy (3365 cm^{-1}) and δ-lactone (1715 cm^{-1}) groups. Comparison of its UV, IR, and ^1H and ^{13}C NMR (Table 1) data with those of **1** indicated that **2** had a similar skeleton to **1**, except that the methoxy (OCH_3) group attached to C-1 in **1** was absent and instead C-1 was attached to the oxygen at C-5 in **2**, forming a δ-lactone ring. By detailed analysis of UV, IR, and 1D and 2D NMR (Figure 1) data, the planar structure of **2** was unequivocally established. The relative configuration of **2** was deduced on the basis of $^3J_{H-H}$ coupling constants and ROESY spectroscopic data. The ROE correlations of H-10 with H_3-12, and H-9 with H-11b, as well as no correlation between H-9 and H-10, suggested the *E* geometry of the C-8/C-9 double bond (Figure 2). The splitting pattern and chemical shifts of H_2-2 (δ_H 2.36, dd, *J* = 16.8, 7.3, H-2ax, and δ_H 2.86, dd, *J* = 16.8, 1.3, H-2eq) of the δ-lactone ring clearly established the *anti* relative configuration of C-3 and C-5.[15] The large vicinal coupling constant (*J* = 7.3) between H-2ax and H-3ax indicated that the 3-OH group was in an equatorial position, and this was confirmed by a ROESY correlation between H-2eq and H-3ax. A *trans*-diaxial coupling (*J* = 10.4)

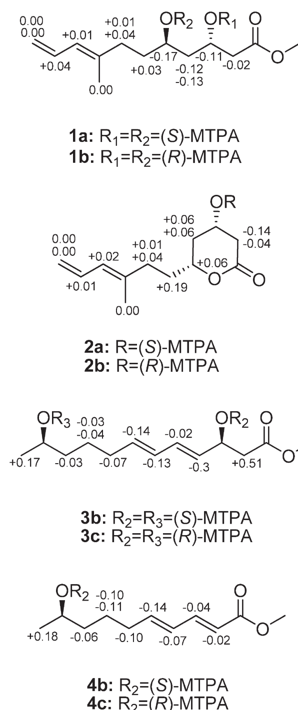

Figure 4. $\Delta\delta_H$ values ($\Delta\delta_H = \delta_S - \delta_R$) for MTPA esters of **1**−**4**.

was observed between H-3ax and H-4ax, which also supported the equatorial position of the 3-OH group. The axial position of H-5 was indicated by its coupling constants with H-4ax (*J* = 7.3) and H-6ax (*J* = 10.4) and also supported by a ROESY correlation between H-4eq and H-5ax (Figure 2). ROESY correlations between H-2ax and H-4ax and between H-3ax and H-5ax confirmed the assignments. These correlation data suggested a chair conformation for the δ-lactone ring.[16] The absolute configuration of **2** at C-3 was determined by application of the modified Mosher's method as for **1**. Acylation of **2** with (*R*)-(−)- and (*S*)-(+)-MTPA-Cl furnished the mono-(*S*)- and (*R*)-MTPA esters (**2a** and **2b**), respectively. Analysis of the ^1H NMR data (Figure 4) for these MTPA esters indicated that the absolute configuration at C-3 was *S*. The configuration of C-5 in compound **2** was determined as *R* based on the *anti* relationship between C-3 and C-5, which was suggested by the coupling constants and ROESY correlations.[16] Comprehensive 1D and 2D spectroscopic data analysis led to the determination of structure of **2** as (3*S*,5*R*,8*E*)-3-hydroxy-5-(8-methylhexa-8,10-dienyl)tetrahydro-1*H*-pyran-1-one (ieodomycin B).

Ieodomycin C (**3**), a pale, amorphous solid, analyzed for $C_{12}H_{20}O_4$ on the basis of its parent ion in the HRESIMS (*m*/*z* 227.1288 $[M - H]^-$) and the ^1H and ^{13}C NMR data (Table 2), indicating three degrees of unsaturation. Its IR spectrum disclosed absorption bands at 3349 and 1711 cm^{-1} assignable to hydroxy (OH) and carbonyl (C═O) groups, respectively. The UV spectrum exhibited an absorption maximum at λ_{max} 230 nm, which was attributed to a conjugated diene. The proton and carbon signals of **3** (Table 2) were unambiguously assigned by 2D NMR experiments ($^1H-^1H$ COSY, HSQC, and HMBC). The resonances in the ^1H and ^{13}C NMR spectra (Table 2) were well separated, and a single spin system from H_2-2 at δ_H 2.45 to H_3-12 at δ_H 1.13 of **3** was determined in a stepwise fashion by analysis of the $^1H-^1H$ COSY spectrum (Figure 1). Both of the

Table 2. NMR Data for Compounds 3 and 4 in CD_3OD

	3			4		
position	δ_C[a]	δ_H, mult. (J in Hz)[b]	HMBC[c]	δ_C[a]	δ_H, mult. (J in Hz)[b]	HMBC[c]
1	175.6			171.5		
2	43.7	2.45, d (6.0)	1, 3, 4	121.3	5.78, d (15.3)	1, 4
3	70.2	4.50, m	1, 2, 4, 5	146.5	7.21, dd (15.3, 10.5)	1, 2, 4, 5
4	133.6	5.60, dd (15.3, 6.5)	2, 3, 6	130.0	6.24, dd (15.3, 10.5)	2, 3, 5, 6
5	132.1	6.22, dd (15.3, 10.5)	3, 6, 7	145.3	6.16, m	3, 4, 6, 7
6	131.0	6.03, dd (15.3, 10.5)	5, 8	34.0	2.20, m	4, 5, 7, 8
7	136.1	5.70, dt (15.3, 7.0)	5, 8, 9	26.2	1.45, m	5, 6, 8, 9
					1.56, m	
8	33.7	2.10, m	6, 7, 9, 10	39.7	1.43, m	7, 9, 10
9	26.7	1.38, m	8, 11	68.4	3.72, m	7
		1.50, m				
10	39.8	1.42, m		23.6	1.14, d (6.0)	8, 9
11	68.5	3.70, m	9			
12	23.6	1.13, d (6.0)	10, 11			

[a] Measured at 125 MHz. [b] Measured at 500 MHz. [c] HMBC correlations are from proton(s) stated to the indicated carbon(s).

double bonds at C-4/C-5 and C-6/C-7 had an *E* geometry, which was supported by large scalar coupling constants between H-4 and H-5 (J = 15.3 Hz) and between H-6 and H-7 (J = 15.3 Hz). This was also confirmed by ROESY correlations between H-4 and H-6 and between H-5 and H-7 (Figure 2). Like compounds **1** and **2**, conversion of compound **3a** into the Mosher's diesters (*S*)-MTPA (**3b**) and (*R*)-MTPA (**3c**) followed by analysis of the ^{1}H NMR data (Figure 4) indicated the *S* and *R* absolute configurations at the C-3 and C-11 stereocenters in **3**, respectively. The structure of **3** was established conclusively as (3*S*,4*E*,6*E*,11*R*)-3,11-dihydroxydodeca-4,6-dienoic acid (iеodomycin C).

Ieodomycin D (**4**) was obtained as an optically active, light yellowish, amorphous solid. The molecular formula of **4** was established as $C_{10}H_{16}O_3$ by HRESIMS and ^{13}C NMR data, having two carbons, four hydrogens, and one oxygen atom less than **3** and the same degrees of unsaturation. Its UV and IR spectra were quite similar to those of **3**, indicating the presence of a carbonyl group (1684 cm^{-1}), a hydroxy group (3365 cm^{-1}), and a conjugated diene moiety (λ_{max} 254 nm). Assignments of all the protonated carbons were done by the analysis of the HSQC spectrum as shown in Table 2. The ^{1}H−^{1}H COSY data of **4** revealed only one spin system starting from H-2 (δ_H 5.78) and ending at H_3-10 (δ_H 1.14). An $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl moiety was established as H-2 at δ_H 5.78 and H-4 at δ_H 6.24 showed long-range correlations with C-1 (δ_C 171.5) and C-3 (δ_C 146.5), respectively. The *E* geometries of the C-2/C-3 and C-4/C-5 double bonds were assigned on the basis of the large coupling constants between H-2 and H-3 (J = 15.3 Hz) and between H-4 and H-5 (J = 15.3 Hz) (Table 2) and were confirmed by ROESY correlations (Figure 2). The absolute configuration of the stereogenic center at C-9 in **4** was addressed by the modified Mosher's method; each mono-(*S*)- and (*R*)-MTPA ester was separately prepared and subjected to ^{1}H NMR data analysis. The *R* configuration of C-9 in **4** was determined on the basis of the guidelines.[11] Compound **4** was named ieodomycin D, and the structure was unambiguously established as (2*E*,4*E*,9*R*)-9-hydroxydeca-2,4-dienoic acid.

The antimicrobial activities of **1**−**4** were tested against *Bacillus subtilis* (KCTC 1021), *Escherichia coli* (KCTC 1923), and *Saccharomyces cerevisiae* (KCTC 7913). Compounds **1**−**4** exhibited activity against *Bacillus subtilis* and *Escherichia coli* with minimum inhibitory concentrations (MICs) of 32−64 μg/mL, but showed only weak growth inhibition against the yeast *Saccharomyces cerevisiae*, with an MIC of 256 μg/mL. In comparison, long-chain and branched polyunsaturated fatty acids isolated from freshwater sponges showed no antimicrobial activity against *E. coli* and *S. cerevisiae* and weak activity against *B. subtilis.*[17] An unsaturated dihydroxy acid isolated from the fungus *Mycosphaerella rubella*, 6*S*,13*R*-dihydroxy-2*E*,4*E*,8*E*-tetradecatrienoic acid, which has a similar structure to **2**, showed weak antibacterial but no antiyeast activities.[12]

Polyunsaturated branched and unbranched fatty acids are widely found in marine organisms including bacteria, cyanobacteria, algae,[18] and dinoflagellates,[19] and most of them are biologically inactive. By comparing the antibacterial activity among different fatty acids, it has been shown that unsaturated and hydroxy fatty acids showed better antibacterial activities.[20−22] Even though there have been several reports regarding the mode of action of long-chain unsaturated fatty acids, the precise mechanism for the antimicrobial activity remains unclear. Recently, it was suggested that the antimicrobial activity of unsaturated fatty acids is related to their inhibition of bacterial fatty acid synthesis.[23]

In conclusion, we discovered four new rare hydroxy unsaturated fatty acids, ieodomycins A−D (**1**−**4**), which showed antibacterial activity against both Gram-positive and Gram-negative pathogens. Interestingly compounds **1**−**4** were produced by this *Bacillus* sp. only in low salinity (12 g/L) but not in high salinity (32 g/L) culture medium.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO (DIP-1000) digital polarimeter. UV spectra were obtained on a Shimadzu UV-1650PC spectrophotometer. IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. 1D and 2D NMR spectra were acquired on a Varian Unity 500 spectrometer in CD_3OD. High-resolution mass spectra were recorded on a hybrid ion-trap time-of-flight mass spectrometer (Shimadzu LCMS-IT-TOF).

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Analytical HPLC was conducted on a PrimeLine binary pump with RI-101 (Shodex) and variable UV detector (M 525). Continuous centrifugation was done on a centrifugal separator (Kansai Centrifugal Separator Manufacturing Co. Ltd.). Semipreparative HPLC was performed using ODS (YMC-Pack-ODS-A, 250 × 10 mm, i.d. 5 μm) and silica (YMC-Pack-SIL, 250 × 10 mm, i.d. 5 μm) columns. Analytical HPLC was conducted on an ODS column (YMC-Pack-ODS-A, 250 × 4.6 mm, i.d. 5 μm). All solvents used were either spectral grade or distilled prior to use.

Isolation and Taxonomy of Strain 09ID194. The strain 09ID194 was isolated from a sediment sample collected from Ieodo, Republic of Korea's southern reef, during an expedition in 2009. In brief, one gram of the sediment sample was diluted in sterilized seawater (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) under aseptic conditions, and 100 μL from each dilution was spread onto modified Bennett's agar (0.1% yeast extract, 0.1% beef extract, 0.2% tryptone, 1% dextrose, 100% natural seawater, and 1.8% agar, pH adjusted to 7.2 before sterilization). The plates were incubated for 14 days at 30 °C, and the resulting colony of strain 09ID194 was isolated and maintained on modified Bennett's agar. The strain 09ID194 formed well-developed, yellowish colonies on modified Bennett's agar. The strain was identified as *Bacillus* sp. on the basis of 16S rDNA sequence analysis. The sequence was deposited in GenBank under accession number JN048684. This strain is currently preserved in the Microbial Culture Collection, KORDI, with the name *Bacillus* sp. 09ID194 under the curatorship of H.J.S.

Seed and Large-Scale Cultures of Strain 09ID194. The seed and large-scale cultures were carried out in modified Bennett's medium (0.1% yeast extract, 0.1% beef extract, 0.2% tryptone, and 1% dextrose, salinity 12 g/L, pH 7.6). The medium (200 mL) was dispensed in a 500 mL conical flask and sterilized. A single colony of 09ID194 strain from the agar plate was inoculated aseptically into the flask and incubated at 24 °C for 2 days on a rotary shaker at 120 rpm. An aliquot (0.2% v/v) from the seed culture was inoculated aseptically into 2 L flasks (total 100 flasks) containing 1 L of sterilized culture medium. The production culture was incubated under the same conditions as the seed culture for 7 days and then harvested.

Extraction and Isolation. The production culture broth (100 L) was centrifuged, and the supernatant was extracted with EtOAc (2 × 100 L). The EtOAc layer was concentrated to dryness using rotary evaporators at 40 °C. The residual suspension (20 g) was subjected to ODS open column chromatography followed by stepwise gradient elution with MeOH−H_2O (v/v) (1:4, 2:3, 3:2, 4:1, and 100:0) as eluent. The fraction eluted with MeOH−H_2O (3:2, v/v) was subjected to further fractionations by semipreparative ODS HPLC (50% MeOH in H_2O; flow rate, 1.5 mL/min; detector, RI) to obtain nine fractions (F-1−9). Compounds **1**−**4** were purified by normal-phase semipreparative HPLC (flow rate, 1.3 mL/min; detector, UV) from F-8, F-6, F-3, and F-4 using the following isocratic programs: 13% MeOH−EtOAc; 5% MeOH−$CHCl_3$; 10% MeOH−$CHCl_3$; 6% MeOH−$CHCl_3$, to yield pure compounds **1** (3.4 mg), **2** (3.4 mg), **3** (3.3 mg), and **4** (5.5 mg), respectively.

*Ieodomycin A (**1**):* yellowish, amorphous solid; $[\alpha]^{23}_{D}$ +19 (*c* 0.9, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 232 (4.08) and 201 (2.92) nm; IR (MeOH) ν_{max} 3400 (br), 2928, 1715, 1251, 1065, 839 cm^{-1}; ^{1}H and ^{13}C NMR data (CD_3OD), see Table 1; HRESIMS *m/z* 243.1587 [M + H]$^+$ (calcd for $C_{13}H_{23}O_4$, 243.1596).

*Ieodomycin B (**2**):* white, amorphous solid; $[\alpha]^{23}_{D}$ +21 (*c* 0.9, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 233 (3.17) and 201 (2.80) nm; IR (MeOH) ν_{max} 3365 (br), 2924, 1715, 1251, 1065 cm^{-1}; ^{1}H and ^{13}C NMR data (CD_3OD), see Table 1; HRESIMS *m/z* 233.1145 [M + Na]$^+$ (calcd for $C_{12}H_{18}O_3Na$, 233.1154).

*Ieodomycin C (**3**):* pale, amorphous solid; $[\alpha]^{23}_{D}$ +18 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.00) and 201 (2.67) nm; IR (MeOH) ν_{max} 3349 (br), 2924, 1711, 1407, 1278, 993 cm^{-1}; ^{1}H and ^{13}C NMR data (CD_3OD), see Table 2; HRESIMS *m/z* 227.1288 [M − H]$^-$ (calcd for $C_{12}H_{19}O_4$, 227.1283).

*Ieodomycin D (**4**):* light yellowish, amorphous solid; $[\alpha]^{23}_{D}$ +15 (*c* 0.8, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 254 (2.98) nm; IR (MeOH) ν_{max} 3365 (br), 2932, 1684, 1255, 1004 cm^{-1}; ^{1}H and ^{13}C NMR data (CD_3OD), see Table 2; HRESIMS *m/z* 183.1029 [M − H]$^-$ (calcd for $C_{10}H_{15}O_3$, 183.1021).

Preparation of Bis-(*S*)-MTPA Ester (1a). Compound **1** (0.6 mg) was dissolved in 200 μL of pyridine and stirred at room temperature (rt) for 10 min. For preparation of the bis-(*S*)-MTPA ester (**1a**) of **1**, 20 μL of (*R*)-(−)-MTPA-Cl was added to the reaction vial, and the mixture was stirred at rt for 16 h. Completion of the reaction was monitored by LC/MS. The reaction mixture was dried *in vacuo*, redissolved in EtOAc, washed with H_2O, and purified by a silica column using 10% MeOH in $CHCl_3$ as eluent to obtain **1a** (0.45 mg).

*Compound **1a**:* amorphous solid; ^{1}H NMR data (CD_3OD) δ_H 2.64 (H_2-2, dd, *J* = 10.0, 6.5 Hz), 5.34 (H-3, m), 1.92 (H-4b, m), 1.94 (H-4a, m), 4.88 (H-5, m), 1.74 (H_2-6, m), 1.91 (H-7b, m), 1.96 (H-7a, m), 5.73 (H-9, d, *J* = 10.5 Hz), 6.51 (H-10, ddd, *J* = 16.5, 10.5, 10.5 Hz), 4.96 (H-11b, d, *J* = 10.5 Hz), 5.04 (H-11a, d, *J* = 16.5 Hz), 1.67 (H_3-12, s), 3.58 (OCH_3, s), 3.54 (2 × OCH_3, s) 7.38−7.64 (10H, m); ESIMS *m/z* 697.34 [M + Na]$^+$.

*Compound **1b**:* In an entirely analogous way, bis-(*R*)-MTPA ester (**1b**) was obtained using (*S*)-(+)-MTPA-Cl. Amorphous solid (0.5 mg); ^{1}H NMR data (CD_3OD) δ_H 2.66 (H_2-2, dd, *J* = 9.0, 6.5 Hz), 5.45 (H-3, m), 2.04 (H-4b, m), 2.07 (H-4a, m), 5.05 (H-5, m), 1.71 (H_2-6, m), 1.90 (H-7b, m), 1.92 (H-7a, m), 5.72 (H-9, d, *J* = 10.0 Hz), 6.03 (H-10, ddd, *J* = 16.5, 10.0, 10.0 Hz), 4.96 (H-11b, d, *J* = 10.0 Hz), 5.04 (H-11a, d, *J* = 16.5 Hz), 1.67 (H_3-12, s), 3.59 (OCH_3, s), 3.60 (2 × OCH_3, s) 7.44−7.57 (10H, m); ESIMS *m/z* 697.45 [M + Na]$^+$.

Preparation of Mono-(*S*)- and (*R*)-MTPA Esters (2a and 2b). Compounds **2a** (0.5 mg) and **2b** (0.62 mg) were prepared in an analogous way from (*R*)-(−)- and (*S*)-(+)-MTPA-Cl, respectively, as described above for **1a** and **1b**.

*Compound **2a**:* amorphous solid; ^{1}H NMR data (CD_3OD) δ_H 2.53 (H-2b, dd, *J* = 16.8, 6.0), 2.06 (H-2a, dd, *J* = 16.8, 6.0), 5.40 (H-3, m), 2.50 (H-4b, m), 2.52 (H-4a, m), 4.41 (H-5, m), 1.80 (H_2-6, m), 2.17 (H-7b, m), 2.26 (H-7a, m), 5.90 (H-9, d, *J* = 11.0), 6.59 (H-10, ddd, *J* = 16.5, 11.0, 11.0), 4.97 (H-11b, d, *J* = 11.0), 5.07 (H-11a, d, *J* = 16.5), 1.77 (H_3-12, s), 3.53 (OCH_3, s), 7.37−7.50 (5H, m); ESIMS *m/z* 449.24 [M + Na]$^+$.

*Compound **2b**:* amorphous solid; ^{1}H NMR data (CD_3OD) δ_H 2.67 (H-2b, dd, *J* = 17.0, 5.5), 3.10 (H-2a, dd, *J* = 17.0, 5.5), 5.56 (H-3, m), 2.44 (H-4b, m), 2.46 (H-4a, m), 4.35 (H-5, m), 1.61 (H_2-6, m), 2.16 (H-7b, m), 2.22 (H-7a, m), 5.88 (H-9, d, *J* = 10.5), 6.58 (H-10, ddd, *J* = 16.5, 10.5, 10.5), 4.97 (H-11b, d, *J* = 10.5), 5.07 (H-11a, d, *J* = 16.5), 1.77 (H_3-12, s), 3.52 (OCH_3, s), 7.40−7.57 (5H, m); ESIMS *m/z* 449.26 [M + Na]$^+$.

Preparation of Compound 3a. Compound **3** (2.0 mg) was dissolved in 1 mL of anhydrous MeOH, and 200 μL of 2 M (trimethylsilyl)diazomethane was added to the solution. The reaction mixture was stirred at rt, and completion of the reaction was confirmed by LC/MS analysis in 1 h. The reaction mixture was dried *in vacuo*, and purification by analytical ODS HPLC using 65% MeOH in H_2O provided the methyl ester of compound **3** (**3a**, 1.8 mg).

*Compound **3a**:* pale, amorphous solid; ^{1}H NMR data (CD_3OD) δ_H 2.50 (H_2-2, d, *J* = 6.5 Hz), 4.51 (H-3, m), 5.59 (H-4, dd, *J* = 15.3, 7.0 Hz), 6.22 (H-5, dd, *J* = 15.3, 10.3 Hz), 6.04 (H-6, dd, *J* = 15.3, 10.3 Hz), 5.71 (H-7, dt, *J* = 15.3, 7.0 Hz), 2.10 (H_2-8, m), 1.38 (H-9b, m), 1.50 (H-9a, m), 1.42 (H_2-10, m), 3.71 (H-11, m), 1.14 (H_3-12, d, *J* = 6.5 Hz), 3.67 (OCH_3, s); ESIMS *m/z* 241.33 [M − H]$^-$.

Bis-(*S*)-MTPA Ester Preparation (3b). Compound **3a** (0.9 mg) was dissolved in 1 mL of pyridine and stirred at rt for 30 min in a vial. A few crystals of 4-dimethylamiopyridine were added, and the solution was stirred for another 30 min. For preparation of the bis-(*S*)-MTPA

ester (**3b**) of **3a**, 10 μL of (*R*)-(−)-MTPA-Cl was added, and the reaction was stirred at rt for 3 h. Completion of the reaction was monitored by LC/MS. The reaction was quenched by adding 200 μL of MeOH. The reaction mixture was dried *in vacuo*, redissolved in H_2O, and extracted with EtOAc (3 × 3 mL). The extract was purified on an ODS analytical column using 67% MeOH in H_2O as eluent to obtain **3b** (0.7 mg).

*Compound **3b**:* pale, amorphous solid; ^{1}H NMR data (CD_3OD) δ_H 3.01 (H_2-2, d, J = 6.5 Hz), 4.51 (H-3, m), 5.55 (H-4, dd, J = 15.0, 7.5 Hz), 6.20 (H-5, dd, J = 15.0, 10.0 Hz), 5.92 (H-6, dd, J = 15.0, 10.0 Hz), 5.56 (H-7, dt, J = 15.0, 6.0 Hz), 1.98 (H_2-8, m), 1.35 (H-9b, m), 1.50 (H-9a, m), 1.48 (H_2-10, m), 3.71 (H-11, m), 1.14 (H_3-12, d, J = 6.5 Hz), 3.67 (OCH_3, s), 3.54 (2 × OCH_3, s), 7.35−7.60 (10H, m); ESIMS m/z 697.33 $[M + Na]^+$.

Bis-(*R*)-MTPA Ester Preparation (3c). Bis-(*R*)-MTPA ester of compound **3a** (0.9 mg) was prepared from (*S*)-(+)-MTPA-Cl similarly to **3b**. Bis-(*R*)-MTPA ester of compound **3a** was purified on an ODS analytical column using 65% MeOH in H_2O as eluent to obtain **3c** (0.6 mg).

*Compound **3c**:* pale, amorphous solid; ^{1}H NMR data (CD_3OD) δ_H 2.50 (H_2-2, d, J = 6.5 Hz), 4.51 (H-3, m), 5.85 (H-4, dd, J = 15.0, 8.5 Hz), 6.22 (H-5, dd, J = 15.0, 10.0 Hz), 6.05 (H-6, dd, J = 15.0, 10.0 Hz), 5.70 (H-7, dt, J = 15.0, 6.0 Hz), 2.05 (H_2-8, m), 1.39 (H-9b, m), 1.53 (H-9a, m), 1.51 (H_2-10, m), 3.71 (H-11, m), 1.13 (H_3-12, d, J = 6.5 Hz), 3.67 (OCH_3, s), 3.54 (2 × OCH_3, s), 7.36−7.60 (10H, m); ESIMS m/z 673.48 $[M - H]^-$.

Preparation of Compound 4a. Compound **4** (3.0 mg) was dissolved in 2 mL of anhydrous MeOH. The methyl ester of compound **4** was prepared according to the above-described method. The reaction mixture was dried *in vacuo* and purified on an analytical ODS HPLC using 60% MeOH in H_2O to obtain the methyl ester of compound **4** (**4a**, 2.3 mg).

*Compound **4a**:* yellowish, amorphous solid; ^{1}H NMR data (CD_3OD) δ_H 5.83 (H-2, d, J = 15.2 Hz), 7.26 (H-3, dd, J = 15.2, 10.3 Hz), 6.25 (H-4, dd, J = 15.2, 10.3 Hz), 6.21 (H-5, m), 2.20 (H_2-6, m), 1.45 (H-7b, m), 1.56 (H-7a, m), 1.42 (H_2-8, m), 3.71 (H-9, m), 1.14 (H_3-10, d, J = 6.5 Hz), 3.70 (OCH_3, s); ESIMS m/z 197.22 $[M - H]^-$.

(*S*)-MTPA Ester Preparation (4b). The (*S*)-MTPA ester of compound **4a** (1 mg) was prepared from (*R*)-(−)-MTPA-Cl according to the above-described method. The reaction mixture was dried *in vacuo*, redissolved in H_2O, and extracted with EtOAc (3 × 3 mL). The extract was purified on an ODS analytical column using 65% MeOH in H_2O as eluent to obtain **4b** (0.8 mg).

*Compound **4b**:* amorphous solid; ^{1}H NMR data (CD_3OD) δ_H 5.82 (H-2, d, J = 15.4 Hz), 7.23 (H-3, dd, J = 15.4, 10.7 Hz), 6.16 (H-4, dd, J = 15.4, 10.7 Hz), 6.06 (H-5, m), 2.11 (H_2-6, m), 1.36 (H-7b, m), 1.56 (H-7a, m), 1.31 (H_2-8, m), 5.13 (H-9, m), 1.33 (H_3-10, d, J = 6.0 Hz), 3.71 (OCH_3, s), 3.54 (OCH_3, s), 7.39−7.57 (5H, m); ESIMS m/z 413.08 ($[M - H]^-$), 414.95 $[M + H]^+$.

(*R*)-MTPA Ester Preparation (4c). The (*R*)-MTPA ester of compound **4a** (1 mg) was prepared from (*S*)-(+)-MTPA-Cl according to the above-described method. The (*R*)-MTPA ester of compound **4a** was purified on a semipreparative silica column using EtOAc−MeOH−*n*-hexane (4:1:1) as eluent to obtain **4c** (0.77 mg).

*Compound **4c**:* amorphous solid; ^{1}H NMR data (CD_3OD) δ_H 5.84 (H-2, d, J = 15.3 Hz), 7.27 (H-3, dd, J = 15.3, 10.0 Hz), 6.23 (H-4, dd, J = 15.3, 10.0 Hz), 6.20 (H-5, m), 2.21 (H_2-6, m), 1.46 (H-7b, m), 1.67 (H-7a, m), 1.37 (H_2-8, m), 5.71 (H-9, m), 1.15 (H_3-10, d, J = 6.0 Hz), 3.71 (OCH_3, s), 3.54 (OCH_3, s), 7.38−7.60 (5H, m); ESIMS m/z 413.07 $[M - H]^-$, 414.93 $[M + H]^+$.

Antimicrobial Assays. The minimum inhibitory concentrations of compounds **1**−**4** were determined by using a conventional broth dilution assay.[24] Compounds **1**−**4** were tested against three microbial strains: *Bacillus subtilis* (KCTC 1021), *Escherichia coli* (KCTC 1923), and *Saccharomyces cerevisiae* (KCTC 7913). Antibacterial and antiyeast tests were performed in nutrient broth and yeast maltose broth, respectively. A serial double dilution of each compound was prepared in 96-well microtiter plates over the range 0.5−512.0 μg/mL. An overnight broth culture of each strain was prepared, and the 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 (20 μL) was added to each dilution of compounds **1**−**4**, the final volume of each well was adjusted to 200 μL using the respective culture medium, and the plates were incubated for 24 h at 37 °C for bacteria and 48 h at 30 °C for the yeast.[25,26] The minimum inhibitory concentration is the lowest concentration of a sample at which the microorganism did not demonstrate visible growth, as indicated by the presence of turbidity.

ASSOCIATED CONTENT

Supporting Information. 1D, 2D NMR and HRESIMS data of compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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